UNIVERSITY OF MIAMI
SCHOOL OF MEDICINE

DEPARTMENT OF
DERMATOLOGY
& CUTANEOUS SURGERY

Report

II

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

REPORT

Evaluation of the effects of a hydrogel wound dressing on *Pseudomonas aeruginosa* multiplication in Second-degree Burn Wounds.

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August 22, 1995

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. Many occlusive dressings have been shown to be effective in stimulating the healing of both acute and chronic wounds, however, their value on burn wounds is less established. The "fear of infection" unfairly associated with occlusive dressings has discouraged their use, especially among burn surgeons.

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 effect of a hydrogel wound dressing on the growth of *Pseudomonas aeruginosa* in second-degree burn wounds.

MATERIALS AND METHODS

Experimental Animals

Three young Specific Pathogen Free (SPF) pigs weighing approximately 28 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 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. Forty-eight burn wounds were made on each animal.

Wound Inoculation

A fresh culture of a pathogenic wound isolate was used in this study. The inoculum strain used was *Pseudomonas aeruginosa (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 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 twelve burn wounds on each animal will be assigned to one of the following treatment groups:

Number of Animals	Number of Wounds	Treatment Groups
3	12	Air Exposed, Untreated
	12	Elasto-Gel
	12	DuoDerm®
	12	ClearSite®

Each treatment will be applied 24 hours after inoculation of the burn wounds to allow for *P. aeruginosa* colonization of the burn wound.

Quantitative Techniques

Bacteria was 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, 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.

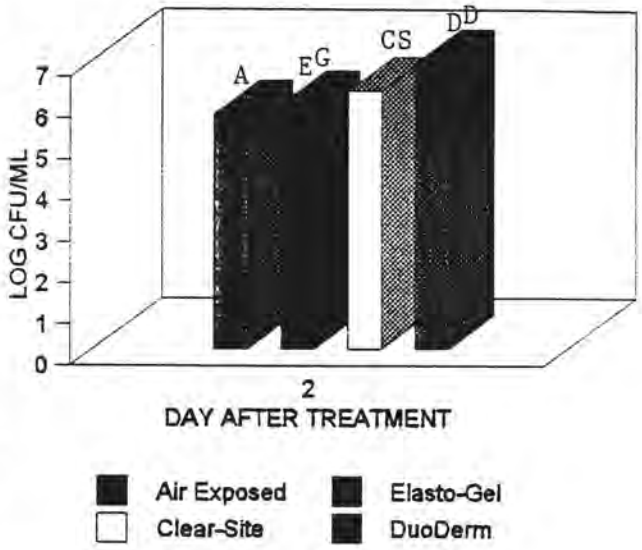
OBSERVATIONS

During the assessment times days 2 and 5 post treatment, the burn wounds underneath all occlusive dressings appeared clinically infected, i.e. edema, erythema and poignant odor. Wounds treated with ClearSite® and Elasto-Gel® appeared macerated during the sampling of the wound area. The wounds treated with DuoDerm® did not appear macerated. DuoDerm® dressing left a sticky residue material on the wound area during removal. On day 7 , the ClearSite® and Elasto-Gel® covered wounds were covered by a thin soft crust. On day 9, the crust from underneath the Elasto-Gel® dressing became detached from the wound during

dressing removal. The wounds underneath the ClearSite® appeared slightly dry by day 9 post treatment. The air exposed wound had a dry crust throughout the entire study. Only slight erythema was noticed with the air exposed wounds on day 2.

RESULTS

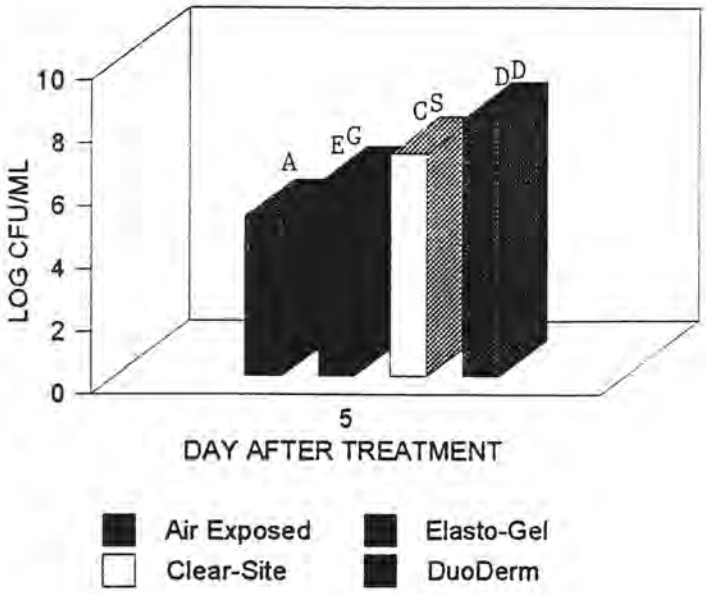
The geometric mean of the Log (CFU/ml) and standard deviation was calculated for each time and treatment. Statistical analysis using unpaired Students T-test was used to detect differences in recovery between treatments (see Figures 1 and 2; Appendices 1 and 2).



