Can Occlusive Dressing Composition Influence Proliferation of Bacterial Wound Pathogens?

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Introduction

Pseudomonas aeruginosa is a gram-negative bacteria that can survive in a semi-dry climate but favors a moist environment. Gram-negative bacteria are relatively rare on normal human skin, but burn wounds present an exudative environment in which *P. aeruginosa* can flourish.[1] This pathogen readily colonizes burn wounds and is considered a major pathogen in burn infections, causing delays in the closure of wounds and increasing the risk of sepsis for the patient.[2–6] *P. aeruginosa* has been consistently associated with the highest morbidity and mortality rates of any

of the gram-negative organisms that cause infection in thermally injured patients. Burn wound care is directed towards the prompt closure of the wound while preventing dehydration and metabolite imbalances due to the highly exudative nature of these wounds.[7,8] This significant loss of water, proteins, and electrolytes may be decreased by the use of occlusive dressings.[9]

Occlusive dressings are used extensively in both acute and chronic wounds. The main advantage of occlusive dressings is their ability to speed healing.[10,11] Other advantages include 1) reduction of pain and tenderness at the wound site, 2) a more cosmetically acceptable wound closure due to decreased scar formation,[12,13] and 3) potential barrier against infection, serving as a substitute for the role of the skin as the first line of defense.[14,15] Dressings keep tissue moist, and this factor can be beneficial for the healing of burn wounds since it has been proven that necrosis in the zone of stasis can be largely reversed by preventing dehydration.[16] Paradoxically, this characteristic is the coinciding factor that has greatly discouraged their use in burn wound patients. Several studies have demonstrated that the total number of bacteria on the skin, as well as the proportion of gram-negative bacilli, are increased by applying occlusive dressings or artificially maintaining a high humidity.[17–19] A wound dressing that could provide the benefits of occlusion without encouraging the growth of *P. aeruginosa* in burn wounds might ultimately result in a more favorable prognosis for burn patients.

The purpose of our study was to evaluate the effect of two hydrogel wound dressings and a hydrocolloid dressing on the multiplication of *P. aeruginosa* in second degree burn wounds *in vivo*. The three dressings evaluated were 1) a hydrogel: 22 percent polyvinyl pyrrolidone [PVP], 18 percent propylene glycol and 60 percent water (ClearSite® Hydrogel Absorptive Wound Dressing, Conmed Corporation), 2) a hydrogel with 65 percent glycerine, 17.5 percent water and 17.5 percent polymeric matrix of acrylamide (Elasto-Gel™, Southwest Technologies, Inc., North Kansas City, Missouri), and 3) a hydrocolloid made of pectin and sodium carboxymethyl-cellulose (DuoDerm® Hydroactive® Dressing, ConvaTec®). Following the *in vivo* study we designed an *in vitro* model to examine the effect of these dressings on various bacteria and to determine the mechanism responsible for our *in vivo* results. The microorganisms we examined in the *in vitro* study are all considered important wound pathogens.

In vivo Study

Experimental animals. Three young Specific Pathogen Free (SPF) pigs weighing 40 to 45 kg were conditioned for two weeks prior to initiating the study. The animals were fed a non-antibiotic chow and housed in our facilities (AALAC approved) with controlled temperature (19–21° C) and controlled light and dark cycles (12L/12D). The animal protocol used in this study was approved by the University of Miami Animal Care Committee, and all procedures followed the federal guidelines for the care and use of laboratory animals (U.S. Department of Health and Human Services). Analgesia was achieved by intramuscular injections of buprenorphine (0.05 mg/kg) after bacterial recovery at each time point.

Burning technique. Each animal was anesthetized with ketamine HCl (20 mg/kg) and xylazine IM (2 mg/kg), 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. Forty-eight burn wounds were made on each animal and the burn wounds were inoculated according to the methods described below.

