



U.S. Army Medical Research Institute of Chemical Defense

USAMRICD-TR-08-08

Test of Surfactant-Based Dermal Wound Cleansers on Sulfur Mustard- Exposed Human Keratinocytes in an In Vitro Wound Healing Model

Claudia L. Henemyre-Harris
Angela L. Adkins
Augustine H. Chuang
John S. Graham

November 2008

Approved for public release; distribution unlimited

U.S. Army Medical Research
Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010-5400

DISPOSITION INSTRUCTIONS:

Destroy this report when no longer needed. Do not return to the originator.

DISCLAIMERS:

The opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army or the Department of Defense.

The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, Publication No. 85-23, 1996), and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

The use of trade names does not constitute an official endorsement or approval of the use of such commercial hardware or software. This document may not be cited for purposes of advertisement.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) November 2008		2. REPORT TYPE Technical Report		3. DATES COVERED (From - To) October 2003 to September 2006	
4. TITLE AND SUBTITLE Test of Surfactant-Based Dermal Wound Cleansers on Sulfur Mustard-Exposed Human Keratinocytes in an In Vitro Wound Healing Model				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Henemyre-Harris, CL, Adkins, AL, Chuang, AH, Graham, JS				5d. PROJECT NUMBER	
				5e. TASK NUMBER 6.2	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) US Army Medical Research Institute of Chemical Defense ATTN: MCMR-CDR-I 3100 Ricketts Point Road Aberdeen Proving Ground, MD 21010-5400				8. PERFORMING ORGANIZATION REPORT NUMBER USAMRICD-TR-08-08	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) US Army Medical Research Institute of Chemical Defense ATTN: MCMR-CDZ-P 3100 Ricketts Point Road Aberdeen Proving Ground, MD 21010-5400				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES Funded by the Defense Threat Reduction Agency, Cutaneous Therapeutics Task Area (Grant # L0007_04_RC_C). Current duty assignment of MAJ Henemyre-Harris is chief, Biochemistry and Stat Labs, Department of Pathology & ALS, Tripler Army Medical Center					
14. ABSTRACT Sulfur mustard is a chemical warfare agent that causes vesication in human skin. These blisters make the victims more susceptible to infection and delay healing of the skin. The first step in treating wounds is to cleanse the wound to prevent infection. Many dermal wound cleaners are commercially available, but little research has been conducted using these products in conjunction with SM-induced cutaneous injury. This study evaluated the impact of using commercially available wound cleansers on SM-induced wounds, in an in vitro wound healing model consisting of normal human epidermal keratinocytes seeded into six-well plates, exposed to sulfur mustard, and wounded (disruption of the cell monolayer) with a sterile wounding instrument. Cells were stained and images captured to measure percent wound fill. Pluronic F-68 surfactant and the dermal wound cleansers Saf-Clens™ and Shur-Clens® were tested. Neither Shur-Clens® nor 10% and 20% Pluronic F-68 surfactant increased wound fill, but the treatments did not harm the cells in the non-wounded areas. Saf-Clens™ killed the cells after one 2-minute treatment. Shur-Clens® may not promote wound healing, but could be safely used, unlike Saf-Clens™, to cleanse sulfur mustard-induced wounds. Other dermal wound cleansers should be used judiciously until proven nontoxic to keratinocytes.					
15. SUBJECT TERMS sulfur mustard, keratinocyte, wound healing, dermal cleansers, cutaneous, chemical warfare agents					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UNLIMITED	18. NUMBER OF PAGES 18	19a. NAME OF RESPONSIBLE PERSON Claudia L. Henemyre-Harris
a. REPORT UNCLASSIFIED	b. ABSTRACT UNCLASSIFIED	c. THIS PAGE UNCLASSIFIED			19b. TELEPHONE NUMBER (include area code) 808-433-6185

Acknowledgments

The authors wish to thank Dr. Bill Smith and Dr. Margaret Martens for scientific guidance and Theresa Nipwoda and Eric Nealley for the NHEK cell preparations. We also thank Kristen Newkirk, Shuqunta Davis, and Tracey Hamilton for technical assistance, Charlene Corun, Juanita Guzman, and Marian Nelson for SM exposures, and Robyn Lee for statistical support. This project would not have been possible without the generous start up funds from USAMRICD commanders Colonel James Romano and Colonel Gennady Platoff. This project was funded by the Defense Threat Reduction Agency, Cutaneous Therapeutics Task Area (Grant # L0007_04_RC_C) and was presented in part at the Medical Defense Bioscience Review, Hunt Valley, MD, May 2004, and at the 19th Annual Clinical Symposium on Advances in Skin & Wound Care, Phoenix, AZ, 2004.

(1) Objective: Sulfur mustard is a chemical warfare agent that causes vesication in human skin. These blisters make the victims more susceptible to infection and delay healing of the skin. The first step in treating wounds is to cleanse the wound to prevent infection. Many dermal wound cleansers are commercially available, but little research has been conducted using these products in conjunction with SM-induced cutaneous injury. The purpose of this study was to evaluate the impact of using commercially available wound cleansers on SM-induced wounds.

(2) Methods: This study used an in vitro wound healing model consisting of normal human epidermal keratinocytes seeded into six-well plates, exposed to sulfur mustard, and wounded (disruption of the cell monolayer) with a sterile wounding instrument. Cells were then stained and images captured to measure percent wound fill. Pluronic F-68 surfactant and the dermal wound cleansers Saf-Clens™ and Shur-Clens® were tested in this model.

(3) Results: Neither Shur-Clens® nor 10% and 20% Pluronic F-68 surfactant increased wound fill, but the treatments did not harm the cells in the non-wounded areas. In sharp contrast, Saf-Clens™ killed the cells after one 2-minute treatment.