Pseudomonas aeruginosa

Day 2

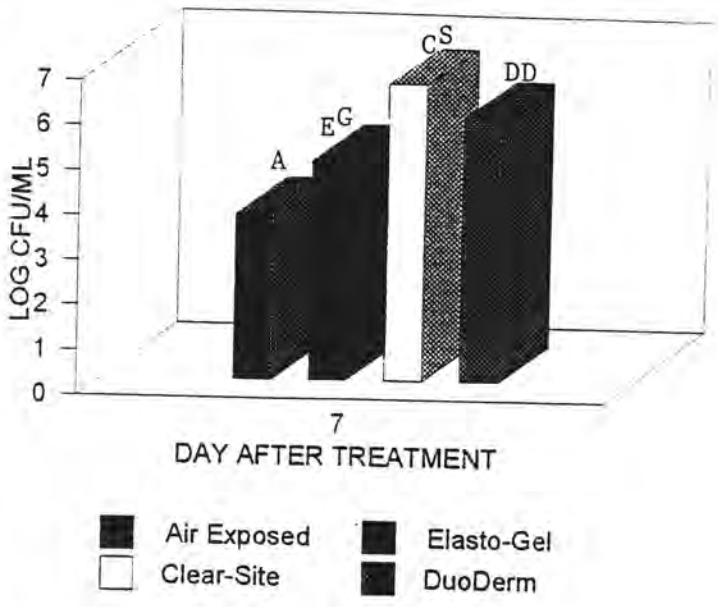
Elasto-Gel treated wounds had similar numbers of *P. aeruginosa* as the air control wounds. Both Elasto-Gel and air exposed wounds had significantly less *P. aeruginosa* than DuoDerm. Clear-Site wounds and DuoDerm wounds did not differ in the amounts of *P. aeruginosa*.



Day 5

Elasto-Gel treated wounds contained significantly less *P. aeruginosa* than either Clear-Site or DuoDerm wounds. Air exposed

wounds had significantly less *P. aeruginosa* than all other treatments.

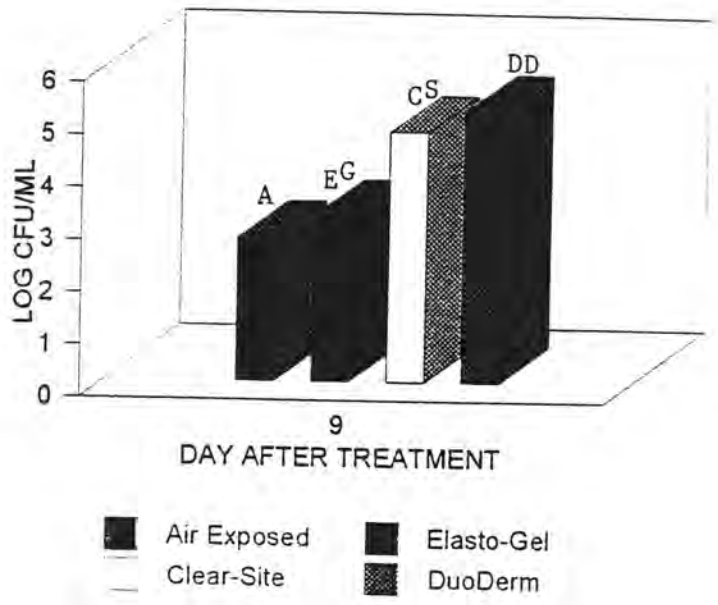


Day 7

Elasto-Gel treated wounds again contained significantly less *P. aeruginosa* than Clear-Site and DuoDerm wounds. Treatment with Clear-Site dressing supported significantly more *P. aeruginosa* (CFU/ml) than DuoDerm. Air exposed wounds continued to have a lower number of *P. aeruginosa* as compared to all other treatments.