Wound inoculation. The inoculation technique has been described previously.[20,21] The inoculum strain used was *P. 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 made by scraping the overnight growth of *P. aeruginosa* from blood agar plates

and placing it in 5 ml of normal saline until the turbidity of the suspension was equivalent to that of a MacFarland #8 Turbidity Standard.[22] This resulted in a suspension concentration of approximately 10^8 colony forming units/mL (CFU/ml). This suspension was serially diluted, and a sample of the suspension was inoculated on a culture plate to quantify the concentration of viable organisms. A 0.05 ml aliquot of the inoculum suspension containing 6.88 ± 0.08 CFU of viable organisms was deposited into a sterile glass cylinder (22 mm diameter) surrounding the wound and the suspension scrubbed into the wound for ten seconds using a sterile teflon spatula. This high concentration of bacteria was used to create a highly challenged environment for the wounds and dressings, not necessarily mimicking the use of these dressings in a clinical environment. (The use of occlusive dressings on clinically infected wounds is contraindicative.)

Treatments. Twenty-four hours after inoculation, 12 burn wounds on each animal were assigned to one of the following treatment groups: 1) air-exposed untreated control, 2) hydrogel dressing containing glycerine, 3) hydrocolloid dressing or 4) hydrogel dressing with proplyene glycol. Treatments were applied 24 hours after inoculation of the burn wounds to allow for adequate colonization by *P. aeruginosa.* All dressings were kept on until assessment time. (Each wound was assessed only once.)

Quantitative Techniques — Recovery Methods

Bacteria were recovered from the burn wounds on days 2, 5, 7 and 9 post treatment (days 3, 6, 8, and 10 post inoculation). At each sampling time three burn wounds from each treatment group were cultured quantitatively using a modified scrub technique.[23] Each wound was encompassed by a sterile glass cylinder (22 mm outside diameter) held in place by two handles. One ml of scrub solution (10% Tween 80 and 3% Asolectin) was pipetted into the glass cylinder and the wound vigorously scrubbed with a sterile teflon spatula for 30 seconds. The scrubbing technique removes not only surface organisms but also tissue-invading organisms and has been shown to be comparable to the tissue biopsy technique for evaluating wound infections.[24] 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.

The recovery media for *P. aeruginosa* was *Pseudomonas* agar base (Oxoid, Columbia, MD) with *Pseudomonas* C-F-C supplement incubated for 24 hours at 37° C aerobically. Strain 27317 is resistant to low concentrations of cetrimide, fucidin, and cephaloridine found in the selective media. The selective media prevents the growth of contaminants and normal pig skin microflora so quantitative results would 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 37° C to quantitate total organisms (including *P. aeruginosa* inoculum present in the sample. All scrub solutions were quantitated using the Spiral Plater system.[25]

In Vitro Study

Bacteria preparation. The bacteria used for our *in vitro* study were *Pseudomonas aeruginosa* (ATCC # 27317), *Escherichia coli* (ATCC #25922), *Streptococcus pyogenes* (ATCC #8668), *Staphylococcus aureus* (ATCC #6538) and MRSA (methicillin-resistant *Staphylococcus aureus* ATCC #33593).

The bacteria were grown and the inoculum prepared as previously described. The MRSA was grown on MRSA screening media with an incubation period of 48 hours. MRSA screening media was prepared by adding 4 percent NaCl and 6 percent µg of Oxacillin to Mueller Hinton agar as recommended by the National Committee of Clinical Laboratory Standards (NCCLS). Two different *in vitro* studies were conducted.

The first study was performed to evaluate the effect of the dressings on a lawn of bacterial growth. The plates were streaked evenly in three directions to assure a uniform growth. This allows zones of inhibition to be observed around the dressings. The concentrations used for this experiment were 10^6, 10^4 and 10^2. The dressing sections were cut with a sterile disposable 8 mm punch biopsy, and the disc-shaped sections of the dressings were placed on top of the inoculated agar. Two disc sections of each dressing were placed on each agar plate. After incubation (24 hours blood agar plates and 48 hours MRSA screening media) the plates were checked for

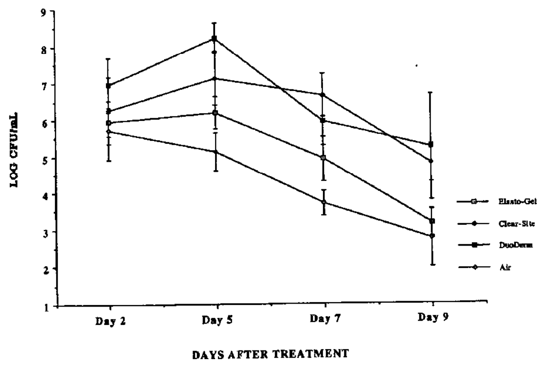

Figure 1. Pseudomonas aeruginosa recovery (log CFU/ml). Inoculum = 6.88 ± 0.08 log CFU/ml