(4) Conclusions: Shur-Clens® may not promote wound healing, but it could be safely used, unlike Saf-Clens™, to cleanse sulfur mustard-induced wounds. However, other dermal wound cleansers should be used judiciously until proven nontoxic to keratinocytes.

Introduction

Sulfur mustard (SM) is an oily, liquid, persistent chemical agent that can cause injury to the airway, eye, and skin. Initial clinical symptoms in the skin include erythema followed by vesication. The extent of blistering is dependent on the level of exposure to SM. Sulfur mustard (2,2'-dichlorodiethyl sulfide) reacts with nucleophilic sites such as DNA, proteins, and membrane components, and several mechanisms for damage have been suggested.¹ The basal epidermal cell is a primary target for sulfur mustard.^{2,3}

Wound healing is a complex and continuous process.⁴ Within hours after dermal injury, keratinocytes migrate into the wound. After several days, migrating keratinocytes proliferate, form a protective monolayer over the wound, and participate in epithelialization, a process that reestablishes contact with the underlying basement membrane.⁵

Current treatments for sulfur mustard injuries include deroofing, debridement, irrigation, topical antibiotics, and sterile dressings.⁶ However, these treatments need further refinement to eliminate infections, enhance cosmetic outcomes, and improve wound healing. Appropriate in vitro screening techniques, prior to animal testing, may speed up the development of clinical treatments.

The purpose of this study was to test commercially available dermal wound care products in an in vitro wound healing model of SM-induced cutaneous injury, prior to animal testing. This study tested two dermal wound cleansers, Saf-Clens™ and Shur-Clens®, and their active ingredient, Pluronic F-68 surfactant.

Materials and Methods

Cell Cultures

Normal human epidermal keratinocytes (NHEK) were obtained from Dr. William Smith's laboratory at the U.S. Army Medical Research Institute of Chemical Defense from commercial sources (Cambrex Bioscience, Walkersville, MD), as previously described.⁷ Cells (third passage) were seeded into 6-well plates (Corning Corporation, Corning, NY) and grown to 30-40% confluency in Keratinocyte Growth Medium (KGM, Cambrex Bioscience).

Sulfur Mustard Wound Production

Cells were exposed to 0, 5, or 10 μ M sulfur mustard (SM), in KGM, in a chemical surety hood for 1 hour.⁸ Cells were transferred to an incubator for an additional 2 hours of SM exposure at 37°C and 5% CO₂ and then used in the wound healing model. The 3-hour total exposure time was chosen to make this model as clinically relevant as possible and to meet laboratory safety standards for working with SM. A 3-mm wide wound (disruption of the cell monolayer) was created with a sterile wounding instrument.^{9,10,11} Cells were rinsed, and the wounded area was examined microscopically to ensure that cellular debris was removed. Wounds were marked on the bottom of the plate with a blue, ultra fine point permanent marker (Sharpie®, Sanford Corporation, Oak Brook, IL), and the wells received fresh media (with or without a surfactant treatment).

“Rinse” Surfactant Treatments

For “rinse” surfactant studies, Pluronic F-68, also known as polyoxamer 188 (P1300, Sigma Chemical Company, St. Louis, MO), was diluted in dH₂O and filter sterilized (0.2 µm). The commercial wound cleansers Saf-Clens™ (#159712, Convatec, Skillman, NJ) and Shur-Clens® (#121222, Convatec), which contain Pluronic F-68, were used at full strength. NHEKs received daily treatments of vehicle (sterile water), 10% Pluronic F-68, 20% Pluronic F-68, Saf-Clens™, or Shur-Clens® for 2 minutes at room temperature. The treatments were removed and fresh cell culture media, warmed to 37°C, was applied. The culture medium was changed daily for additional control NHEKs that received no treatments.

“Constant” Surfactant Treatments

For “constant” surfactant studies, Pluronic F-68 (P1300, Sigma Chemical Company) was diluted in KGM and filter sterilized (0.2 µm) to prepare working stocks. The surfactant was diluted to its final concentrations (0.0125, 0.025, or 0.05 % [wt/vol]) in KGM. Half volume media changes (with fresh surfactant) were performed daily. Control NHEKs received daily half volume medium changes with KGM alone. Half volume media changes were conducted to provide fresh surfactant to cells and to reduce disruption of the cellular environment within the well in the event that cells secreted autocrine factors.

Crystal Violet Stain

Cells were stained with the cytoplasmic stain crystal violet (#C3886, Sigma-Aldrich, St. Louis, MO), as previously described.¹¹ Briefly, cells were fixed with 4% paraformaldehyde, rinsed three times with 0.1M phosphate buffer, and stained for 1 minute in 0.1% crystal violet (wt/vol) in dH₂O. Cells were washed with dH₂O and air dried.

Wound Fill Measurements

Images of stained cells were captured using a digital camera (Coolpix, Nikon Instrument Inc., Lewisville, TX) and a dissecting microscope (#SZX12, Olympus, Center Valley, PA). Images were then analyzed with Image-Pro® Plus 5.0 software (Media Cybernetics, Bethesda, MD) to determine percent wound fill. Each image was analyzed three times.

Statistical Analyses

Data are reported as mean ± standard error of the mean (SEM). Statistical significance was defined as $P \leq 0.05$ for all tests. Analyses were conducted using Statistical Analysis System software (SAS Institute, Inc., Cary, NC). Depending on the number of variables involved in a particular study (i.e., SM concentration, drug treatment, day cells stained), a two-sample t-test or an ANOVA was conducted. A Tukey’s test was then used to compare the pairs of treatment groups. See figure legends for the specific statistical test conducted.

