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SILVER-ZINC COUPLED BIOELECTRIC DRESSING DISRUPTS BACTERIAL BIOFILM BY TARGETING QUORUM SENSING AND ANTIBIOTIC RESISTANCE

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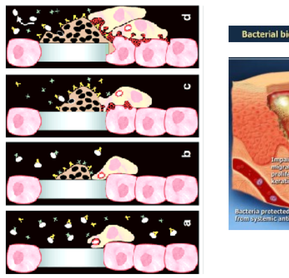
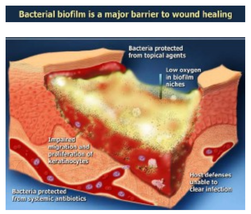
Background

- In the United States, 6.5 million patients are affected by chronic wounds adding an estimated US$25 billion annually to heath care costs
- Of all the chronic wounds, about 60% are associated with biofilm infection.
- Bacteria may establish social networking and structurally organize in aggregates known as biofilms
- In the biofilm form, bacteria are encased within an extracellular polymeric substance (EPS) and become recalcitrant to antimicrobials and host defenses, rendering them recalcitrant to antimicrobials and host defenses, rendering them extremely challenging and costly to treat





In order to be effective against biofilms, 500 to 5000 times greater antibiotics than those required for killing planktonic (floating) strains of the same bacterial species, is necessary

N Wellman, S M Fortun, and B R McLeod. 1996, *Bacterial biofilms and the bioelectric effect*. Antimicrob Agents Chemother. 40(9):2012-4.

A number of research efforts have been concentrated on the problem of the resistance of biofilm bacteria to antimicrobial agent, the efficacy of antibiotics was shown to be increased through the application of weak electric fields –Wellman *et. al* coined the term BIOELECTRIC FIELD For this phenomenon. With the combined application of electric fields and antibiotics, the concentrations of antibiotics needed to be effective against biofilm bacteria fell to only 1.5 to 4.0 times those necessary for planktonic bacteria.

Bioelectric dressing – Procellera™ - improves wound healing

PLoS One. 2014 Mar 3;9(3):e89239. doi: 10.1371/journal.pone.0089239. eCollection 2014.

Improvement of human keratinocyte migration by a redox active bioelectric dressing.

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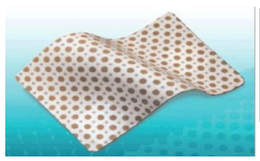
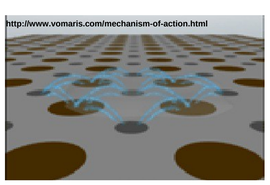
Abstract

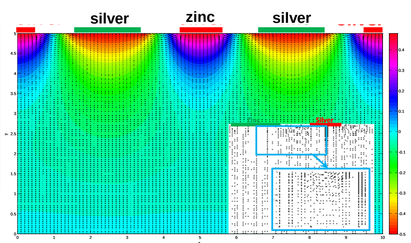
Exogenous application of an electric field can direct cell migration and improve wound healing; however clinical application of the therapy remains elusive due to lack of a suitable device and hence, limitations in understanding the molecular mechanisms. Here we report on a novel FDA approved redox-active Ag/Zn bioelectric dressing (BED) which generates electric fields. To develop a mechanistic understanding of how the BED may potentially influence wound re-epithelialization, we direct emphasis on understanding the influence of BED on human keratinocyte cell migration. Mapping of the electrical field generated by BED led to the observation that BED increases keratinocyte migration by three mechanisms: (i) generating hydrogen peroxide, known to be a potent driver of redox signaling, (ii) phosphorylation of redox-sensitive IGF1R directly implicated in cell migration, and (iii) reduction of protein thiols and increase in integrinαv expression, both of which are known to be drivers of cell migration. BED also increased keratinocyte mitochondrial membrane potential consistent with its ability to fuel an energy demanding migration process. Electric fields generated by a Ag/Zn BED can cross-talk with keratinocytes via redox-dependent processes improving keratinocyte migration, a critical event in wound re-epithelialization.