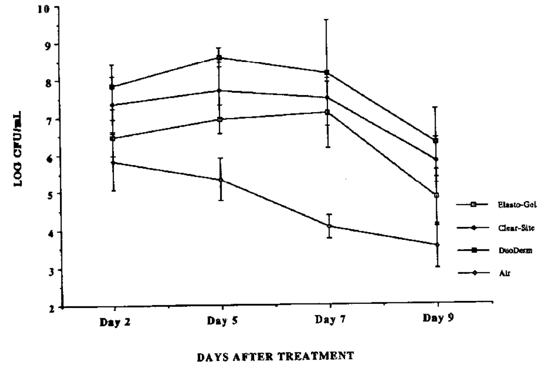

Figure 2. Total bacteria including Pseudomonas aeruginosa recovery (log CFU/ml). Inoculum = 6.88 ± 0.08 log CFU/ml.

Table 1
Bacteria Recovery *In Vitro* (Log CFU/ml)

	P. aeruginosa		*E. coli*	
Dressing	Agar	Dressing	Agar	Dressing
Inoculation size → (Log CFU/ml)	10^2		10^2	
Elasto-Gel	1.42 ± 0.00†	3.90 ± 1.68	1.42 ± 0.00†	1.43 ± 0.01†
ClearSite	5.29 ± 0.35	6.75 ± 0.12*	6.81 ± 0.33*	9.32 ± 0.39
DuoDerm	7.46 ± 0.02	9.74 ± 0.17	8.82 ± 0.11	10.40 ± 0.49
Inoculation size → (Log CFU/ml)	10^4		10^4	
Elasto-Gel	1.42 ± 0.00†	2.89 ± 0.01†	1.42 ± 0.00†	8.44 ± 0.58*
ClearSite	6.30 ± 0.24	9.46 ± 0.01	8.43 ± 0.57	8.82 ± 0.01
DuoDerm	8.76 ± 0.89	9.43 ± 0.33	8.06 ± 0.04	10.37 ± 0.39

† = $p < 0.05$ significance compared to ClearSite and DuoDerm
* = $p < 0.05$ significance compared to DuoDerm
√ = $p < 0.05$ significance compared to ClearSite

Table 2
Bacteria Recovery *In Vitro* (Log CFU/ml)

	S. aureus		*Strep. pyogenes*	
Dressing	Agar	Dressing	Agar	Dressing
Inoculation size → *(Log CFU/ml)*	*10^2*		*10^2*	
Elasto-Gel	4.86 ± 1.32✓	9.31 ± 0.01✓	1.42 ± 0.00✓	1.42 ± 0.00†
ClearSite	9.59 ± 0.77	11.69 ± 0.21	7.05 ± 0.23	7.57 ± 0.44
DuoDerm	9.36 ± 0.11	9.73 ± 0.06	4.77 ± 0.48✓	7.87 ± 0.59
Inoculation size → *(Log CFU/ml)*	*10^4*		*10^4*	
Elasto-Gel	2.08 ± 0.93†	8.75 ± 0.28†	1.42 ± 0.00†	1.78 ± 0.50†
ClearSite	9.36 ± 0.09	11.76 ± 0.26	9.51 ± 0.18	7.78 ± 0.06
DuoDerm	10.10 ± 0.30	11.08 ± 0.36	8.91 ± 0.46	7.50 ± 0.21

† = $p < 0.05$ signiicance compared to ClearSite and DuoDerm
* = $p < 0.05$ significance compared to DuoDerm
✓ = $p < 0.05$ significance compared to ClearSite

inhibition zones around the different dressing discs.

A second *in vitro* experiment was designed to evaluate the effect of the dressings on agar plates inoculated with known amounts of bacteria. The inoculum concentrations used were 10^2 and 10^4. Higher concentrations were not used since the aim of this experiment was to mirror wounds colonized by low numbers of microorganisms, not showing clinical signs of infection, that would be covered by occlusive dressings in a clinical setting.