Results

Test of Commercial Dermal Wound Cleansers

Experiments were performed to evaluate the dermal wound cleansers Saf-Clens™ and Shur-Clens®, and one of the cleaners' ingredients, Pluronic F-68 surfactant (Figs. 1-3). Cells were exposed to either 0 or 10 μ M SM, wounds created, and cells treated with 10% Pluronic F-68, 20% Pluronic F-68, Saf-Clens™, or Shur-Clens®. Additional control cells received the F-68 vehicle (H₂O) or no daily 2-minute treatments (None). Wells stained immediately after wounding revealed no significant differences between the starting wounds for each treatment group (data not shown).

In the first experiment, cells were stained on day 6 after wounding (Fig. 1). For the 0 μ M SM concentration, the 10% and 20% F-68 groups had significantly greater* wound fill than the Saf-Clens™ and vehicle groups (93.62 and 92.19% vs. 10.96 and 0.13%, respectively). The vehicle group had a significantly greater^ wound fill than the Saf-Clens™ group. Similar results were found for the 10 μ M SM concentration. The 10% and 20% F-68 groups had significantly greater* wound fill than the Saf-Clens™ and vehicle groups (67.78 and 76.07% vs. 3.52 and 1.4%, respectively). The 20% F-68 group had significantly different^ wound fill than the 10% F-68 group. Microscopic observations revealed that Saf-Clens™ killed the cells after just one 2-minute treatment.

In the second experiment, cells were stained on days 3, 6, and 9 after wounding (Fig. 2). For the 0 and 10 μ M SM concentrations, the vehicle control had significantly less*^ wound fill than the Shur-Clens®, 10% and 20% F-68 groups at all days tested. Significant improvements in wound healing were also found on day 9 for the 10 μ M SM-exposed cells. Shur-Clens® had significantly greater* wound fill than the 20% F-68, 10% F-68, and vehicle groups (61.59, 34.5, 15.11, and 7.43%, respectively). The 20% F-68 group also had significantly greater* wound fill than the 10% F-68 and vehicle groups.

In the third experiment, NHEK cells were stained on days 3, 6, and 9. For the 0 μ M SM concentration, the vehicle and Saf-Clens™ group had significantly less* wound fill than the 10% F-68, 20% F-68, Shur-Clens®, and no treatment groups (0.97 and 2.15% vs. 31.54, 80.17, 75.43, and 81.74%, respectively). The 10% F-68 group also had significantly less* wound fill than the 20% F-68, Shur-Clens®, and no treatment groups. For the 10 μ M SM concentration, there was no significant difference in wound fill between any of the F-68 containing products on day 3. On day 6 for the 10 μ M SM concentration, the vehicle and Saf-Clens™ had significantly less wound fill than the Shur-Clens®, 20% F-68, and no treatment groups (0.26 and 0.07% vs. 23.52, 29.89, and 38.17%, respectively). Also, the 10% F-68 group had significantly less* wound fill than the Shur-Clens® and no treatment groups (13.36% vs. 29.89 and 38.17%, respectively), and the 20% F-68 group had a significantly less* wound fill than the no treatment group. On day 9 for the 10 μ M SM concentration, the vehicle and Saf-Clens™ groups had significantly less+ wound fill than the 20% F-68 and no treatment groups (1.01 and 0.93% vs. 18.29 and 32.19%, respectively). Also, the 10% F-68 and Shur-Clens® groups had significantly less+ wound fill than the no treatment groups (7.13 and 11.55% vs. 32.19%, respectively).

Test of Continuous Surfactant Presence

Additional studies were conducted to see if the addition of Pluronic F-68 to the medium (i.e., “constant” presence) could improve wound healing. In two separate studies, NHEK cells were

exposed to 0 or 5 μM SM (Fig. 4) and 0 or 10 μM SM (Fig. 5), wounded, and treated daily with KGM containing 0.0125, 0.025, or 0.05% F-68. Additional control cells received KGM alone (0% F-68). No significant differences in wound fill were found between any of the F-68 treatment groups on day 8 (Fig. 4) or days 3, 5, 9, and 12 (Fig. 5).

Figure 1. NHEK cells exposed to 0 or 10 μM SM and treated (2-min rinse) daily with 10% Pluronic F-68, 20% Pluronic F-68, or Saf-Clens™. Additional control cells received the F-68 vehicle (H₂O). Cells were stained on day 6 after wounding. For the 0 μM SM concentration, the 10% and 20% F-68 groups had significantly different* wound fill than the Saf-Clens™ and vehicle groups. The vehicle group had a significantly different^ percent wound fill than the Saf-Clens™ group. For the 10 μM SM concentration, the 10% and 20% F-68 groups had significantly different* wound fill than the Saf-Clens™ and vehicle groups. The 20% F-68 group had a significantly different^ percent wound fill than the 10% F-68 group. Data points represent mean values $\pm$ SEM of 3 determinations. A two factor ANOVA was used to compare the SM concentrations and four treatment groups. If a significant interaction of SM concentration and treatment was observed, then a one factor ANOVA at each SM concentration was used to compare the treatment groups. Next, a Tukey's test was used to compare all pairs of treatment groups for significant differences. Statistical significance was defined as $P \leq 0.05$ for all tests.

