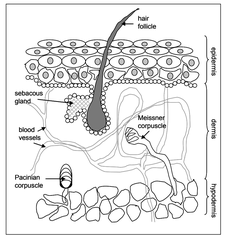
Improvement of Human Keratinocyte Migration and Wound Healing by a Redox Active Bioelectric Dressing (BED)

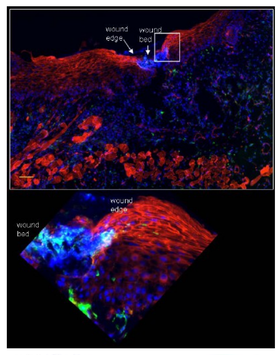
Jaideep Banerjee[1], Piya D. Ghatak[1], Savita Khanna[1], Sashwati Roy[1], Emily Sequin[2], Vish Subramaniam[2], Chandan K. Sen[1]

Dept. of Surgery[1], Dept. of Mech. & Aerospace Eng.[2], The Ohio State University, Columbus, OH

Wexner Medical Center

A) Key to wound healing – Re-epithelialization



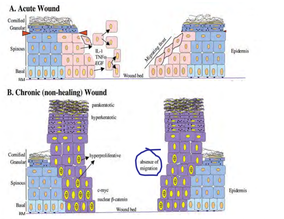


Phases Of Wound Healing

1. Inflammatory phase
2. Proliferative phase
   - Angiogenesis
   - Granulation tissue
   - Collagen deposition
   - Re- Epithelialization
3. Remodeling phase

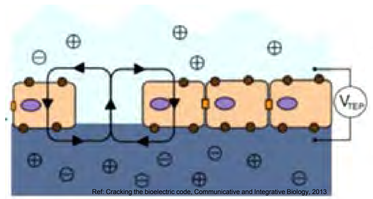
Chronic Wounds

1. Does not heal in the orderly set of stages.
2. Does not heal in a predictable amount of time like most wounds



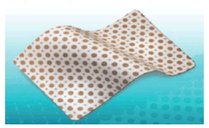
Ref: Epidermal stem cells: the cradle of epidermal determination, differentiation and wound healing. Morasso MI, Tomic-Canic M. Biol Cell. 2005 Mar;97(3):173-83.

B) The bioelectric dressing - BED



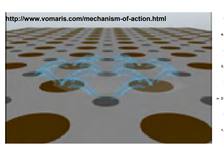
Wound epithelium
Trans-epithelial potential
Tissue Level

Ref: Cracking the bioelectric code. Communicative and Integrative Biology. 2013

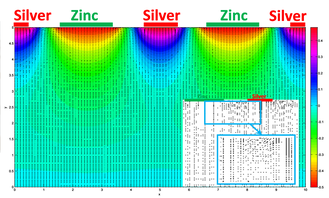


Procellera™ With PROSIT™ Technology

Ag/Zn Bioelectric Dressing

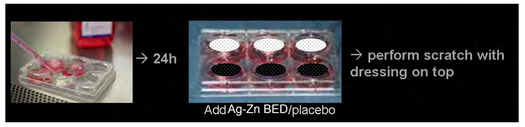


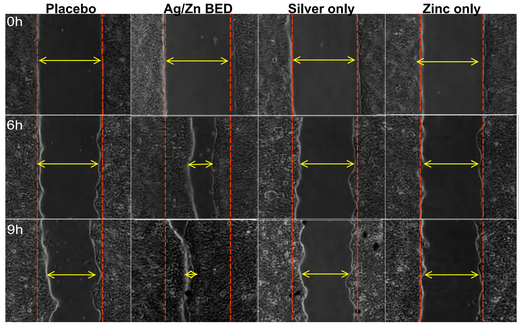
- Dots of silver oxide and zinc
- Contact with conducting fluid (fluid containing ions) such as wound exudate activates E field

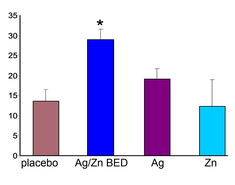


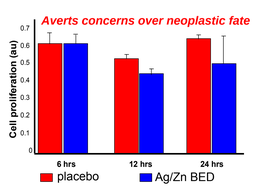
Potential difference = 0.2V to 1V
Peak electric field = 2.7V/cm to 13.5V/cm

C) Ag/Zn BED improves human keratinocyte migration



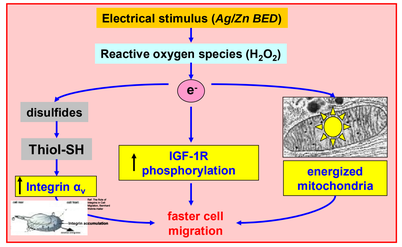






Ag/Zn BED increases keratinocyte migration. Human keratinocyte cells were plated under placebo or Ag/Zn BED dressing. After 24h, a scratch assay was performed and cell migration was observed at 6h and 9h post-scratch. Ag/Zn BED demonstrated significant increase in rate of migration while no significant difference was observed under only-silver or only-zinc dressings implicating the importance of electrical coupling for increase in cell migration. No effect on cell proliferation was observed.

