IMPROVED HEALING IN WEANLING PIG MODEL OF VESICANT INJURY SHOWN BY HIGH FEATURE ANTIMICROBIAL DRESSING

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Abstract

Quick-Med Technologies, Inc. (QMT) has developed a dressing for the treatment of vesicant injuries following debridement under a US Army SBIR contract. This dressing was designed to enable optimal wound healing by providing a moist wound healing environment with antimicrobial protection, protease inhibiting properties through sustained delivery of an antibiotic (doxycycline) that also acts as a protease inhibitor, as well as antioxidants and a growth factor (EGF). Technical properties and results of tissue culture experiments were previously reported - we now report new data based on animal clinical studies.

Wound healing of vesicant injuries on weanling pigs was tested by Battelle Memorial Institute (Columbus, OH). Injuries were created by liquid phase sulfur mustard exposure, with dressings applied on day 2, removed on day 9 and final evaluation and tissue sectioning performed on day 16. Clinical evaluation was based on pathophysiological observations. Histopathology of tissue sections was used to assess wound healing progress. Histological techniques included routine H&E as well as Masson's Trichrome staining. Results showed that relative to controls, NIMBUS-SAP dressings, and NIMBUS-SAP dressings with protease inhibitor and/or growth factor enhanced healing (in both speed and quality of tissue formed). Histopathology showed marked improvement in terms of neoepithelialization and recovery of appropriate epidermal and underlying dermal juncture.

Background

Sulfur Mustard (SM) is a vesicant (blistering) agent that was widely used in WWI, and was more recently used in Iraq by Saddam Hussein. The mechanisms by which sulfur mustard creates injury on skin is thought to involve proteases (and likely other inflammatory agents), which illustrates significant similarity in the biochemistry of chemical and thermal burns.

Exposure of skin to sulfur mustard (also often called HD or SM) is thought to disrupt the balance between basement membrane protein synthesis by keratinocytes and their degradation by proteinases. This disturbance causes a loss of adherence between epidermis and dermis due to more net protein degradation than synthesis. This effect is observed macroscopically as vesication. Mortality resulting from direct SM exposure is relatively low, but blistering predisposes victims to secondary bacterial infection, which represents the greatest hazard to SM victims.

This research is the result of a US Army SBIR solicitation for a treatment that provides a moist wound healing environment combined with fluid handling, antimicrobial protection and protease inhibition while delivering nutritive factors and growth factor to stimulate healing. We previously reported upregulated tissue proliferation in chemically insulted tissue culture models. The research project concluded with a wound healing study using a weanling pig model, chemically injured with sulfur mustard by liquid exposure as detailed and analyzed below.

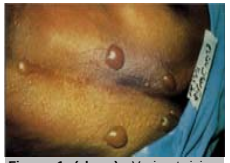


Figure 1 (above). Vesicant injury after sulfur mustard exposure. Blisters on the back 16 h after SM exposure. (from Willems, Annals Med Militar Belg, 1989).

	Doxycycline-SAP	NIMBUS-SAP
Day 1	7.48*	5.65
Day 2	7.64*	7.64*
Day 3	7.90*	6.23
Day 4	8.82*	8.82*
Day 5	9.03*	6.93
Day 6	8.99*	8.99*
Day 7	9.01*	4.31

* Denotes full kill. Kill level variation due to individual controls

Figure 2 (above). Repeated inoculation with *P. aeruginosa (PA)* per AATCC method 100-1999.

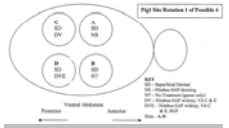


Figure 3 (above and below). Top panel shows exposure sites on weanling pigs. Sites were rotated to provide each animal every treatment. Bottom panel shows excised lesion tissue as recovered from sites.

Antimicrobial dressings. Antimicrobial dressings are of particular utility in protecting patients with vesicant injuries, since these injuries make the patient more susceptible to bacterial infections. The base material used to prepare the high feature dressing described here (which we commercially call the Nimbus Advanced Active system), is NIMRUS-SAP antimicrobial superabsorbent dressing (a proprietary non-leaching superabsorbent dressing material prepared from a rayon base). The NIMBUS-SAP dressing treated with doxycycline has redundant dual-mode microbial control capacity, as well as providing protease inhibition. The MMPI doxycycline is a potent antibiotic, while the base dressing material provides intrinsic antimicrobial efficacy. Experiments have shown that the NIMBUS-SAP, both on its own and when loaded with doxycycline, is effective against [daily] repeated inoculation with *Pseudomonas aeruginosa,* identified by CDC as one of the most common burn wound pathogens, for at least 7 consecutive days (**Figure 2**)

Experimental Treatments

Controls: Control sites were treated with rayon gauze.