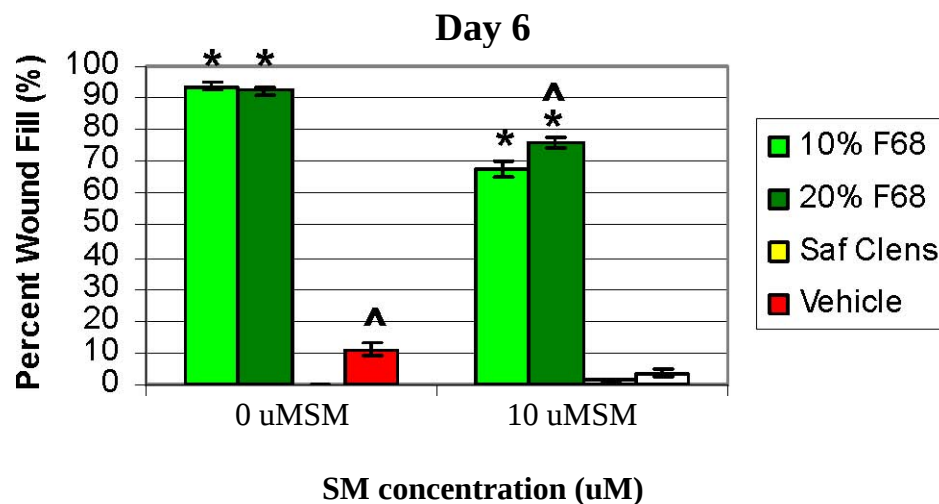


Figure 2. NHEK cells exposed to 0 or 10 μ M SM and treated (2-min rinse) daily with 10% Pluronic F-68, 20% Pluronic F-68, or Shur-Clens® and stained on days 3, 6, and 9 after wounding. Additional control cells received the F-68 vehicle (H₂O). Significant interaction between SM concentration and treatment groups was observed at days 3 and 9. (a) For 0 μ M SM, on days 3 and 9, a significant difference* in wound healing was observed between the vehicle and the 10% F-68, 20% F-68, and Shur-Clens® treatment groups. (b) For 10 μ M SM on day 3, a significant difference* in wound healing was observed between the vehicle and the 10% F-68, 20% F-68, and Shur-Clens® treatment groups. For 10 μ M SM on day 9, significant differences* in wound healing were observed between the Shur-Clens® and the 10% F-68, 20% F-68, and vehicle treatment groups, and between the 20% F-68 and the 10% F-68 and vehicle groups. For both 0 and 10 μ M SM, no significant interaction between SM concentration and treatment groups was observed for day 6. However, there were significant differences^ in wound healing between the vehicle and the 10% F-68, 20% F-68, and Shur-Clens® treatment groups and between the 0 and 10 μ M SM concentrations. Data points represent mean values $\pm$ SEM of 3 determinations. A two factor ANOVA was used to compare the SM concentrations and four treatment groups. If a significant interaction of SM concentration and treatment was observed, then a one factor ANOVA at each SM concentration was used to compare the treatment groups. Next, a Tukey's test was used to compare all pairs of treatment groups for significant differences. Statistical significance was defined as $P \leq 0.05$ for all tests.

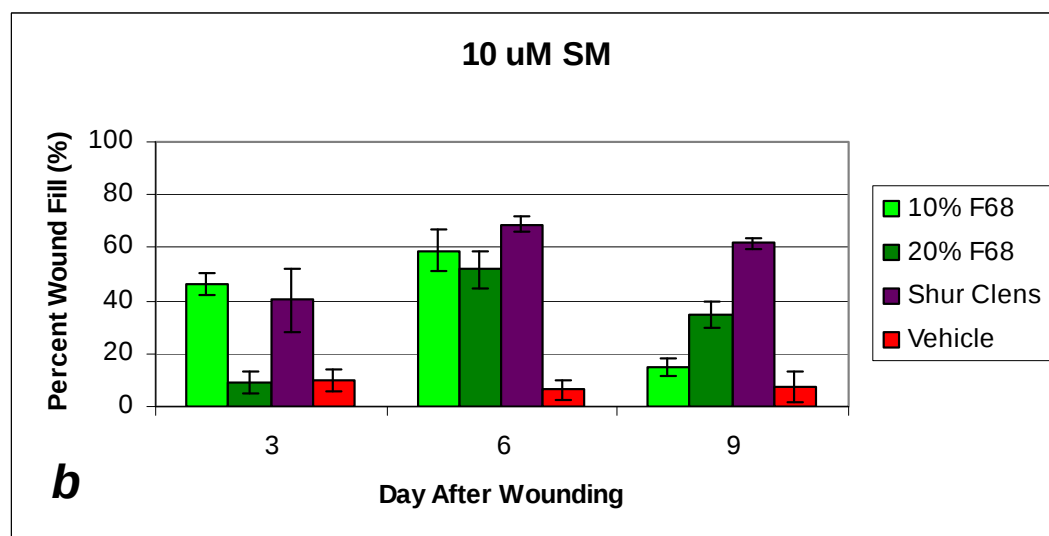
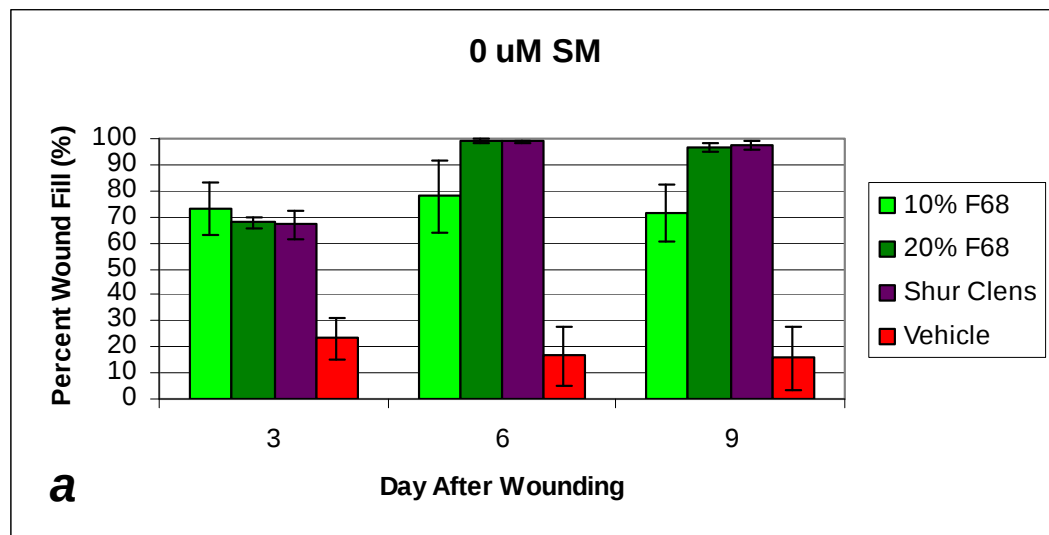


