



The Effects of BIOGUARD® vs. Kerlix® AMD Dressings when used in combination with Dakin's® Solution or SANTYL Ointment



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Abstract

An in vitro analysis of the performances of SANTYL® Ointment and Dakin's Solution® has brought to light some important information on the chemical interaction with antimicrobial dressings such as BIOGUARD® and Kerlix® AMD. This is a brief summary of that evaluation. The experimental work summarized here sought to evaluate the effect of two commercial antimicrobial gauze dressings on the enzymatic activity of SANTYL Ointment and the effect on the dressings' efficacy when Dakin's Solution is applied. In summary the

data shows that 1) PHMB, the microbicide in Kerlix AMD, which is released from the dressing, reacts with SANTYL, which is a collagenase enzyme. This reaction leads to a significant loss of activity of the collagenase enzyme. 2) Dakin's solution reacts with the Kerlix AMD antimicrobial agent. This reaction significantly diminishes the microbicidal effectiveness of the Kerlix AMD. BIOGUARD does not result in either of these adverse effects as evidenced when comparing its performance using the same test methods.

The Role of Wound Care Therapeutic Agents SANTYL Ointment and Dakin's Solution

SANTYL® Ointment is a petrolatum formulation of a collagenase debriding enzyme derived from the fermentation of *Clostridium histolyticum*. It is the most commonly used debriding agent for the treatment of chronic infected wounds which contain debris and necrotic tissue that can interfere with healing. It is a blend of 250 collagenase units per gram of USP grade white petrolatum. It is indicated for the debridement of pressure, diabetic and venous leg ulcers.

SANTYL is an active debriding agent which continuously removes necrotic tissue and maintains a clean wound bed. It has been shown to be superior to a placebo in the debridement of dermal ulcers. It also has been shown to be superior to standard antimicrobial therapy alone and in combination with topical antimicrobial therapy. It is used until the wound is cleared of cellular debris and granulation is firmly established. Debridement by SANTYL Ointment acts by destroying the collagen that binds the necrotic tissue to healthy tissue thus reducing the tendency toward inflammation.

The activity of the collagenase in SANTYL Ointment is adversely affected by a pH outside the range of 6-8. The presence of heavy metal ions such as silver, mercury and lead as well as povidone iodine and some detergents are also known to reduce the effectiveness of the collagenase enzyme. The use of silver release dressings is contraindicated with SANTYL treatment.

Dakin's Solution®, sodium hypochlorite, is an antiseptic wound cleansing agent that was first used during World War I. It can safely be applied to human skin and wounds and is sometimes used prior to or after surgery. Dakin's Solution is often used as an irrigant to flood the wound or applied from a saturated sponge so as to preserve a low bioburden. Because it is capable of separating necrotic tissue from healthy tissue it is used to prevent and treat skin and tissue infections that result from cuts, scrapes and pressure sores. Dakin's Solution does not damage healthy tissue like stronger disinfectants such as

carbolic acid and iodine, and the activity of Dakin's solution is not diminished in the presence of blood serum.

In water a low concentration of hypochlorous acid, a weaker cousin of hydrochloric acid, is released from the solution. Consequently the solution has a short shelf life and is not used after a few days storage. It is effective against bacteria, spores, fungi and viruses, and it may be used as an irrigant to loosen and dissolve necrotic tissue⁷.

While the application of Dakin's solution has somewhat diminished in recent years, it is still used by some clinicians who are attempting to debride or disinfect a wound that appears to be chronically infected.

Antimicrobial Wound Dressings

BIOGUARD with NIMBUS® is medical grade gauze to which a polyquaternary polymer (poly Diallyl dimethyl ammonium chloride or polyDADMAC) has been attached such that it does not leach or migrate from the dressing. This very large polymer has numerous active quaternary sites with the ability to disrupt bacterial cell walls and destroy their functionality. It renders the gauze as a bacterial barrier that prevents the increase of bacterial burden in the dressing.

