UNIVERSITY OF MIAMI
SCHOOL OF MEDICINE

DEPARTMENT OF
DERMATOLOGY
& CUTANEOUS SURGERY

Report

Number I

Effect of Elasto-Gel on *Pseudomonas aeruginosa* Proliferation in Burn Wounds

REPORT

Evaluation of the Multiplication of *Pseudomonas aeruginosa* in Second-degree Burn Wounds on Domestic Swine treated with a hydrogel wound dressing.

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INTRODUCTION

The ultimate objective of burn wound care is to close the wound in a timely fashion in order to help prevent subsequent infection and/or mortality. The concept of moist wound healing has been proven to be effective in some cases for the healing of both acute and chronic wounds. However, the "fear of infection" unfairly associated with occlusive dressings has discouraged their use.

Pseudomonas aeruginosa (*P. aeruginosa*) is one of the major pathogens related to burn wound infections. Although *P. aeruginosa* survives in a semi-dry climate, this pathogen favors a moist environment. The aim of this study was to evaluate the ability of a hydrogel wound dressing to discourage the growth of *Pseudomonas aeruginosa* in second-degree burn wounds.

MATERIALS AND METHODS

Experimental Animals

Three young Specific Pathogen Free (SPF) pigs weighing 18-22 kg were conditioned for two weeks prior to initiating the study. The animals were fed a non-antibiotic chow *ad libitum* and housed in our animal facilities (AAALAC approved) with controlled temperature (19-21°C) and light and dark (12L/12D). This protocol has been approved by the University of Miami, School of Medicine Animal Review Committee and the procedures were performed according to the Guide for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services).

Burning Technique

Each animal was anesthetized with ketamine HCl (20 mg/kg) and xylazine (2.0 mg/kg) I.M., followed by mask inhalation of an isoflurane and oxygen combination. The hair on the backs of the

pigs was clipped with standard animal clippers. The skin on both sides of the animal was prepared for burning by washing with a non-antibiotic soap. Five specially designed cylindrical brass rods weighing 358 g each were heated in a boiling water bath to 100°C. A rod was removed from the water bath and wiped dry before it was applied to the skin surface to prevent water droplets from creating a steam burn on the skin. The brass rod was held in a vertical position on the skin, for six seconds, with all pressure supplied by gravity to make a burn wound 8.5 mm diameter x 0.8 mm deep. Immediately after burning, the roof of the burn blister was removed with a sterile spatula. Thirty-three burn wounds were made on each animal. After bacteria inoculation, nine burn wounds were assigned to one of the treatment groups.

Wound Inoculation

A fresh culture of a pathogenic wound isolate was used in this study. The inoculum strain used was *Pseudomonas aeruginosa* ATCC 27317. This strain was stored at -70°C on glass beads. To obtain a fresh culture, one glass bead was removed and placed in a nutrient broth, incubated overnight and cultured. All inoculum suspensions were cultured and placed into 5 ml of normal saline until the turbidity of the suspension is equivalent to that of a MacFarland #8 Turbidity Standard. This results in a suspension concentration of approximately 10^8 colony forming units/ml (CFU/ml). The 10^8 suspension was then serially diluted to make an inoculum suspension with a concentration of 10^6 CFU/ml. A small amount of the inoculum suspension was plated onto culture media to quantitate the exact concentration of viable organisms. The inoculum suspension was used directly to inoculate the wounds. A 0.05 ml aliquot of the suspension was deposited into a sterile

glass cylinder (22 mm diameter) surrounding the wound. The suspension was scrubbed into the wound for ten seconds using a sterile teflon spatula.

Experimental Design

Twenty-four hours after inoculation nine burn wounds on each animal was assigned to one of the following treatment groups: 1) untreated, air exposed control, 2) Elasto-Gel®, or 3) Clear-Site . Each treatment was applied 24 hours after inoculation of the burn wounds to allow for colonization of the invading organism.

Quantitative Techniques

Bacteria was recovered from the burn wounds at 24 hrs, 48 hrs, and 72 hrs post treatment (48 hrs, 72 hrs and 96 hrs post inoculation, respectively). At each sampling time, burn wound areas were cultured quantitatively. Each burn area was encompassed by a sterile glass cylinder (22mm outside diameter) held in place by two handles. One ml of buffered tween 80 scrub solution was pipetted into the glass cylinder; and the area encompassed by the cylinder was then vigorously scrubbed with a teflon spatula for 30 seconds. The scrub solution was aspirated from the burn wound and placed in a sterile vial for quantitative analysis which was performed within one hour of sampling. All scrub solutions were quantitated using the Spiral Plater System which deposits a small defined amount (40 µl) of suspension over the surface of a rotating agar plate. After an incubation period, colonies on the plate were counted and colony forming units per ml (CFU/ml) were calculated.

Recovery Media

Pseudomonas aeruginosa ATCC 27317

The recovery media for *P. aeruginosa* was Pseudomonas agar base (Oxoid, Columbia, MD) with Pseudomonas C-F-C supplement incubated for 24 hours at 35°C aerobically. Strain 27317 is resistant to low concentrations of cetrimide, fucidin, and cephaloridine found in the selective media. The selective media was used to prevent the growth of contaminants and normal pig skin microflora so quantitative results will not be affected by competitive inhibition. In addition to the selective media used, a non-selective media (tryptic soy agar with 5% sheep's blood) was inoculated with each recovery sample and incubated aerobically at 35°C to quantitate total organisms present in the sample.

pH MEASUREMENTS

Six additional wounds were made in each treatment group (these wound were not cultured for quantitation). Two wounds per day per treatment group were measured for pH using a Orion pH meter and flat electrode which allowed the measurement of the pH at the wound surface.