In the second study, two sites on the agar plates were inoculated with 25 µL of inoculum suspension containing 10^2 or 10^4 CFU of bacteria or sterile saline solution. A 2 cm^2 section of dressing was used to cover each inoculated site. Six separate 2 cm^2 sections of each dressing per bacterial strain were used. Four of the cut dressings, divided into pairs, were placed immediately on top of an agar plate inoculated with duplicates of each of the two concentrations of bacteria. The two remaining sections served as control and were placed on top of agar plates inoculated with sterile saline. The plates were then incubated for 24 hours (MRSA screening media plates for 48 hours) at 37° C. After the incubation period, the dressings were removed aseptically and placed in sterile homogenizer bags (Whirl-Pak, Nasco) containing 5 ml of scrub solution to evaluate bacterial content. The inoculated agar was also evaluated. The center of the inoculated site, which had been covered with the dressing, was biopsied using an 8 mm sterile disposable punch biopsy. The agar was then placed in sterile homogenizer bags with 5 ml of scrub solution. All homogenizer bags containing the dressing or the agar in the scrub solution were processed using a Colworth stomacher (Tekmark Co.) for two 30 second intervals. The stomacher is a specialized machine equipped with two paddles that beat against the sample and remove bacteria from it by a sponging action.

The dressing and agar homogenate obtained from the stomacher were serially diluted and the bacterial burden quantified using the Spiral Plater System.

Data analysis. After the 24 hour incubation period (48 hours for MRSA), colonies on the

plates were counted and the colony forming units per mL (CFU/ml) calculated.

The geometric mean of the log CFU/ml and the standard deviation were calculated for each sample analyzed. Analysis of variance and student t-tests were used to detect significant differences in recovery between the different dressings evaluated.

Results

Pseudomonas aeruginosa and total bacteria recovery for each time point and treatment for the *in vivo* study are shown in Figures 1 and 2. The results from the *in vitro* study, showing the growth from the dressings and agar, are in Tables 1 to 3.

***In vivo* study.** All of the wounds covered with the occlusive dressings appeared clinically infected on observation days 2 and 5. The wounds treated with the hydrogel and the hydrogel containing glycerine were moist and macerated (days 2 and 5). Wounds covered with the hydrocolloid dressing did not appear macerated but had a sticky residue on days 2 and 5. On day 7 all the wounds had a foul odor, but by day 9 none of the wounds appeared clinically infected. Air-exposed wounds were dry and covered by a thick eschar and did not show any clinical signs of infection at any time point.

Pseudomonas was recovered from all of the wounds. However, wounds covered with the hydrogel containing glycerine had a significantly lower number of CFU/ml of *P. aeruginosa* than those covered by the hydrocolloid dressing at all time points, and than those treated with the hydrogel dressing on days 5, 7 and 9 (Figure 1). The recovery from the air-exposed wounds was significantly lower than that from all of the occlusive dressing treatments including the hydrogel containing glycerine on days 5 and 7 after treatment. Total bacteria recovery (including *P. aeruginosa* from the wounds treated with the hydrogel containing glycerine) was significantly lower than those of the wounds covered with the hydrocolloid and the hydrogel dressing on days 2, 5, and 9 (Figure 2). No significant differences in recovery were observed between the hydrocolloid and the hydrogel dressing except on day 5. At this time point the total bacteria recovery from the wounds treated with the hydrocolloid dressings was significantly higher than that of the hydrogel dressing treated wounds. Total bacteria recovery from air-exposed control wounds was significantly lower than that of all occluded wounds at all time points evaluated. Wound healing was not evaluated in this study.

Table 3
Bacteria Recovery *In Vitro* (Log CFU/ml)

	MRSA	
Dressing	Agar	Dressing
Inoculation size → *(CFU/ml)*	10^2	
Elasto-Gel	2.02 ± 0.83	3.90 ± 1.68†
ClearSite	8.11 ± 0.13	6.75 ± 0.12*
DuoDerm	1.42 ± 0.00✓	9.74 ± 0.17
Inoculation size → *(CFU/ml)*	10^4	
Elasto-Gel	1.42 ± 0.00✓	4.18 ± 0.89†
ClearSite	6.92 ± 0.03*	9.90 ± 0.96
DuoDerm	1.73 ± 0.43	10.39 ± 0.38

