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Preliminary Evidence on the Barrier Properties of a PHMB-Impregnated Acellular Biologic Grafts for Treatment of Third-Degree Burns

Stephen C. Davis[1], Joel Gil[1], Alexander Higa[1], Michael Solis[1], Emily Buck[2], William M. Heim[2] and Nicholas C. Pashos[2]

[1]Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, Miller School of Medicine, University of Miami, Florida, [2] BioAesthetics Corporation, Durham, NC, United States.

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Abstract:

Introduction:
The wound environment is a fertile breeding ground for pathogens.[1] Some products such as wound dressings, have the ability to prevent the entrance of these pathogens, while others are formulated to reduce the bacterial load. The present study investigated the ability of a PHMB-Impregnated Acellular Biologic Grafts to act as a barrier using a 3rd degree burn porcine model.[2]

Methods:
Twenty-four (24) third degree burn wounds were created on pig and after 3 days of burn progression, all wounds were debrided with 4mm currete.[3,4] Six (6) wounds were randomly treated with one of the following treatments: 1) PHMB-Impregnated Acellular Biologic Graft (Graft+PHMB)^, 2) Acellular Biologic Graft (Graft Alone)+, 3) Bilayer Matrix Wound Dressing (BMWD)*, or 4) Untreated Control●. All treated wounds were inoculated with *Pseudomonas aeruginosa* ATCC27312 (PA) and then covered with a polyurethane film dressings until assessment on days 7 and 14.

Results:
On days 7 and 14, Graft+PHMB showed a significant ($p \leq 0.05$) reduction compared to all treatment groups with the highest values in reduction being 3.51±0.11 and 4.60±0.05 Log CFU/g compared to Untreated Control, respectively. These values represent more than 99.9% reduction in bacterial load. Comparing both assessment times, Graft+PHMB was the only treatment able to reduce PA bacterial count significantly ($p \leq 0.05$). Graft Alone resulted in a significant reduction of PA compared to BMWD and Untreated Control on both assessment days. No statistical significance resulted comparing BMWD and Untreated Control on both assessment days.

Discussion:
This study demonstrated that the Graft+PHMB sequestered and impeded bacterial penetration into the wounds. These results may have important implications clinically, especially for controlling the bacterial load within a wound and the prevention of wound infections.

^PHMB-Impregnated Acellular Biologic Grafts (BioAesthetics Corporation, Durham, NC USA), +Acellular Biologic Grafts (BioAesthetics Corporation, Durham, NC USA), *Integra® (Integra LifeSciences, Princeton, NJ, USA), ●Tegaderm™ (3M, St. Paul, MN USA)

Introduction:

Preventing the entrance of wound pathogens that can impede the healing process, is very important.[5] Several products have been used as physical barriers to prevent wound infection.[6,7] We have previously demonstrated the effectiveness of a polyhexamethylene biguanide (PHMB) gauze to significantly reduce the entrance of *Pseudomonas aeruginosa* using a porcine model.[8] PHMB is a broad-spectrum antimicrobial agent which does not appear to inhibit the healing process.[9] In this study we evaluate the ability of PHMB-Impregnated Acellular Biologic Grafts to prevent the entrance of *P. aeruginosa* in 3rd Degree Burn wounds.

Materials and Methods:

1. Experimental Animals:

- Swine were used as our experimental animal due to the morphological, physiological, and biochemical similarities between porcine skin and human skin.[10]

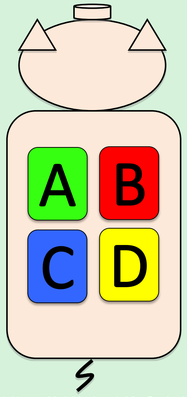
2. Wounding Technique:

- Twenty-four (24) 3rd degree burn wounds were created using a branding iron (L & H Manufacturing Company Mandan, North Dakota 58554).
- Branding iron at 300°C was placed for 15 seconds to create wounds with 27mm diameter and a depth of approximately 3mm.
- After 72 hours all wounds were debrided until punctate bleeding occurred using a Weck knife (as performed clinically).

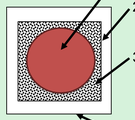
3. Inoculation:

- *Pseudomonas aeruginosa* ATCC 27312 (PA27312) with 10^6 CFU/ml was used to inoculate the areas to challenge each treatment.

4. Experimental Design:



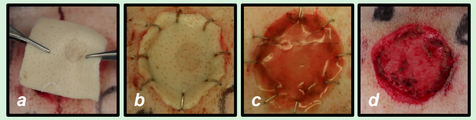
Treatment Groups
A - PHMB-Impregnated Acellular Biologic Graft^
B - Acellular Biologic Graft+
C - Bilayer Matrix Wound Dressing*
D - Untreated Control●



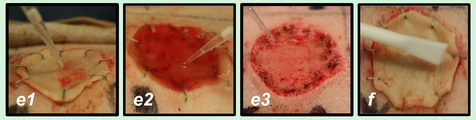
1) Wounds were created and after 72 hours debrided.
2) Wounds were covered with each treatment ≅ 4.5x4.5cm.
3) Treatments were then challenged on the top surface and surrounding area with 25µL of PA27312.
4) Treatments were covered with polyurethane film dressing.