Day 9

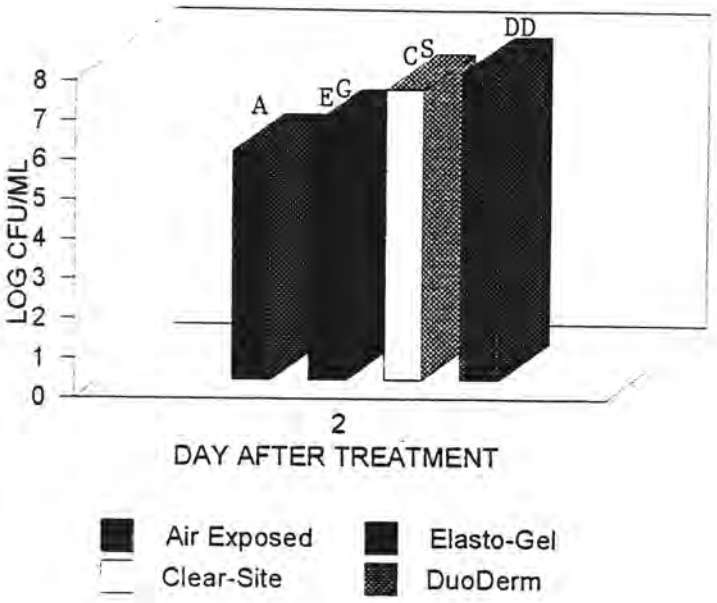
Elasto-Gel treated wound continued to contain significantly less *P. aeruginosa* than both Clear-Site and DuoDerm treated wounds and were comparable to air exposure.



Total Bacteria (including *Pseudomonas aeruginosa*)

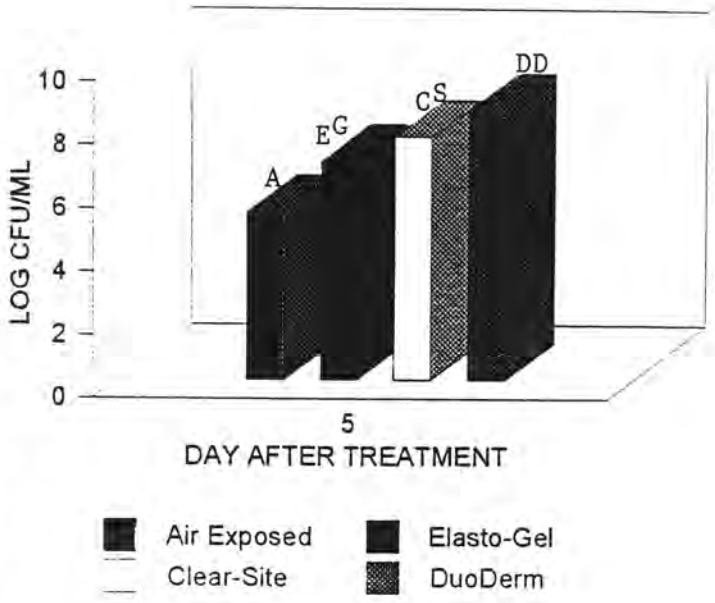
Day 2

The Elasto-Gel treated wounds had significantly less total bacteria (including *P. aeruginosa)* than both Clear-Site and DuoDerm. Air exposed wounds had significantly less total bacteria than all other treatment groups.



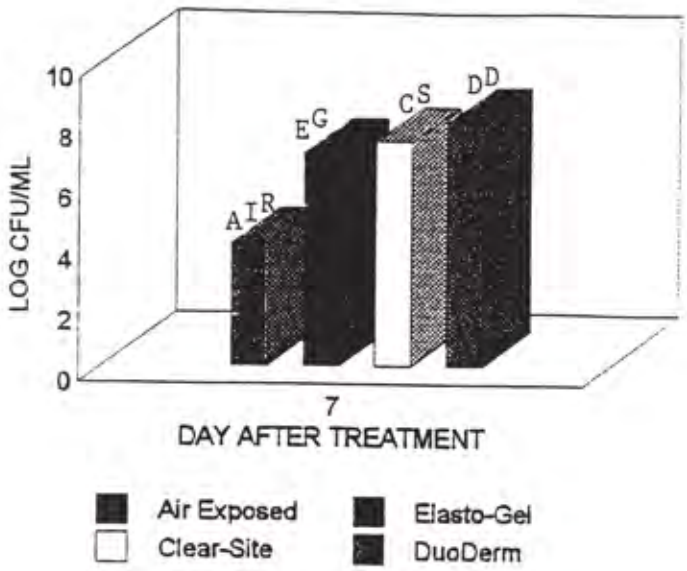
Day 5

Elasto-Gel treated wounds had significantly less total bacteria (including *P. aeruginosa*) than both Clear-Site and DuoDerm wounds. The Clear-Site treated wound had a lower total bacteria count than DuoDerm treated wounds. The air exposed wounds had a lower total number of bacteria (including *P. aeruginosa*) as compared to wounds treated with all other treatment groups.



