Assessment of the effects on wound healing and gene expression of a bio-electric dressing (CMB) using a porcine wound model and real time RT-PCR

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Abstract:

Wounds are a major cause of morbidity and impaired quality of life. Non-healing wounds can lead to prolonged periods of distress, permanent debilitation, and death.[1] Every year, 6.5 million Americans are afflicted with chronic wounds due to pressure, venous stasis, or diabetes.[2] Electrical stimulation has been shown to be beneficial to non-healing wounds.[3-5]
Using a well established porcine model for wound healing[6] we evaluated the effects of a bio-electric (CMB) wound dressing on deep partial thickness wounds using an epidermal migration assay and real-time RT-PCR with porcine specific oligonucleotides. Six female specific pathogen free animals were used in our experiments. Wounds (10 x 10 x 0.5mm LxWxD) were created using a specialized electrokeratome. Wounds were treated with sterile dressings (CMB versus non-active) and assessed using an epidermal migration assay beginning on day 4 (post-wounding). Biopsies were also taken for molecular analysis.
Treatment with the CMB dressing resulted in 65% of wounds being completely epithelialized on day 5 as compared to 20% of wounds in the control group (p-value < 0.001). Gene expression analysis of interleukin 1α (IL-1α) indicated that treatment with active dressing results in delayed peak expression. Although an initial inflammatory response is normal in adult fibrotic healing, scarless fetal wound healing is characterized by the absence of the inflammatory phase of healing. Comparative analysis of matrix metalloproteinase-9 (MMP-9) indicated that peak gene expression was reduced in the active dressing group. Over expression of MMP-9 has been associated with chronic wounds. Others have shown MMP-9 levels in acute wounds are upregulated in scarless wound healing. These results suggest that the CMB bio-electric dressing may have important clinical implications. Additional studies, including clinical are warranted.

Introduction and Objectives:

Wounded skin has been shown to have natural bioelectrical currents.[7] Electrical fields are important in the wound healing process and can potentially be manipulated therapeutically to enhance wound healing.[8,9,10] The possibility of delivering electrical fields through active dressings (without external currents) is very attractive. In addition to providing a moist environment, this material produces a microcurrent voltage that is very close to the voltage that occurs at areas of skin injury in normal hosts. The physiologic current of injury is necessary for the initiation of wound healing and the transport of cells to the healing wound margins. Domestic swine were chosen as our experimental animal since porcine skin is physiologically and biochemically homologous to human skin.

Our study was designed to determine the effects of a bio-electric (CMB) wound dressing on:
- The rate of epithelialization of deep partial thickness wounds
- The inflammatory response using interleukin 1α (IL-1α) as a reporter marker
- Wound proteases using matrix metalloproteinase 9 (MMP-9) as the reporter marker
- Early repair strength using type-1 and type-3 collagen expression as indicators

References:

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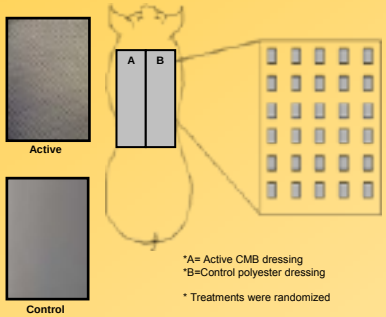
Methods and Materials:

1. Animals/Wounding/Treatment:
- 6 pigs were used
- 80 deep partial thickness wounds (10x10x0.7mm) were created on each animal
- Wounds were randomly assigned to 2 groups (A or B) consisting of either moistened CMB dressings or sterile polyester dressings
- Dressings were changed on day 1, 4, and 7 post-wounding (day 0).



*A= Active CMB dressing
*B=Control polyester dressing

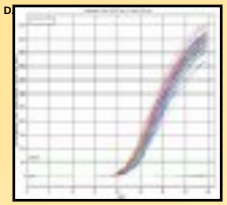
* Treatments were randomized

2. Epidermal Migration Assay:
A. Beginning on day 4, five wounds were excised, using a 22mm blade, from each group.
B. Tissue samples were incubated in 0.5M NaBr at 37ºC, overnight.
C. Tissue were placed on a glass plate and
D. Separated at the basement membrane zone.
E. The recovered epidermis was macroscopically assessed for defects. Mature epidermal layers which survived the separation completely intact were considered completely epithelialized.