Kerlix AMD, on the other hand, is antimicrobial-treated gauze with a leaching antimicrobial agent as evidenced by a significant zone of inhibition in standard diffusion plate assays. That is to say the bactericide migrates from the dressing. The active agent in Kerlix AMD is PHMB, i.e. poly (hexamethylene biguanide), a much smaller molecule than the polyDADMAC used in BIOGUARD.

Results with SANTYL Ointment

BIOGUARD antimicrobial rolled gauze (Derma Sciences) was compared to Kerlix AMD antimicrobial gauze (Covidien) and

untreated gauze. The effectiveness of the SANTYL collagenase enzyme was assessed by Azocoll, a bovine collagen containing a sequestered dye that is released as the collagen is degraded by collagenase. The collagenase activity is measured by UV absorption/ transmission. Because the petrolatum based ointment resisted the transfer of the collagenase into the aqueous phase, an experiment was designed using the pure *Clostridium histolyticum* derived collagenase.

Experimental Detail

A calibration curve (final concentrations in analyte) was constructed using collagenase of 10^{-6} , 10^{-7} , and 10^{-8} . All test samples were exposed to a concentration of 10^{-5} collagenase (dilution by analyte yields a final concentration of 10^{-6}). Test samples were 50 mg of substrate in 500 μ l of PBS. These were incubated in collagenase/PBS solution for 1h. 200 μ l of solution were withdrawn and added to 1800 μ l of Azocoll in PBS (5mg/ml Azocoll concentration). UV transmission was measured at 575 nm after 1h and 4h. The 1h and 4h data is shown and compared to calibration curves.

Experimental Results

The activity of collagenase enzyme exposed to the test samples was assessed at 1h and 4h using an Azocoll assay. Test data

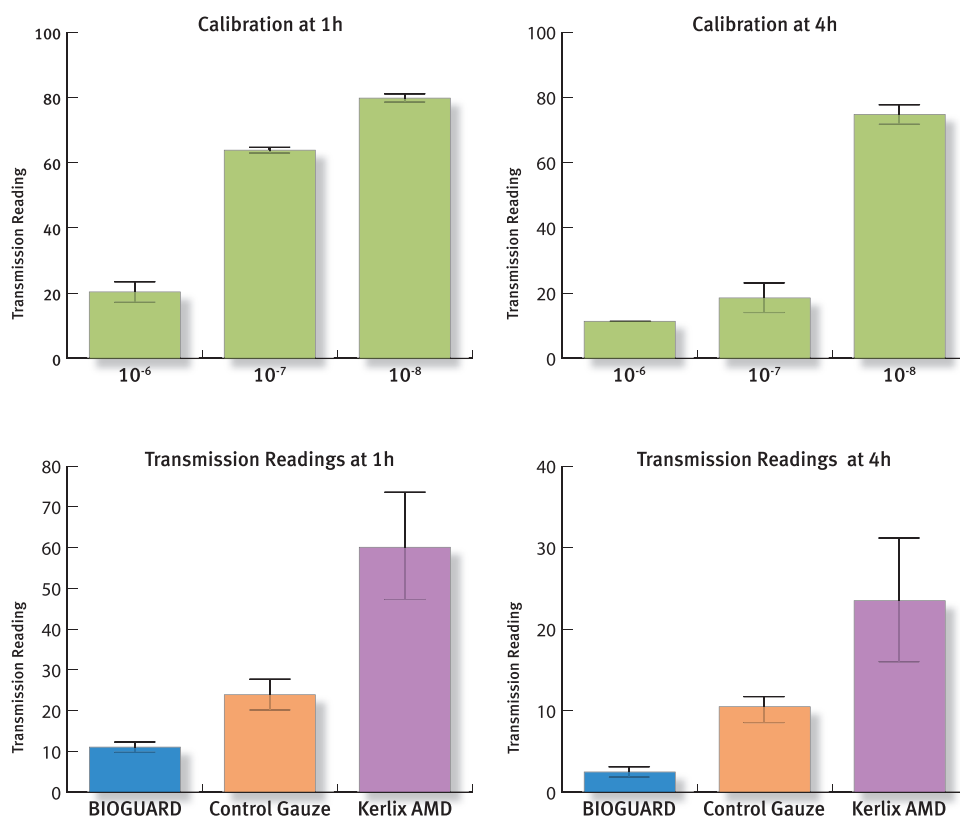
are presented in absorption readings, with accompanying calibration curves for concentrations of pure collagenase. Azocoll is dissolved by collagenase to release a dye; higher dye concentrations indicate more collagenase activity by giving a higher absorption reading.