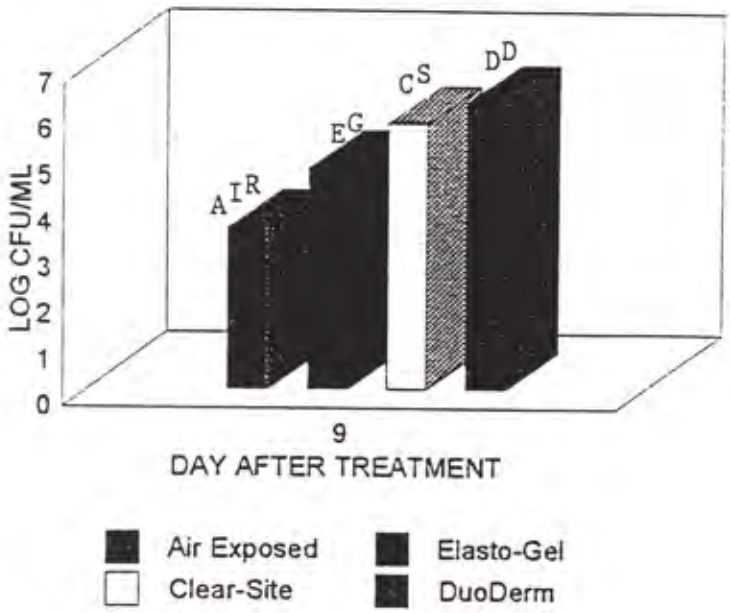
Day 7

On day 7 post treatment a decrease in the total number of bacteria (including *P. aeruginosa)* was seen with air exposed wounds as compared to all other treatment groups. No other statistical differences were seen between treatment groups.



Day 9

Elasto-Gel treated wounds had significantly less total number of bacteria (including *P. aeruginosa)* than both Clear-Site and DuoDerm treated wounds. The air exposed wounds had a lower total number of bacteria as compared to all treatments. No statistical differences were seen between Clear-Site and DuoDerm treated wounds.