3. Gene Expression Analysis:
A. On days 4 through 6, 4mm punch biopsies were taken from one wound from each treatment group on four animals.
B. Tissue samples were incubated in RNAlater at 4ºC, overnight.
C. RNA was extracted from stabilized samples using the Qiagen Rneasy Mini Kit
D. Total RNA was used with specific porcine primers to assess the relative expression of molecular markers to the geometric mean of actβ and β-2-M expression from the corresponding sample. All reactions were conducted using one-step RT-PCR and SYBR green.

A.

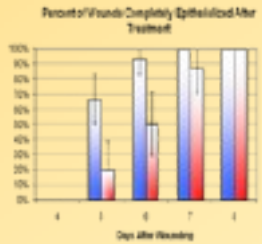
D



Results:

Active CMB dressing *Polyester dressing*

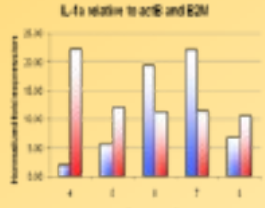
Results of epidermal migration assay combined from 6 animals. P-value for day 5 were p<0.001, day 6 p=0.006, and day 7 p= 0.04.

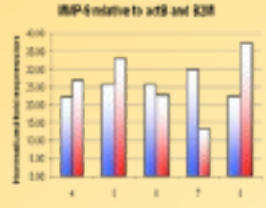


Epidermal Migration Assay Results:

Day 4: none of the wounds had healed
Day 5: CMB dressing had a mean complete epithelialization rate of 67% and the polyester dressing had a rate of 20%. This difference was shown to be significant using a paired sample T-test (p-value < 0.001).
Day 6: a mean 93% of wounds treated with the CMB dressing had completely re-epithelialized as opposed to only 50% in the polyester treated group. This difference was also found to be significant upon statistical analysis (p-value = 0.006).
Day 7: all wounds treated with CMB dressing had completely healed while 87% had healed in the polyester treated group. This difference was significant (p-value < 0.05).
Day 8: all wounds healed

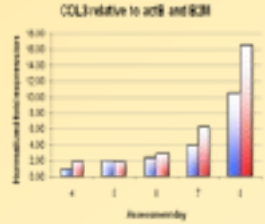
Results of molecular analysis from 4 animals. All genes were normalized to the geometric mean of the corresponding β-actin (actB) and β-2-microglobulin (B2M) expression. All values are scaled to the lowest value.





Interleukin 1 alpha (IL-1α): was upregulated in the polyester treated wounds on days 4, 5 and 8 compared to CMB treated wounds. CMB treated wounds had higher levels of IL-1α gene expression on days 6 and 7. Maximal IL-1α expression was greater for polyester treated wounds than CMB treated wounds. Polyester treated wounds had the highest IL-1α gene levels on day 4 while CMB treated wounds had maximal expression on day 6, indicating that expression of the cytokine in CMB treated wounds was both suppressed and delayed by comparison to polyester treated wounds.

Matrix metalloproteinase-9 (MMP-9): was upregulated in wounds treated with polyester dressing on days 4, 5, and 8 and was downregulated on days 6 and 7 by comparison to expression levels in wounds treated with CMB dressing. Expression levels in CMB treated wounds did not fluctuate greatly throughout the experiment. Gene levels in the polyester treated wound varied peaking at day 5, dropping on day 7, and spiking again at day 8. Such variation is not unusual in MMP expression since the enzymes are involved in initial degradation of the extracellular matrix (ECM) in early wound healing and play major roles in wound remodeling during late wound healing. Despite the differences in expression patterns, polyester treated wounds showed higher maximal expression levels than did wounds treated with the CMB dressing.



Type-1 collagen (COL1): was consistently higher in wounds treated with polyester dressing at every assessment time. Additionally, polyester treated wounds had higher maximal expression of COL1. Although type-1 collagen is the most abundant form of dermal collagen, upregulation of protein synthesis has been associated with both normal fibrotic healing and excessive scarring.

Type-3 collagen (COL3): did not vary greatly until day 7. Analysis of wounds sampled on days 7 and 8 indicated that COL3 was upregulated in polyester treated wounds. Upregulation of COL3 in adults has been associated with lower wound strength resulting in delayed healing and recurrent injury.

Conclusions:

- Wounds treated with active (CMB) wound dressing had a stimulated rate of re-epithelialization as compared to control dressing.
- Gene expression analysis of CMB treated wounds indicate a delayed inflammatory response.
- Gene expression analysis indicated a less intense MMP-9 expression in CMB treated wounds.
- Type 1 and type 3 collagen expression was reduced in the CMB treated wounds.
- These results warrant further analysis into the mechanism of action of CMB dressings and their effects on gene expression associated with chronic and acute wounds.

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