Treatment dressings. All three treated dressings are based on NIMBUS-SAP antimicrobial superabsorbent dressing: base NIMBUS-SAP dressing, ents, NIMBUS-SAP dressing with doxycycline + antioxidants (vitamins C and E) integrated, and finally the previous plus epidermal growth factor.

Experimental Evaluation methods. Clinical observation included pathophysiological evaluation based on established observation parameters. In addition to the clinical observations, histological examinations were performed on tissue excised and either preserved in formalin or flash frozen in nitrogen.

The weanling pig model

Injury Model. Battelle Memorial Institute used a previously developed injury model with an established exposure protocol, treatment administration and evaluation time points and protocols. Exposures were performed by placing 400µl of undiluted HD (sulfur mustard in liquid form) onto a PTFE filter paper disc, which was applied onto the target surface for 8 min, held in place by a 300g weight. On day 2 following exposure, debridement of sites took place and the dressings were placed according to treatment plan. Dressings were removed on day 9, and a preliminary evaluation was made. Another evaluation was made on day 16, after which animals were sacrificed and tissue samples were collected for evaluation.

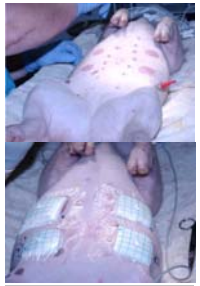


Figure 4 (above). Weanling pigs with SM lesions. Day 2: before and after dressing application.

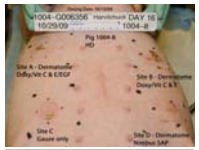
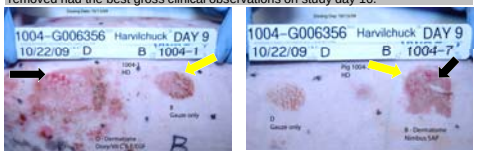


Figure 6 (above). Weanling pigs with SM lesions, day 16. Sites shown prior to tissue collection.

Figure 5 (below). Lesions after dressing removal on day 9 (Black arrows mark where the impregnated dressing was unable to be removed. Yellow arrows mark where the removal of the Nimbus-SAP dressings led to the disruption of the healing tissue.) Nearly all NIMBUS-SAP dressings (with and without active agents) had some adherence to the lesions due to ingrowth of regenerating neo-epithelium. In some cases dressing fragments were left in place rather than further damaging regrown skin. The lesions whose dressings were most easily removed had the best gross clinical observations on study day 16.



Clinical Observations

No adverse reactions to the treatment materials were observed in the animals during the study. Clinicians noted some edema, irritation and localized infection in areas away from the dressings under adhesives / staples (**Fig. 5**). All histology was done on lesion tissue and immediately adjacent tissue, so these effects were noted only in gross pathology observations, and were attributed to environmental effects not based on treatments/dressings. Clinical scoring is shown for average lesion size in **Fig 7**.

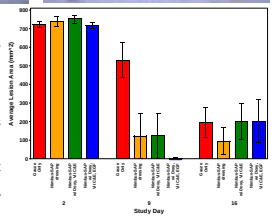


Figure 7 (above). Clinical evaluation by lesion size on days 2, 9 and 16. Dressing removal from NIMBUS-SAP sites induced localized scab and erythema, evident for day 16 gradings.

Histology

The main macroscopic feature of all sites was the presence of neoepithelium, indicating full closure of the wound. All sites had re-established the epithelial layer covered with stratum corneum, but maturity of epithelium varied greatly: NIMBUS SAP + doxy + EGF sites reestablished a thick epidermal layer, compared to untreated gauze. Inflammatory cell infiltrates were prominently present in all dermal layers of the control tissue, in most parts of the papillary dermis of the NIMBUS SAP treated tissue, and to a lesser degree in the tissue protected by NIMBUS SAP + DOXY dressing (with or without EGF).

Control dressings showed loosely organized and enlarged collagen fibrils, consistent with scar formation, while particularly the NIMBUS-SAP + doxy + vitamins + EGF dressing demonstrated very well ordered deposition of collagen (see **Fig 8** and magnified view presented in **Fig 9** comparing with control)

Figure 8 (below). Histology by H&E staining on tissue sections, 40x magn. The Epidermal-dermal separation is marked by arrows. Legend: **EP**-epidermis; **DM**-dermis, **II** = Inflammatory infiltrate.