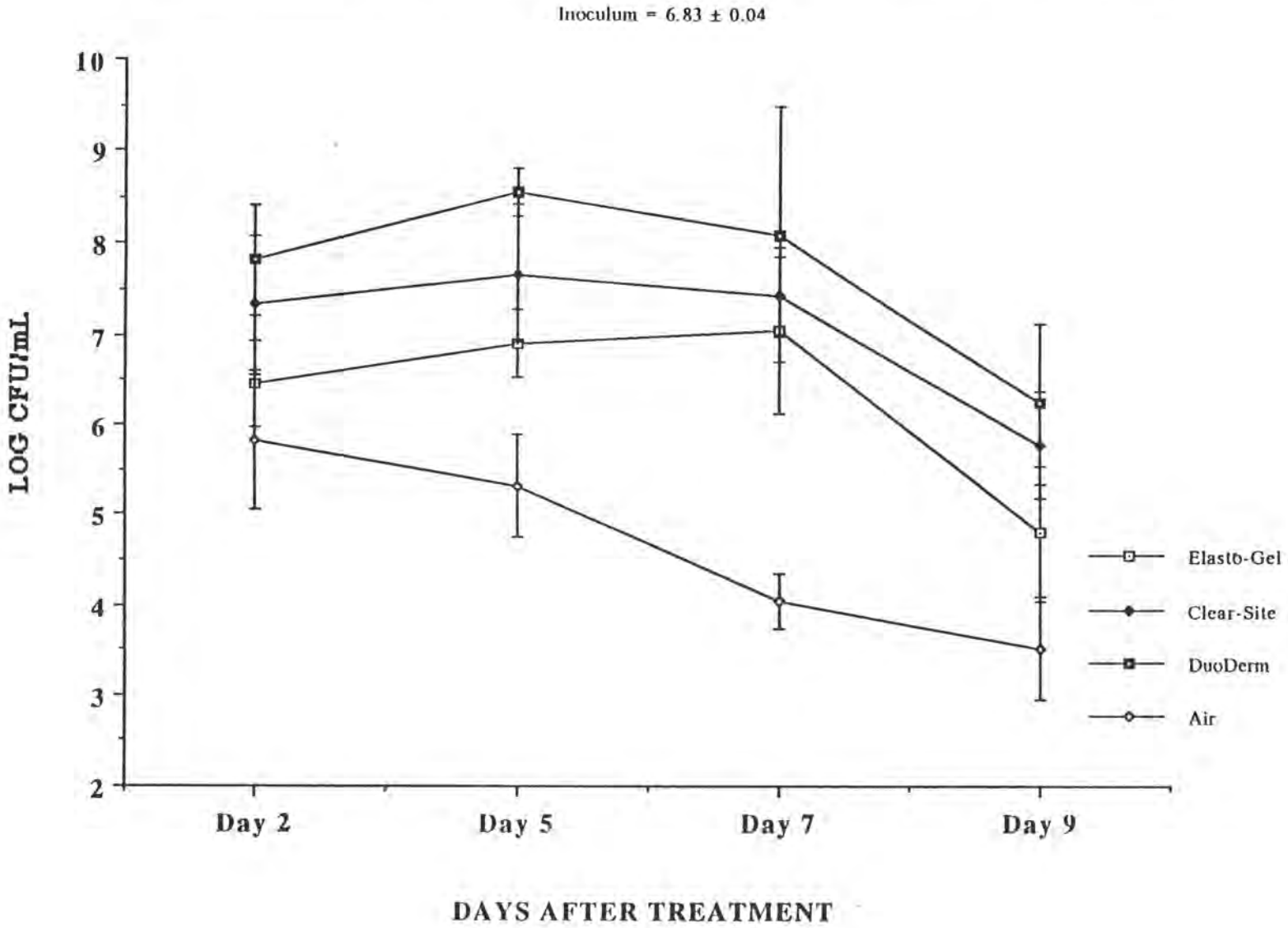
CONCLUSIONS

Elasto-Gel was effective in reducing both the number *P. aeruginosa* and total number of bacteria (normal skin and gram positive flora including the challenge *P. aeruginosa*) when compared to both DuoDerm and Clear-Site dressings. In some instances Elasto-Gel treated wounds had comparable numbers of *P. aeruginosa* as air exposure. These data suggest that Elasto-Gel occlusion does not favorable support *P. aeruginosa* proliferation and may have significant implications for

clinical use. The decrease in bacteria count for the air exposed wounds was expected since *P. aeruginosa* favors a moist environment for proliferation. IT is interesting that the other hydrogel dressing (Clear-Site), enhanced bacteria growth as compared to Elasto-Gel. The mechanism responsible for the total bacteria growth reduction under Elasto-Gel is unknown. We can speculate that either one of the materials used in making the gel has a bacteriostatic or bactericidal effect or the moist wound environment created by Elasto-Gel does not favor bacterial proliferation.

It would be possible to determined if an individual component of Elasto-Gel was responsible for the reduction in numbers of *P. aeruginosa*. An assay system to make these determination could be conducted *in vitro*.

Figure 2: TOTAL BACTERIA RECOVERY (INCLUDING P. AERUGINOSA)



Appendix 1: Statistical comparison of *Pseudomonas aeruginosa* recovered in CFU/ml

DAY 2	Air Exposed 5.71± 0.81	Elasto-Gel® 5.94± 0.39	Clear-Site® 6.26±0.91	DuoDerm® 6.95±0.74
Air Exposed 5.71± 0.81		NS	p = 0.0028	p = 0.0021
Elasto-Gel® 5.94± 0.39			NS	p = 0.0067
Clear-Site® 6.26±0.91				NS
DuoDerm® 6.95±0.74				

DAY 5	Air Exposed 5.12± 0.51	Elasto-Gel® 6.19± 0.44	Clear-Site® 7.11±0.72	DuoDerm® 8.20±0.40
Air Exposed 5.12± 0.51		p = 0.0008	p = 0.0008	p = 0.0001
Elasto-Gel® 6.19± 0.44			p = 0.0082	p = 0.0001
Clear-Site® 7.11±0.72				p = 0.008
DuoDerm® 8.20±0.40				

DAY 7	Air Exposed 3.69± 0.34	Elasto-Gel® 4.90± 0.61	Clear-Site® 6.63±0.58	DuoDerm® 5.93±0.67
Air Exposed 3.69± 0.34		p = 0.0008	p = 0.0001	p = 0.0001
Elasto-Gel® 4.90± 0.61			p = 0.0001	p = 0.0009
Clear-Site® 6.63±0.58				p = 0.0078
DuoDerm® 5.93±0.67				

DAY 9	Air Exposed 2.74± 0.76	Elasto-Gel® 3.16± 0.37	Clear-Site® 4.78±0.52	DuoDerm® 5.21±1.44
Air Exposed 2.74± 0.76		N.S.	p = 0.0001	p = 0.0014
Elasto-Gel® 3.16± 0.37			p = 0.0002	p = 0.0027
Clear-Site® 4.78±0.52				N.S.
DuoDerm® 5.21±1.44				

N.S. = Not Significant

Appendix 2: Statistical comparison of total bacteria (including *Pseudomonas aeruginosa*) recovered in CFU/ml