OBSERVATIONS

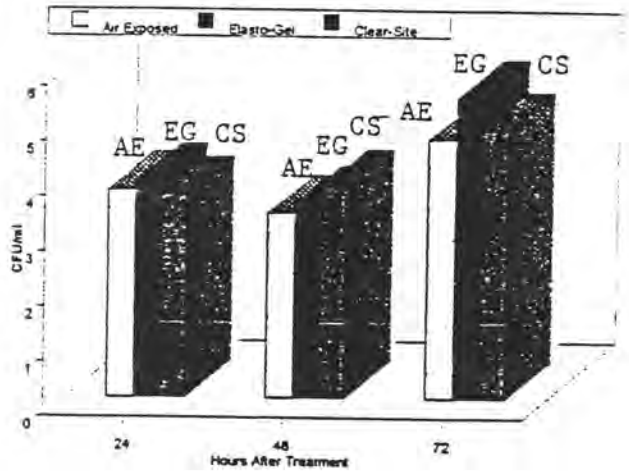
During all assessment times (24, 48, and 72 hours) erythema was noticed surrounding the wounds under both occluded treatments. The air exposed wounds had slight erythema at 24, 28 and 72 hours.

RESULTS

The geometric mean of the Log (CFU/ml) and standard deviation was calculated for each time and treatment. Statistical analysis using unpaired Students T-test was used to detect differences in recovery between treatments.

Pseudomonas aeruginosa

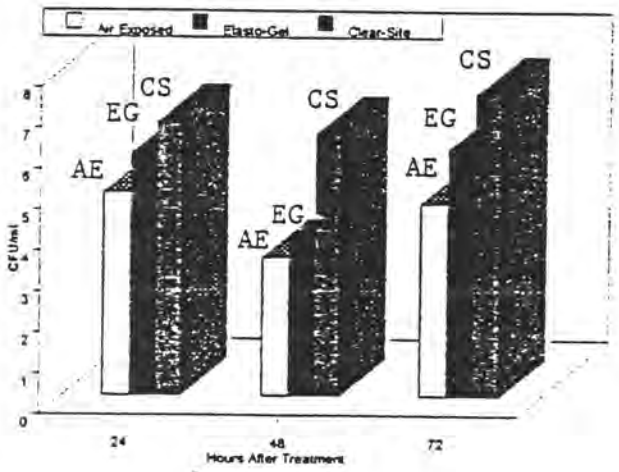
No differences were seen with any of the treatment groups at the 24 , 48 or 72 hour assessment times. The data including standard deviations is presented in Table 1 and Figure 1.



Total Bacteria Recovery

(including *Pseudomonas aeruginosa*)

The total bacteria recovered include normal gram positive skin flora and the challenge *P. Aeruginosa*. The recovery of the total bacteria from wounds treated with Elasto-Gel and air exposed wounds were significantly lower than Clear-Site treated wounds at the 24, 48 and 72 hour assessment times. No differences were seen between air exposed and Elasto-Gel treated wounds. The data including standard deviations is presented in Table 2 and Figure 2.



pH MEASUREMENTS

The pH of the wounds treated with both dressings remained fairly constant over the course of the experiment. No differences were seen between the two treatments. The pH of the air exposed wounds dropped from 8.49 (24 hour) to 6.74 (72 hour). These results are presented in Table 3.

CONCLUSIONS

Elasto-Gel was effective in reducing the total number of bacteria (normal skin and gram positive flora including the challenge *P. aeruginosa*) when compared to Clear-Site. Although the Elasto-Gel was not able to significantly lower the *P. aeruginosa* counts, it did not enhance the proliferation of this pathogen. It is possible that a smaller *P. aeruginosa* inoculum challenge proliferation could be controlled by Elasto-Gel. The mechanism responsible for the total bacteria growth reduction under Elasto-Gel is not understood. It appears from our results that the pH is not responsible for this decrease. It is our experience that all the occlusive dressing we have previously examine have enhanced the proliferation of normal flora over air exposed control. This study was the first time we did not see an increase in bacteria in occluded wounds.

TABLE 1: RECOVERY OF *PSEUDOMONAS AERUGINOSA* *
(Inoculum = 4.57 ± 0.25 Log CFU/ml)

TREATMENT	24 HOURS	48 HOURS	72 HOURS
ELASTO-GEL	3.92 ± 0.21	3.53 ± 0.26	5.47 ± 0.77
CLEAR-SITE	3.69 ± 0.24	3.83 ± 0.67	4.89 ± 0.68
AIR EXPOSED	3.77 ± 0.29	3.36 ± 0.64	4.72 ± 0.88

* Data is represented as mean Log CFU/ ml ± Standard Deviation

TABLE 2: TOTAL BACTERIA RECOVERY INCLUDING *P. AERUGINOSA**
(Inoculum = 4.96 ± 0.11 Log CFU/ml)

TREATMENT	24 HOURS	48 HOURS	72 HOURS
ELASTO-GEL	5.90 ± 0.29	3.80 ± 0.36	6.04 ± 0.50
CLEAR-SITE	6.68 ± 0.27	6.40 ± 0.96	7.40 ± 0.31
AIR EXPOSED	4.96 ± 0.91	3.39 ± 0.22	4.72 ± 0.88

* Data is represented as mean Log CFU/ ml ± Standard Deviation

TABLE 3: pH OF WOUNDS*

TREATMENT	24 HOURS	48 HOURS	72 HOURS
ELASTO-GEL	7.64 ± 0.22	7.44 ± 0.21	7.24 ± 0.39
CLEAR-SITE	7.40 ± 0.30	7.34 ± 0.38	7.31 ± 0.34
AIR EXPOSED	8.49 ± 0.34	7.53 ± 0.62	6.74 ± 0.32

* Data is represented as mean pH ± Standard Deviation