Figure 3. NHEK cells exposed to SM and treated (2-min rinse) daily with 10% Pluronic F-68, 20% Pluronic F-68, Saf-Clens™, or Shur-Clens®. Additional control cells received the F-68 vehicle (H₂O) or no daily 2-minute treatments (None). (a) NHEK cells were exposed to 0 μ M SM and stained on day 6. The vehicle and Saf-Clens™ groups had significantly different* wound fill than the 10% F-68, Shur-Clens®, 20% F-68 and no treatment groups. Also, the 10% F-68 group had a significantly different* wound fill than the Shur-Clens®, 20% F-68 and no treatment groups. (b) NHEK cells were exposed to 10 μ M SM and stained on days 3, 6, and 9 after wounding. On day 3, a significant difference^ in wound fill was observed between the vehicle and no treatment group. On day 6 for 10 μ M SM, the vehicle and Saf-Clens™ groups had significantly different* wound fill than the Shur-Clens®, 20% F-68 and no treatment groups. Also, the 10% F-68 group had significantly different* wound fill than the Shur-Clens® and no treatment groups, and the 20% F-68 group had a significantly different* wound fill than the no treatment group. On day 9, the vehicle and Saf-Clens™ groups had significantly different+ wound fills than the 20% F-68 and no treatment groups. Also, the 10% F-68 and Shur-Clens® groups had significantly different+ wound fills than the no treatment groups. Data points represent mean values $\pm$ SEM of 3 determinations. A one factor ANOVA was used for each staining day/SM concentration combination to compare the treatment groups. If the treatment effect in the ANOVA was significant, then a Tukey's test was used to compare all pairs of treatment groups for significant differences. Statistical significance was defined as $P \leq 0.05$ for all tests.

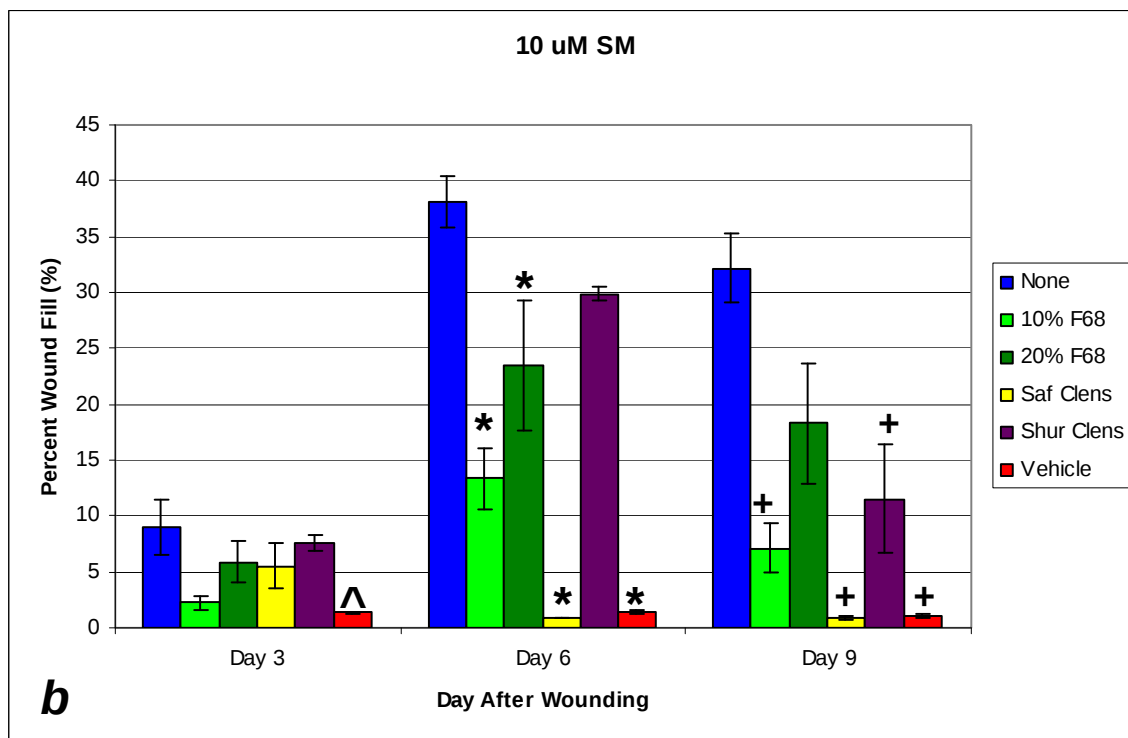
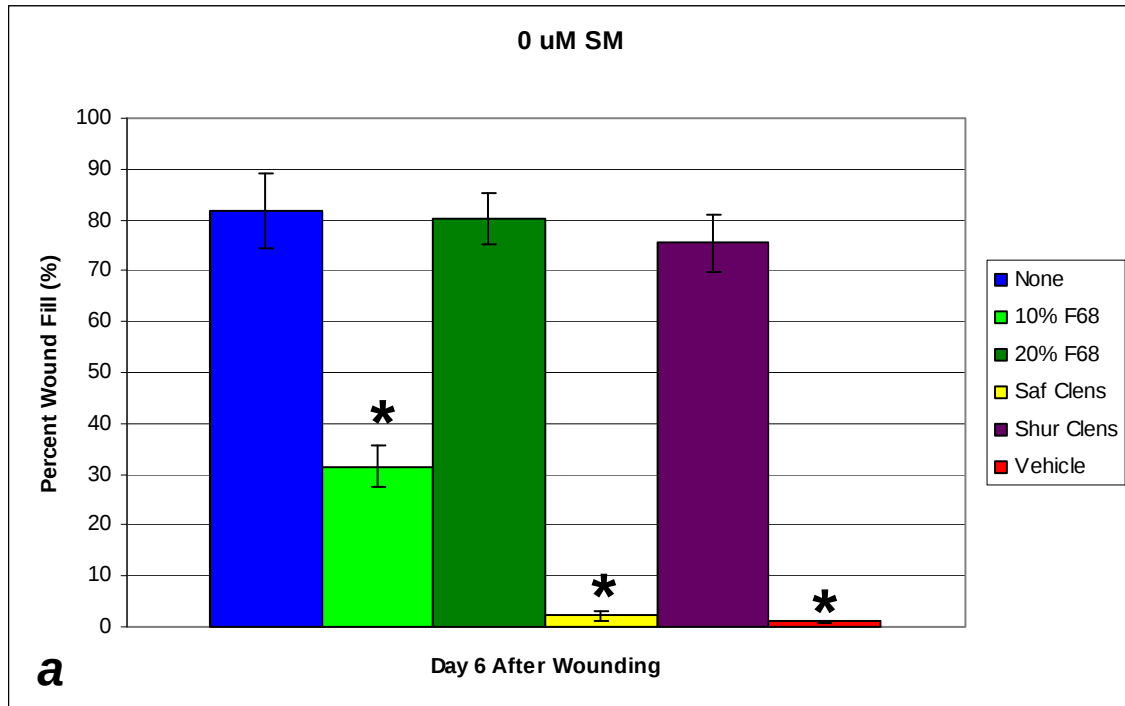