Procellera™ With PROSIT™ Technology

Ag/Zn Bioelectric Dressing





- **Dots of silver oxide and zinc**
- **Contact with conducting fluid (fluid containing ions) such as wound exudate activates E field**

Potential difference = 0.2V to 1V
Peak electric field = 2.7V/cm to 13.5V/cm

Observations

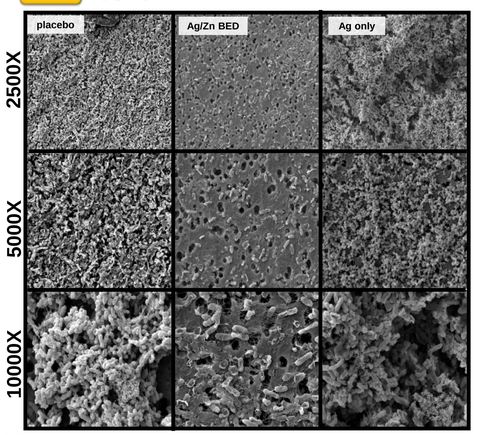
1 Ag/Zn BED has potent bacteriostatic activity

Ag/Zn BED is more potent than silver alone

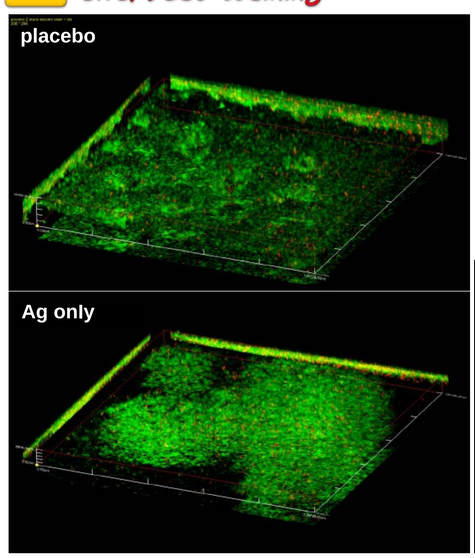
A. Agar plate with dressings embedded inside them, showing zone of clearance after PAO1 was inoculated and kept for 24h, **B.** OD measurement after adding the inoculation and dressing together. **C.** CFU count from 6h time point from the same experiment

Ag/Zn BED effectively kills bacteria in an in-vitro biofilm model demonstrated by