DAY 2	Air Exposed 5.81± 0.75	Elasto-Gel® 6.45± 0.48	Clear-Site® 7.03±0.60	DuoDerm® 7.83±0.60
Air Exposed 5.81± 0.75		p = 0.004	p = 0.0001	p = 0.0001
Elasto-Gel® 6.45± 0.48			p = 0.0022	p = 0.0001
Clear-Site® 7.03±0.60				NS
DuoDerm® 7.83±0.60				

DAY 5	Air Exposed 5.32± 0.58	Elasto-Gel® 6.92± 0.39	Clear-Site® 7.69±0.75	DuoDerm® 8.57±0.26
Air Exposed 5.32± 0.58		p = 0.0002	p = 0.0004	p = 0.0001
Elasto-Gel® 6.92± 0.39			p = 0.0072	p = 0.0001
Clear-Site® 7.69±0.75				p = 0.0072
DuoDerm® 8.57±0.26				

DAY 7	Air Exposed 4.04± 0.31	Elasto-Gel® 7.06± 0.92	Clear-Site® 7.44±0.44	DuoDerm® 8.12±1.40
Air Exposed 4.04± 0.31		p = 0.0001	p = 0.0001	p = 0.0001
Elasto-Gel® 7.06± 0.92			N.S.	N.S.
Clear-Site® 7.44±0.44				N.S.
DuoDerm® 8.12±1.40				

DAY 9	Air Exposed 3.51± 0.58	Elasto-Gel® 4.80± 0.75	Clear-Site® 5.78±0.60	DuoDerm® 6.25±0.90
Air Exposed 3.51± 0.58		p = 0.0001	p = 0.0001	p = 0.0001
Elasto-Gel® 4.80± 0.75			p = 0.0017	p = 0.0016
Clear-Site® 5.78±0.60				N.S.
DuoDerm® 6.25±0.90				