5. Treatment Regimen:

a. After debridement, treatments were randomly assigned to wounds with six (6) wounds per group. Acellular Biologic Graft with or without PHMB treatments were carefully placed on top of their assigned wounds.
b. Each graft was cut to fit the wound and stapled in place.
c. Bilayer Matrix Wound Dressing was also placed carefully onto its respective wounds, cut to fit, and stapled in place.
d. Untreated control wounds did not receive treatment.



e. All treated areas were challenged with 25 µL of *Pseudomonas aeruginosa* ATCC 27312. Acellular Biologic Graft with or without PHMB (**photo e1**), Bilayer Matrix Wound Dressing (**photo e2**), and Untreated control wounds (**photo e3**).
f. After inoculation the inoculum was spread with sterile spatula over the treatments and surrounding normal skin.



6. Wound Recovery:

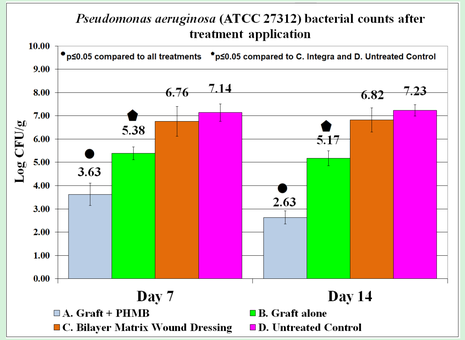
- On days 7 and 14 after treatment application, treated areas were recovered using a 6mm punch biopsy (**photo g**).
- Biopsies were homogenized and combined with a scrub solution.
- Serial dilutions were made (**photo h**) and quantified using the Spiral Plater System, which deposits a defined amount (50µl) of suspension over the surface of a rotating agar plate (**photo i**).
- Pseudomonas Agar-base with CN supplement was used to isolate PA27312. Plates were incubated at 37±2°C for 24 hours (**photo j**).The colony forming units per g (CFU/g) were calculated.

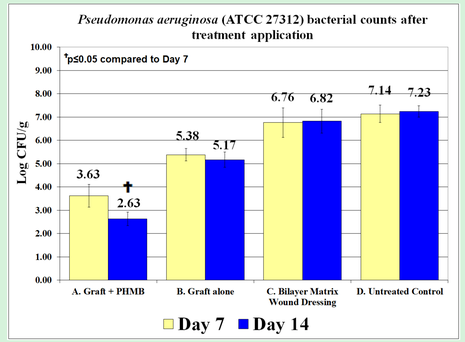
7. Statistical Analysis:

- A two-way analysis of variance (ANOVA) was used for statistical analysis for the microbiology and histology results. A p-value ≤ than 0.05 was considered significant.

Results:

- On day 7, Untreated wounds showed the highest amount of PA27312 at 7.14±0.37 Log CFU/g. Wounds treated with BMWD were slightly lower than Untreated Control at 6.76±0.64 Log CFU/g. Wounds treated with Graft alone had a bacterial count of 5.38±0.27 Log CFU/g, having significantly ($p \leq 0.05$) lower counts than those wounds treated with BMWD and Untreated Control.
- Wounds treated with Graft + PHMB showed a significantly ($p \leq 0.05$) lower PA27312 count (3.63±0.48 Log CFU/g) than all other groups on day 7. When this result was compared against Untreated Control, there was a PA27312 reduction of 99.97%, being the highest reduction on day 7.
- Wounds treated with Graft alone had substantially lower PA27312 counts than BMWD and Untreated Control; however, those treated with Graft + PHMB had a significant PA27312 reduction ($p \leq 0.05$) compared to those treated with Graft alone (1.76±0.21 Log CFU/g), this value represents a 98.26% of reduction.
- On day 14, Untreated wounds had the highest PA27312 count (7.23±0.24 Log CFU/g), which was consistent for the entire study. Those wounds treated with BMWD had similar PA27312 counts on days 7 and 14 (6.76±0.64 and 6.82±0.24 Log CFU/g, respectively).
- Wounds treated with Graft alone had significantly ($p \leq 0.05$) lower PA27312 recovered (5.17±0.32 Log CFU/g) than those treated with BMWD and Untreated Control.
- Wounds treated with Graft + PHMB exhibited the lowest PA27312 counts for the entire study at 2.63±0.29 Log CFU/g. Significantly lower ($p \leq 0.05$) on day 14 as compared to day 7.
- The largest % bacterial reduction for the entire study was observed when Graft + PHMB was compared against Untreated Control (99.99%), followed by Graft alone and BMWD (99.71 and 99.99%, respectively).





Conclusions:

- Overall, the trends on days 7 and 14 mirror each other with those wounds treated with Graft + PHMB providing significantly superior barrier properties against *Pseudomonas aeruginosa* ATCC 27312 than Graft alone and Bilayer Matrix Wound Dressing. While this set of wounds continued reducing the bacterial activity as days progressed, those wounds treated with Bilayer Matrix Wound Dressing and Untreated Control groups increased their bacterial counts from day 7 to day 14.
- Graft + PHMB exhibited substantially better results than Graft without PHMB, and significantly less bioburden (4 Log/CFU difference) than those wounds protected with Bilayer Matrix Wound Dressing.
- The ability of the Graft + PHMB to reduce the entrance of this common wound pathogen may have significant clinical implications and a promising future for patients needing treatment.

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Contact Information: Stephen C. Davis / Research Professor
Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery.
Miller School of Medicine, University of Miami
sdavis@med.miami.edu. Ph: 305.243.4897