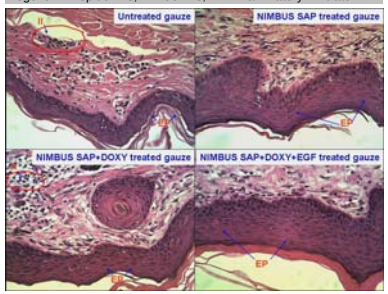


Figure 9 (top right). H&E stained dermis showing structural organization for (left to right):
(A) untreated gauze control,
(B) NIMBUS-SAP
(C) NIMBUS –SAP + doxy + vitamins + EGF
Note the difference in organization, and absence of inflammatory cells in (C). Neovascularization of varying degrees is evident for the different treatments.

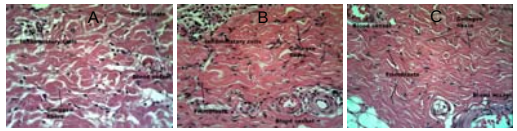


Figure 10 panels (below). Histology included both H&E and collagen specific Masson's Trichrome staining. Masson's Trichrome stain can better illustrate the degree of collagen matrix remodeling in tissue by showing old collagen fibers as greenish-blue, and new collagen fibers light blue-purplish (some light and contrast settings shows this as brown). Variations in collagen remodeling between treatments include the thickness of neoepidermal formations and rate of neodermal proliferation, specifically as observed in the NIMBUS treated wounds. The set of panels below also features an untreated and unexposed section of skin tissue for comparison to wound sites (panel A).

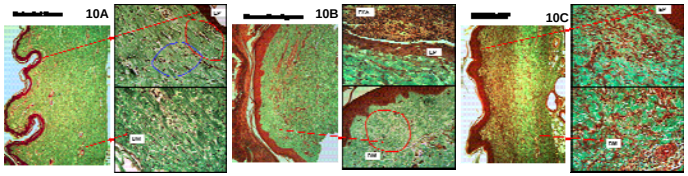


Figure 10A (above). Normal (unexposed and untreated) porcine skin tissue. Notice well differentiated epithelial layer, with semi parallel collagen bundles in lattice form. Note abundance and the even distribution of normal dermal cells with small round nucleus stained dark brown – characteristic of fibroblasts in resting state.

Figure 10B (above). Untreated conventional gauze, exposed tissue. Epithelial layer (EP) is formed, but with little differentiation. Prominent eschar (ESh) covers poorly developed epidermis. Papillary dermis is granular and disorganized. Many cells are notably devoid of nuclear structures. This structure suggests active wound healing, but also is indicative of scar tissue formation.

Figure 10C (above). NIMBUS-SAP, exposed tissue. Shows extensive staining, with abundant new collagen in both papillary and deep dermis. Note inflammatory cells with intensely stained, granular nuclei, decreased diameter of the deposited collagen fibers, and lack of the normal lattice arrangement. These indicate active wound healing, at a more advanced stage than controls.

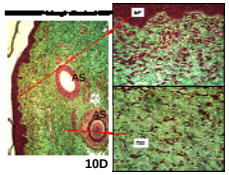


Figure 10D (left). NIMBUS SAP + doxycycline + vitamins + EGF shows fully reformed epidermis over organized dermis with fully developed collagen matrix featuring hair follicles and multiple blood vessels. The papillary dermis shows new collagen deposits, incorporated into the mature collagen matrix. Deep dermis has collagen fibers in relatively orderly fashion, and shows well organized cells surrounding adnexal structures **(AS).** Collagen fibril size, arrangement and inter-fibril interaction are all very similar to non-perturbed tissue, indicating near completion of wound healing.

Conclusions

The NIMBUS-SAP based dressings all demonstrated excellent results in the animal wound healing model, showing improved tissue structure over the wounds treated with control dressings. The NIMBUS-SAP dressing with doxycycline, vitamins and EGF showed the strongest effect on wound healing, with improved tissue maturity, more rapid formation of well structured collagen deposition, pronounced neo-vascularization, and migration of progenitor cells from adnexal structures driving epithelial reformation. In addition, the NIMBUS-SAP dressings did not induce rigid scar formation. The control sites showed disordered collagen in the dermis consistent with scarring, and inferior structural maturity in the connective tissue underlying the epithelium.

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