Figure 4. NHEK cells exposed to 5 μ M SM and replenished daily (constant presence) with 0, 0.0125, 0.025, or 0.05% F-68 in KGM. Cells were stained on day 8 after wounding. No significant differences were observed between F-68 doses and the 0% F-68 (no treatment) group on day 8. Data points represent mean values $\pm$ SEM of 3 determinations. A one factor ANOVA followed by a Tukey's test was used to compare all pairs of treatment groups for significant differences. Statistical significance was defined as $P < 0.05$ for all tests.

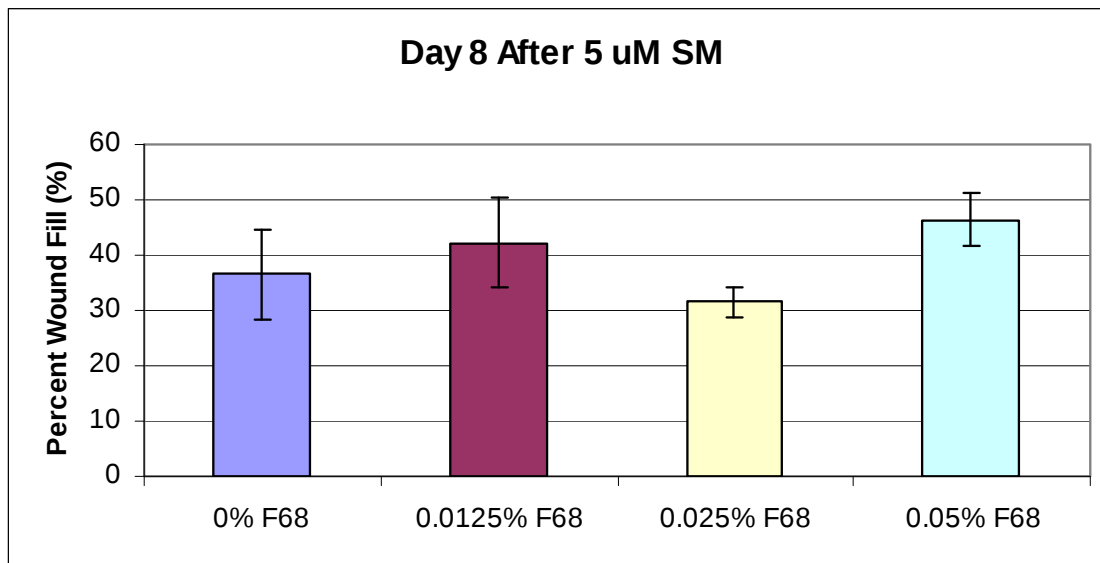
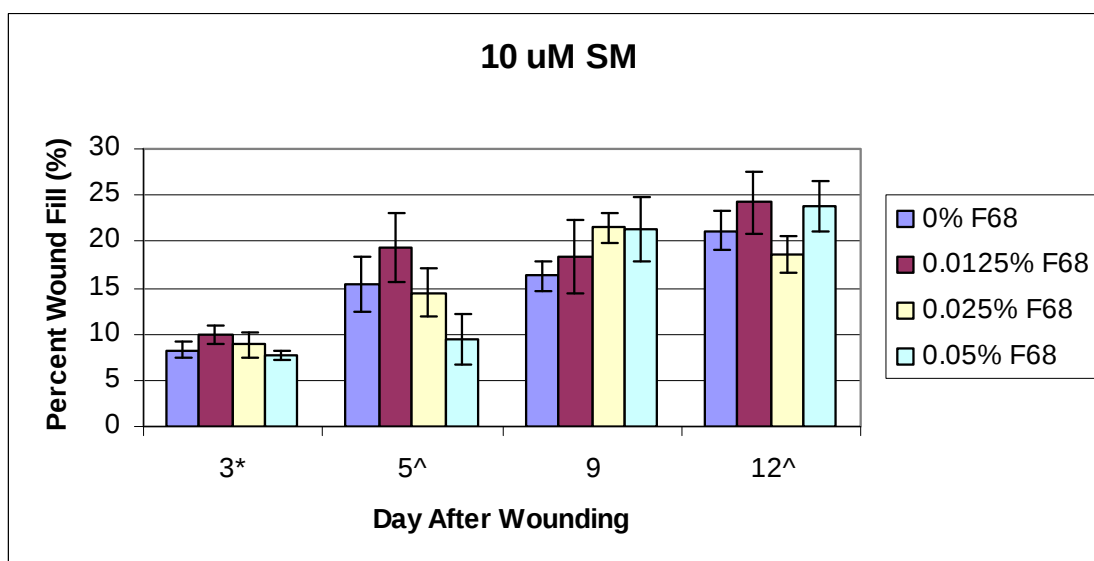