N.S. = Not Significant

Figure 1: PSEUDOMONAS AERUGINOSA RECOVERY

Inoculum = 6.88 ± 0.08

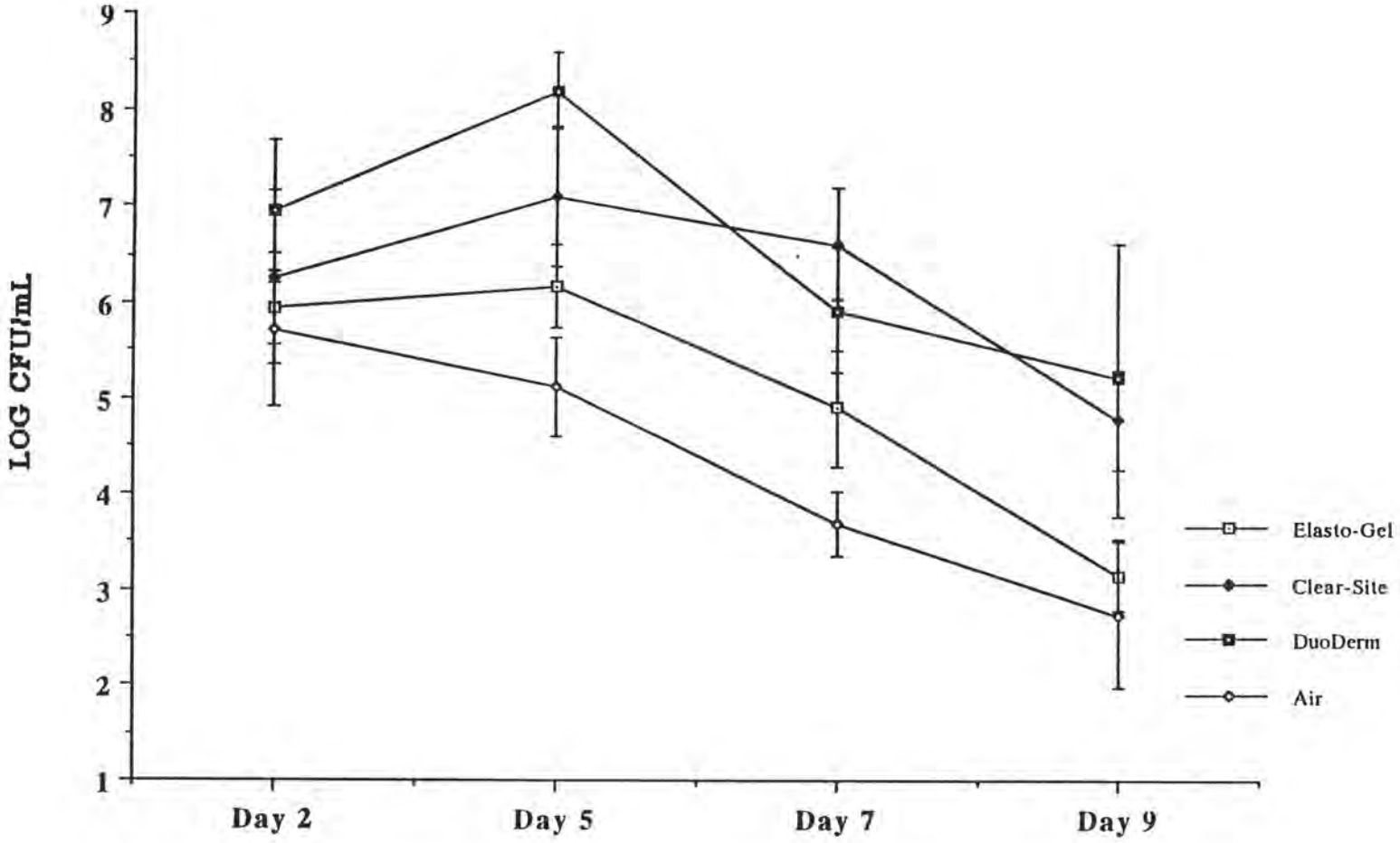
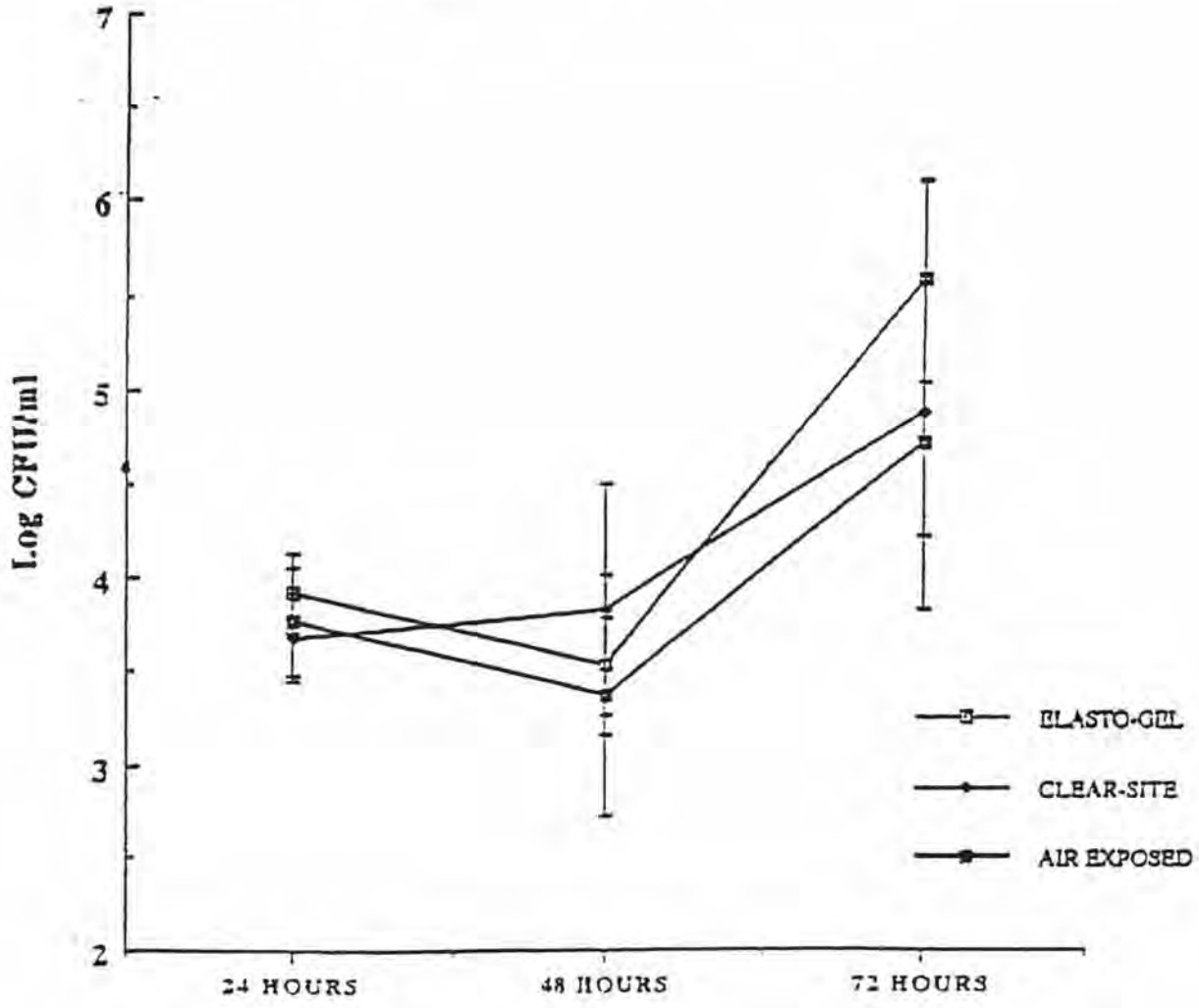