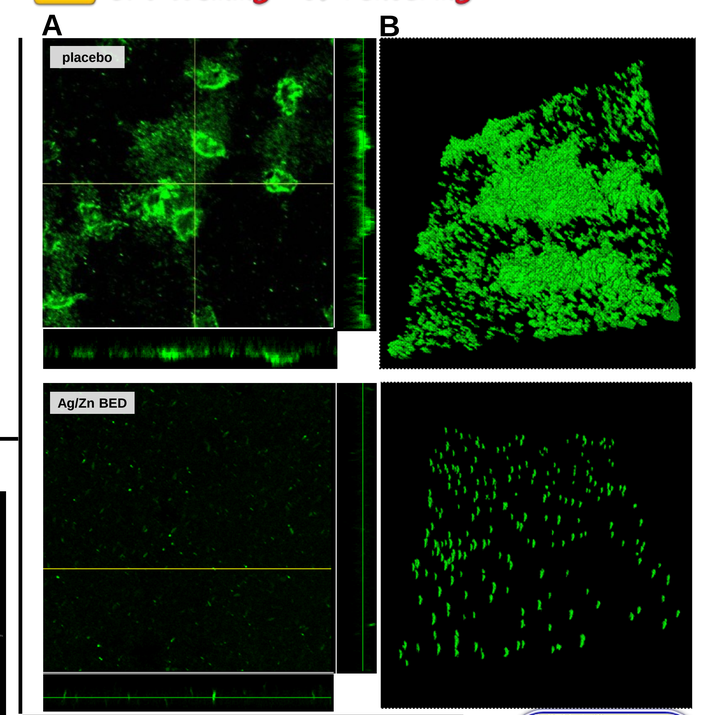
2 SEM

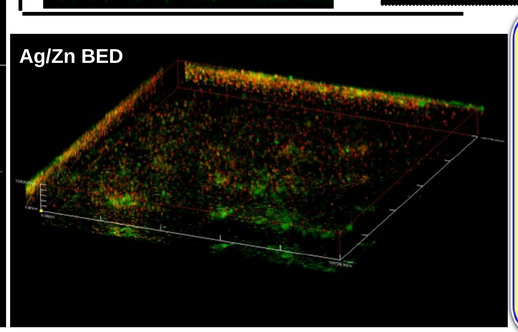


3 Live/Dead staining



4 EPS staining – 3D rendering





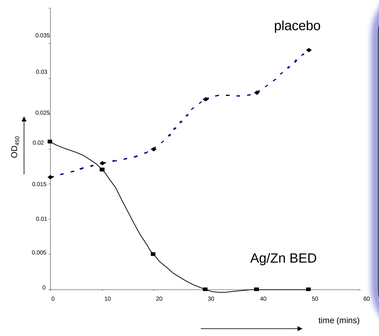
2. SEM at different magnifications of in vitro biofilm exposed to Ag/Zn BED,Ag only and Placebo dressings for 24h.

3. Representative images from biofilm stained with the BacLight kit,to observe bacterial viability in the biofilm.Live bacteria are stained green and dead are stained red

4. Z-stack image created by merging serial scans of the *in vitro* biofilm viewed under 60x magnification confocal laser scanning microscopy showing clumps of *Pseudomonas* in the placebo group

Mechanism of action

Down-regulation of GPDH activity

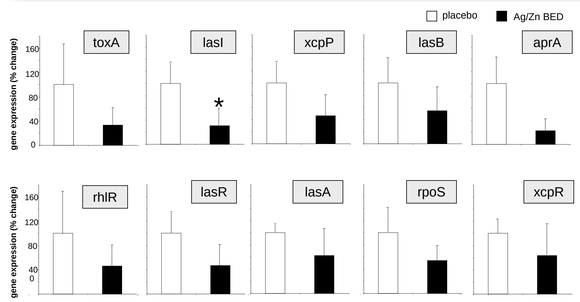


- GPDH (glycerol-3P-dehydrogenase) is affected by electric field. (Giladi,*et al, Antimicrobial agents and Chemotherapy, 2008*)
- GPDH is involved in bacterial metabolism.
- GPDH is required for phospholipid biosynthesis.
- GPDH converts glycerol 3-phosphate to dihydroxyacetone phosphate and passes on electron to ubiquitone and oxygen. (Christopher J. Southey-Pillig *et al., Journal of Bacteriology, 2005)*

Measurement of Glycerol-3-Phosphate Dehydrogenase activity in biofilm treated with placebo and Ag/Zn BED.

BED silences quorum sensing genes

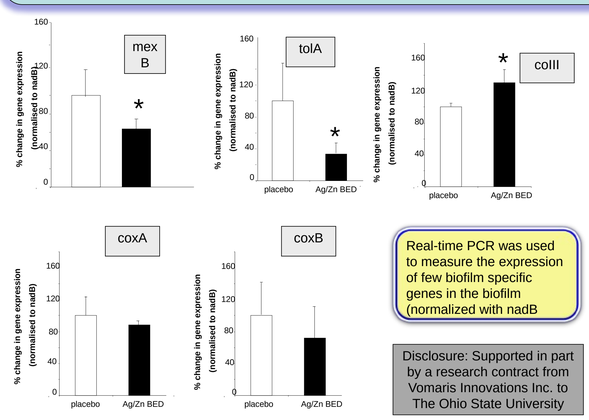
PAO1 exhibit quorum sensing using two distinct acylhomoserine lactone (AHL) based pathways: the rhlI/rhlR pathway, which uses butyryl acyl homoserine lactone (C4-HSL), and the lasI/lasR pathway that uses 3-oxo-dodecanoyl homoserine lactone (3-oxo C12-HSL).



Real-time PCR was used to measure the expression of QS genes in the biofilm (normalized with nadB)

BED down-regulates anti-biotic resistance genes

- mexB-product of this gene contribute to the active efflux of several antibiotic
- tolA- Enables the directed movement of substances (such as macromolecules, small molecules, ions) into, out of or within a cell, or between cells.
- coxA-cytochrome C oxidase subunit I, required for respiration
- coxB-cytochrome c oxidase subunit II, required for respiration
- colII-cytochrome c oxidase subunit III, involved in energy coupling, has catalase activity



Real-time PCR was used to measure the expression of few biofilm specific genes in the biofilm (normalized with nadB

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