Figure 5. NHEK cells exposed to 10 μ M SM and replenished daily (constant presence) with 0, 0.0125, 0.025, or 0.05% F-68 in KGM. Cells were stained on days 3, 5, 9, and 12 after wounding. A significant day effect was observed between day 3* and days 5, 9, and 12, and between day 5^ and day 12. No significant differences were observed for the F68 doses. Data points represent mean values $\pm$ SEM of 3 determinations. A two factor ANOVA was used to compare the wound healing of F-68 doses and stained days, followed by a Tukey's post hoc comparison of pairs of F-68 doses and pairs of days. Statistical significance was defined as $P < 0.05$ for all tests.



Discussion

The first step in treating wounds is to cleanse the wound to prevent infection. Migrating keratinocytes also assist in the fight against infection by forming a protective monolayer over the wound.⁵ Many dermal wound cleansers are commercially available, but little research has been conducted using these products in conjunction with SM-induced cutaneous injury. The purpose of this study was to use an in vitro wound healing model to evaluate the impact of using commercially available wound cleansers on SM-induced wounds. Normal human epidermal keratinocytes were chosen as they appear to be a good model for basal epidermal cells,¹² a principal target for SM-induced skin lesions.^{2,13} The SM concentrations selected for these experiments were based on a previously published study.¹¹

Pluronic F-68 surfactant showed promise as an effective wound cleanser in an animal model. Mechanical cleansing of wounds with sponges soaked in Pluronic F-68 prevented wound infections and minimized tissue damage in rabbits.¹⁴ In the present study, two commercially sold dermal wound cleansers, Shur-Cleans® and Saf-Cleans™, were evaluated in the NHEK wound healing model. Shur-Cleans® is a 20% Pluronic F-68 solution made in purified water. Saf-Cleans™ contains these main ingredients (although the exact concentration of F-68 is unknown due to proprietary information restrictions) as well as Sorbitol Solution, USP; coco phosphatidyl PG-dimonium chloride; sodium chloride, USP; potassium phosphate monobasic, NF; DMDM Hydantoin; citric acid, USP; and sodium hydroxide, NF added for pH adjustment. Ten and 20% Pluronic F-68 solutions in water were also tested in the model.

Two-minute treatments were conducted to mimic how a patient might use the cleansers (i.e., spray the cleanser on, wait a few minutes for the solution to dry, and then apply a dressing). The Shur-Cleans® and F-68 treatments did not improve wound healing, but they also did not harm the NHEK cells in the non-wounded areas. In contrast, Saf-Cleans™ damaged and/or killed the NHEK cells just after one 2-minute treatment. The sterile water vehicle control also killed the cells. These findings are consistent with previous studies that showed that Shur-Cleans® was nontoxic.^{15,16} Wilson et al. used a human infant keratinocyte culture to show that Shur-Cleans® was nontoxic and that Saf-Cleans™ had to be diluted 10-fold before it reached nontoxic levels in an MTS cell viability assay.¹⁷ Since the Pluronic F-68 solutions did not harm the NHEKs, one can conclude that the toxicity differences between Shur-Cleans® and Saf-Cleans™ can be attributed to one or more of the extra ingredients contained in Saf-Cleans™. One candidate for toxicity is the preservative DMDM Hydantoin (1,3-dimethylol-5,5dimethylhydantoin). In one clinical study, 33% of formaldehyde-allergic patients developed dermatitis after application of a cream containing 1% DMDM Hydantoin.¹⁸ Each ingredient and/or a combination of ingredients must be further tested to definitely state the cause of keratinocyte toxicity. In conclusion, one can concur with Wilson et al. when they suggest that “judicious use of these supposedly innocuous agents (i.e. dermal wound cleansers) should be considered in a clinical setting.”¹⁷

Previous cell culture studies suggested that the constant presence of surfactant might promote wound healing. Very low dosages of Pluronic F-68 in cell culture media stimulated an increase in the attachment of human gingival fibroblasts (hGF) on plastic and dentin surfaces, suggesting that F-68 may promote early wound healing by facilitating the attachment, spread, and growth of hGFs.¹⁹ The addition of Pluronic F-68 to cell culture media protected periodontal cells from the harmful effects of alcohol. Human gingival fibroblasts were fed with DMEM media containing 0, 1, 2, or 4% ethanol with or without 0, 0.025, 0.05, or 0.1% Pluronic F-68. Cells treated with 0.025 and 0.05% F-68 showed protection of the non-wounded areas of hGFs, with less cell loss

and morphological changes than the ethanol-exposed but untreated hGFs. However, there were no statistically significant improvements in wound fill between the study groups.²⁰ Similar results were obtained in a study of periodontal ligament fibroblasts fed with DMEM containing 10-fold lower doses of ethanol with or without the Pluronic F-68 concentrations above.²¹ In the present study, NHEK cells were exposed to SM, wounded, and replenished daily (constant presence) with 0, 0.0125, 0.025, or 0.05% F-68 in KGM. No significant differences in wound fill were found between any of the F-68 and control treatment groups.

