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 disability, and death. Every year, 0.3 million Americans are afflicted with chronic wounds due to pressure, venous, arterial, or diabetic occlusive disease. In order to determine the effects of a bio-electric (CMB) wound dressing on deep partial thickness wounds, the University of Miami, Miller School of Medicine, Department of Dermatology and Cutaneous Surgery, Miami, FL evaluated the effects of a bio-electric (CMB) wound dressing on deep partial thickness wounds using an epidermal migration assay and measuring RT-PCR with positive specific oligonucleotides. Six female specific pathogenic free animals were used in our experiment. Wounds (10 x 10 x 0.5mm) in the left ear were created using a specialized electromicrotome. Wounds were treated with sterile white dressings (CMB treatment) or redressed using an epidermal migration assay on day 4 (post-wounding). Biopsy was then taken for molecular analysis.

Methods and Materials:

1. Animals/Wounding/Treatment:
- 6 pigs were used
- Wounds were randomly assigned to 2 groups of 3 pigs (treated with either active (CMB) dressings or sterile polyester dressings)
- Wounds were excised, using a 22mm blade, from each group
- Tissue samples were incubated in 0.5M PBS
- DNA was extracted from excised tissue using the Qagen DNA mini kit

2. Epidermal Migration Assay:
A. Beginning on day 4, 5 wounds were excised, using a 22mm blade, from each group.
B. Tissue samples were incubated in 0.5M PBS
C. Tissue were placed on a glass plate and
D. Separated at the basement membrane zone.

3. Gene Expression Analysis:
A. On days 4 through 6, 4mm punch biopsies were taken from one wound in each treatment group on days 4, 5, and 6.
B. RNA was extracted from excised tissue using the Qagen DNA mini kit
C. Total RNA was used with specific primer pairs to assess the relative expression of molecular markers to the geometric mean of actB and β-2-microglobulin expression from the corresponding sample. All reactions were conducted using one-step RT-PCR and SYBR green.

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 shown in the table below.

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.

References:

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