Figure 1: RECOVERY OF PSEUDOMONAS AERUGINOSA

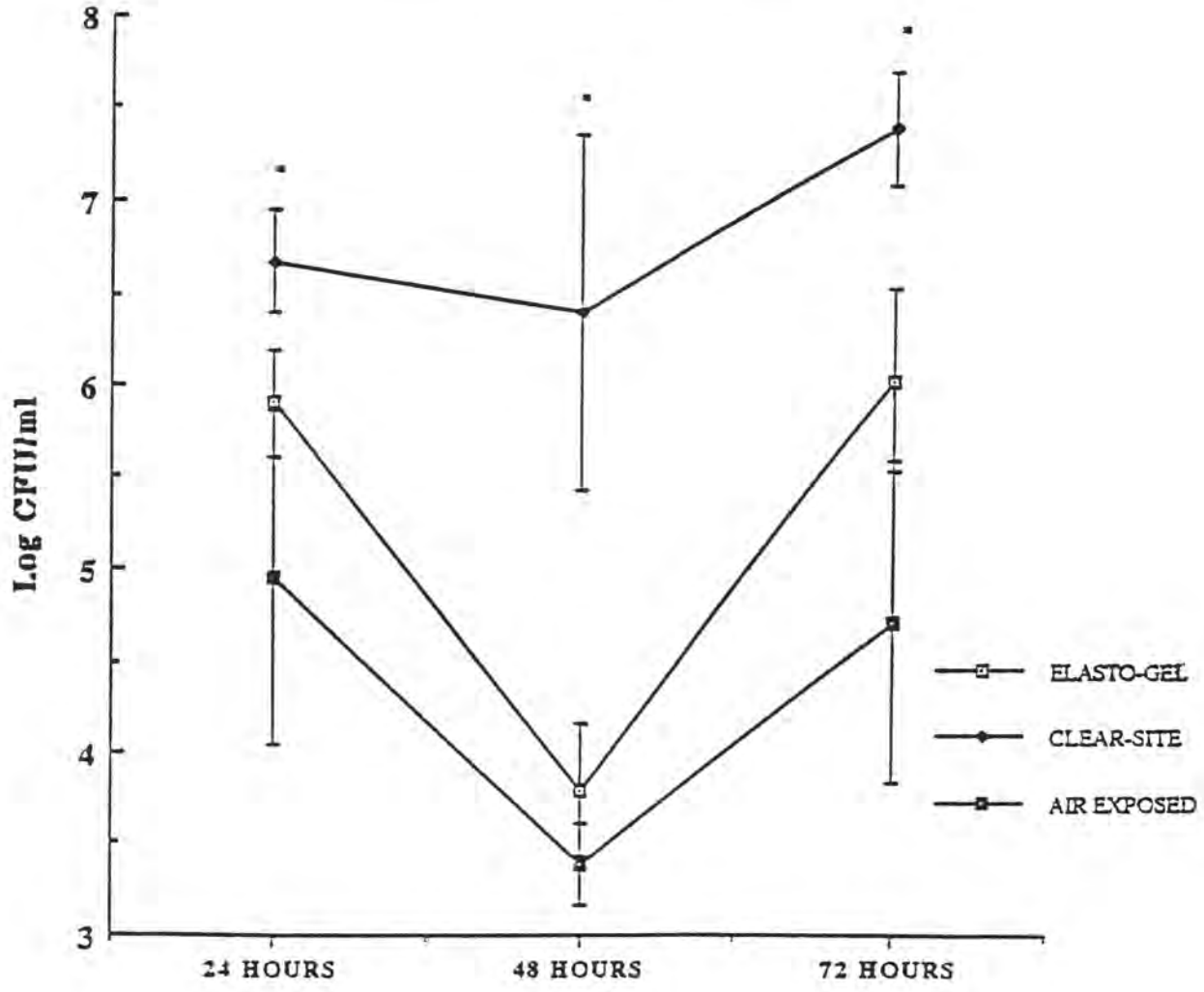
(Inoculum = 4.57 ± 0.25 Log CFU/ml)



HOURS AFTER TREATING

Figure 2: TOTAL RECOVERY OF BACTERIA INCLUDING P. AERUGINOSA

(Inoculum = 4.96 ± 0.11 Log CFU/ml)



HOURS AFTER TREATING

* $p < 0.05$ Elasto-Gel and Air Exposed vs Clear-Site