In conclusion, surfactant studies found no improvement in wound fill by Pluronic F-68 surfactant or the dermal wound cleansers Saf-Clens™ and Shur-Clens®. Pluronic F68 and Shur-Clens® did not impair wound healing, but Saf-Clens™ killed the NHEK cells after just one 2-minute treatment. Caution must be used in accepting the advertised safety of products marketed for delicate wound tissue. This in vitro wound healing model will be a useful tool for evaluating additional commercially available wound healing products to combat cutaneous sulfur mustard injuries.

References

1. Martin P. Wound healing-aiming for perfect skin regeneration. *Science* 1997; 276:75-81.
2. Papirmeister B, Gross CL, Meier HL, Petralli JP, Johnson JB. Molecular basis for mustard-induced vesication. *Fundamental App Toxicol* 1985; 5:S134-S149.
3. Petralli JP, Oglesby SB, Mills KR. Ultrastructural correlates of sulfur mustard toxicity. *J Toxicol Cutan Ocular Toxicol* 1990; 9:193-204.
4. Hunt TK, Hopf H, Hussain Z. Physiology of wound healing. *Adv Skin Wound Care* 2000; 13(Suppl 2):6-11.
5. Bensing KA, Brill LR, Cohen IK, Kantner TR, Kerstein MD, Krasner D, Levin ME. *The Physiology of Wound Healing*. Oxford Institute for Continuing Education, Yardley, PA, 1998;1-47.
6. Sidell FR, Urbanetti JS, Smith WJ, Hurst CG. Vesicants. In: Sidell FR, Takafuji ET, Franz DR (ed). *Textbook of Military Medicine, Part I: Warfare, Weaponry, and the Casualty Medical Aspects of Chemical and Biological Warfare*. Washington, D.C., Office of the Surgeon General at TMM Publications, Borden Institute, Walter Reed Army Medical Center, 1997. pp197-228.
7. Gross CL, Nealley EW, Nipwoda MT, Smith WJ. Pretreatment of human epidermal keratinocytes with D,L-Sulforaphane protects against sulfur mustard cytotoxicity. *Cutan Ocul Toxicol* 2006; 25:155-163.
8. Smith WJ, Gross CL, Chan P, Meier HL. The use of human epidermal keratinocytes in culture as a model for studying the biochemical mechanisms of sulfur mustard-induced vesication. *Cell Biol Toxicol* 1990; 6(3):285-291.
9. Lackler KP, Cochran DL, Hoang AM, Takacs V, Oates TW. Development of an *in vitro* wound healing model for periodontal cells. *J Periodontol* 2000; 71:226-237.
10. Oates TW, Mumford JH, Carnes DL, Cochran DL. Characterization of proliferation and cellular wound fill in periodontal cells using an *in vitro* wound model. *J Periodontol* 2001; 72:324-330.
11. Henemyre-Harris CL, Adkins AL, Chuang AH, Graham JS. Addition of epidermal growth factor improves the rate of sulfur mustard wound healing in an *in vitro* model. *Open Access Journal of Plastic and Reconstructive Surgery (ePlasty)*, 2008, 8:136-150.
12. Smith WJ, Gross CL, Chan P, Meier HL. The use of human epidermal keratinocytes in culture as a model for studying the biochemical mechanisms of sulfur mustard toxicity. *Cell Bio Toxicol* 1990; 6:285-291.

13. Vogt RF, Dannenberg AM, Schofield BH, Hynes NA, Papirmeister B. Pathogenesis of skin lesions caused by sulfur mustard. *Fundamental App Toxicol* 1984; 4:S71-S83.
14. Rodeheaver G, Smith S, Thacker J, Edgerton M, Edlich R. Mechanical cleansing of contaminated wounds with a surfactant. *Am J Surg* 1975; 129:241-245.
15. Dire DJ, Welsh AP. A comparison of wound irrigation solutions used in the emergency department. *Ann Emerg Med* 1990; 19:704-708.
16. Bryant CA, Rodeheaver GT, Reem EM, Nichter LS, Kenney JG, Edlich RF. Search for a nontoxic surgical scrub solution for periorbital lacerations. *Ann Emerg Med* 1984; 13:317-321.
17. Wilson JR, Mills JG, Prather ID, Dimitrijevič SD. A toxicity index of skin and wound cleansers used on in vitro fibroblasts and keratinocytes. *Adv Skin Wound Care* 2005; 18(7):373-378.
18. de Groot AC, van Joost T, Bos JD, van der Meeren HL, Weyland JW. Patch test reactivity to DMDM hydantoin. Relationship to formaldehyde allergy. *Contact Dermatitis* 1988; 18(4):197-202.
19. Hokett SD, Cuenin MF, O'Neal RB, Brennan WA, Strong SL, Runner RR, McPherson III JC, Van Dyke TE. Pluronic polyol effects on human gingival fibroblast attachment and growth. *J Periodontol* 2000; 71:803-809.
20. Chuang AH, McPherson III, JC, McPherson Jr, JC, Michel G, Henemyre CL. The protective effect of Pluronic F-68 on wound healing in an alcohol condition. *Ga J Sci* 2002; 60:54.
21. Chuang AH, Runner R, Lapp C, Bhatt B, McPherson, III, JC. The effect of Pluronic F-68 on wound healing of periodontal ligament fibroblasts in low alcohol conditions. *Ga J Sci* 2005; 63:74.