Conclusion

The data indicates for both time points that the collagenase activity remained at or above the level of the control gauze when collagenase was exposed to BIOGUARD. The Kerlix AMD dressing depressed collagenase activity significantly. Comparison with the calibration curve for pure collagenase shows that collagenase activity after exposure to Kerlix AMD was depressed by at least 10-fold (an order of magnitude). Therefore, Kerlix AMD dressings should not be used with SANTYL.

It appears that the free biguanide polymer that has migrated from the dressing interacts with the collagenase such that a significant level of activity is lost rendering SANTYL nearly ineffective. The activity of the SANTYL enzyme was not affected by the presence of BIOGUARD.

Exhibit 1: Comparison of the Concentrations of Collagenase in the Presence of BIOGUARD Gauze vs. Kerlix AMD



Results with Dakin's Solution

The test protocol was designed to measure the efficacies of BIOGUARD and Kerlix AMD in the presence of Dakin's Solution. The evaluation of the effect of Dakin's Solution was carried out using an *in vitro* measurement of the antimicrobial efficacies of BIOGUARD Wound Dressing and Kerlix AMD dressing in the presence of the Dakin's Solution.

Experimental Results

The method chosen to determine the antimicrobial efficacies of BIOGUARD, Kerlix and an untreated gauze control was Quick-Med Technologies' modified version of Test Method AATCC-100, "Assessment of Antibacterial Finishes on Textile Materials" (QMT 01-2010). The modification to the standard version is that the testing of all dressings is carried out in the presence of 10% fetal bovine serum to help simulate the wound environment. Additional sample preparations included pre-soaking the substrates in various concentrations of Dakin's Solution and drying them at 110° C for two hours to inactivate any residual chlorine from the Dakin's Solution. The test organism used was *E. coli* (ATCC# 8739).

Various concentrations of Dakin's solution were tested against the Kerlix AMD antimicrobial substrate at the 0.025% concentration. The substrate appeared to be inactivated at

these conditions. A follow-up assay was performed under the same conditions with the BIOGUARD antimicrobial gauze in parallel and the comparative results can be observed in Exhibit 2.

Conclusion

The analysis demonstrated that the BIOGUARD gauze sponges soaked in 0.025% Dakin's Solution retained their initial efficacy of 7.7 log reduction vs. *Escherichia coli*. In marked contrast, the efficacy of the Kerlix AMD that had an initial efficacy of 7.7 log reduction, ended up with a 1.21 log reduction after soaking with Dakin's Solution.

As discussed earlier, the antimicrobial polymer is bonded to BIOGUARD such that it doesn't migrate away from the gauze sponge. Kerlix AMD has a measurable zone of inhibition. The PHMB polymer is slowly released to a place where it is available and free to interact with the hypochlorous acid in Dakin's Solution which is an oxidizing agent. Oxidation of the PHMB by hypochlorous acid results in a reduction in the availability of the biguanide polymer and thus the suppression of the efficacy of Kerlix AMD. BIOGUARD is not affected in the same way because it does not migrate from the dressing and is thus unavailable for reactions with Dakin's.

Exhibit 2: Comparison of the Efficacies of BIOGUARD Gauze vs. Kerlix AMD in the Presence of Dakin's Solution

Sample versus <i>E. coli</i>	Average Log Reduction Overnight Controls	Standard Deviation
Kerlix AMD, Presoaked in 0.025% Dakin's	1.21	0.28
BIOGUARD Gauze, Presoaked in 0.025% Dakin's	7.70*	0.00
Control Gauze, Presoaked in 0.025% Dakin's	0.73	0.20
Kerlix AMD, as is, positive control	7.70*	0.00
BIOGUARD Gauze, as is, positive control	7.70*	0.00

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