† = $p < 0.04$ signiicance compared to ClearSite and DuoDerm
* = $p < 0.03$ significance compared to DuoDerm
✓ = $p < 0.09$ significance compared to ClearSite

***In vitro* study.** The results from the first *in vitro* study in which we examined bacterial inhibition using dressing sections placed on top of a lawn of bacterial growth showed no detectable antimicrobial effect. In the second *in vitro* study where we inoculated the blood plates then immediately covered an area with the hydrogel containing glycerine, a significant reduction of *P. aeruginosa* and *E. coli* counts were seen. Both concentrations (10^2 and 10^4) of the two pathogens were reduced when compared to the hydrogel and the hydrocolloid dressing (Table 1). The hydrogel containing glycerine significantly reduced both concentrations of *S. pyogenes* and *S. aureus* counts in the agar when compared to the agar covered with hydrogel dressing only. The hydrogel containing glycerine significantly

reduced the higher concentration of these two bacteria (10^4) when compared to the hydrocolloid dressing (Table 2). When MRSA was covered with the various dressings, lower counts were seen with the hydrogel containing glycerine as compared to the hydrogel dressing for the higher inoculum concentration of MRSA (Table 3).

Significant differences were also observed when the dressings were evaluated. The hydrogel dressing had a significantly higher count of bacteria for both concentrations of *S. pyogenes*, *S. aureus* and MRSA when compared to the hydrogel dressing containing glycerine (Table 2). The same was true for the lower concentration of *E. coli* (10^2) and the higher concentration of *P. aeruginosa* (10^4) (Table 1). The hydrocolloid dressing also had significantly higher counts of *S. pyogenes*, MRSA and *E. coli* at both inoculum concentrations. The higher concentration of *S. aureus* and *P. aeruginosa* had significantly higher counts in hydrocolloid dressing when compared to the hydrogel dressing containing glycerine (Tables 1 to 3).

Discussion

Overall, we found that the hydrogel dressing with glycerine was able to reduce the number of organsims (*in vivo* and *in vitro*) as compared to the other dressings that were examined. The use of an occlusive dressing that does not enhance the proliferation of *P. aeruginosa* is a definite advantage when treating wounds (especially burns). In the *in vivo* study the burn wounds were heavily colonized and appeared clinically infected by *P. aeruginosa*. In clinical circumstances occlusive dressings would not be applied to an infected wound.

The higher number of CFU/ml of total bacteria recovered from all occluded wounds as compared to air-exposed was expected. This increase is due to the typical proliferation of normal flora under occlusive dressings and is not considered to be detrimental to wound repair.[19] Furthermore, a review of the literature has shown that wounds covered with occlusive dressings had better infection rates than those covered with conventional dressings (i.e. gauze, non-adherent layers and paste bandages).[26] The decrease in bacteria count for the air-exposed wounds was also expected since *P. aeruginosa* favors a moist environment for multiplication.

In the agar diffusion assay, which measures the ability of an agent to diffuse and produce a zone of inhibition, no antimicrobial activity could be demonstrated for the dressing biopsies. Many variables can influence these results, such as solubility of the agent, inoculum density, pH and the stability of the antimicrobial agent. Conversely, our *in vitro* and *in vivo* studies seem to substantiate that the hydrogel containing glycerine does not enhance the proliferation or create an adequate environment for the maintenance of *P. aeruginosa* in burn wounds. The mechanism of action is not yet known. Glycerine has been reported to have an antimicrobial effect, which is attributed to dehydration.[27] Ahmed, et al. reported that a solution of ten percent glycerine and ichthammol showed limited *in vitro* activity against gram negatives and particularly *P. aeruginosa*.[28] The results from these studies demonstrate that the composition of the dressings can influence the proliferation of the common bacterial pathogens *in vitro*. These results suggest that not only does the hydrogel containing glycerine provide a wound environment that does not support multiplication of *P. aeruginosa*, but it is also capable of reducing the multiplication of this pathogen in the dressing itself as compared to the hydrogel and hydrocolloid dressings. It is important to note that the total bacteria (including *P. aeruginosa*) was reduced with the glycerine dressing, which is very clinically relevant since an excessive bioburden impairs wound healing.

The clinical significance of these results has not been determined. However, the suggestion that a hydrogel containing glycerine might reduce the bacterial burden of a chronic colonized wound is intriguing.

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