D) Ag/Zn BED generates reactive oxygen species which trigger signaling pathways



(1) 2 H_2O (l) → $O_2(g)$+4H^++4e^- [?1.23 *V*]
(2) 2 H_2O (l)+2e^- → $H_2(g)$+2 OH^- [?0.83 *V*]
(3) O_2+2H^++2e^- → H_2O_2 [+0.70 *V*]

Oxygen-Derived Hydrogen Peroxide as a Key Signal for Wound Healing

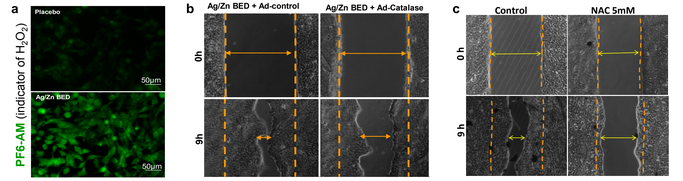
Chandan K. Sen*

Comprehensive Wound Center, The Ohio State University Medical Center, Columbus, Ohio.

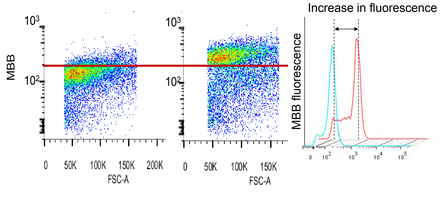
ADVANCES IN WOUND CARE, VOLUME 2

Molecular Therapy

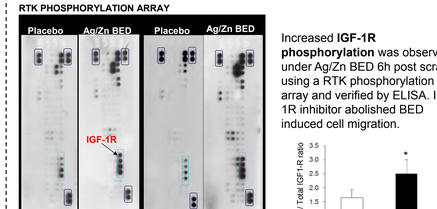
Dermal Wound Healing Is Subject to Redox Control



(a) Increased fluorescence from H_2O_2 indicator Pf6-AM under the effect of Ag/Zn BED demonstrates generation of reactive oxygen species. (b,c) Improved migration observed under Ag/Zn BED is abolished with Catalase or N-Acetyl Cysteine which decrease cellular H_2O_2.



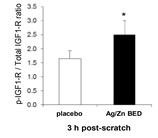
Ag/Zn BED increases cellular protein sulfhydryl (thiol) levels. Human keratinocyte cells were cultured under placebo or Ag/ZN BED dressing. After 24h, cells were treated with monobromo bimane (MBB) at room temp. and fluorescent emission from UV excited cells were collected. Differential estimation of thiol-specific cellular MBB fluorescence is shown in this contour plot. Increased expression in integrin α_v was also observed.

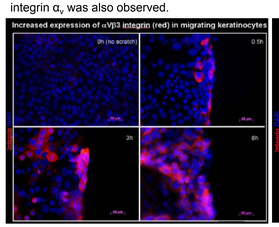


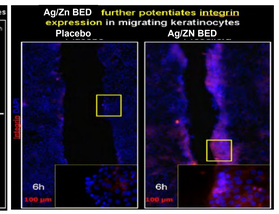
Insulin-Like Growth Factor-1 Promotes Wound Healing in Estrogen-Deprived Mice: New Insights into Cutaneous IGF-1R/ERα Cross Talk

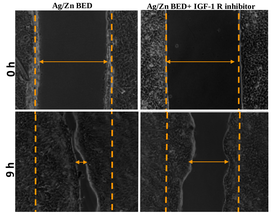
Journal of Investigative Dermatology (2012)

Increased **IGF-1R phosphorylation** was observed under Ag/Zn BED 6h post scratch using a RTK phosphorylation array and verified by ELISA. IGF-1R inhibitor abolished BED induced cell migration.

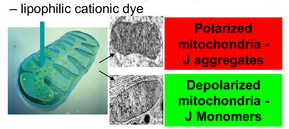


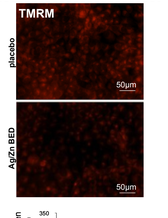


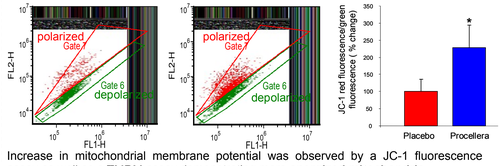




JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimi-dazolylcarbocyanine Iodide) – lipophilic cationic dye



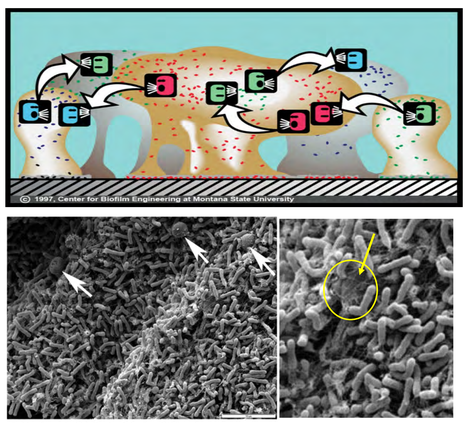




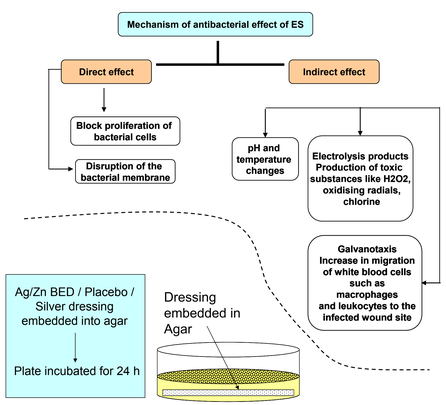
Increase in mitochondrial membrane potential was observed by a JC-1 fluorescence assay as well as a TMRM assay demonstrating more **energized mitochondria.**

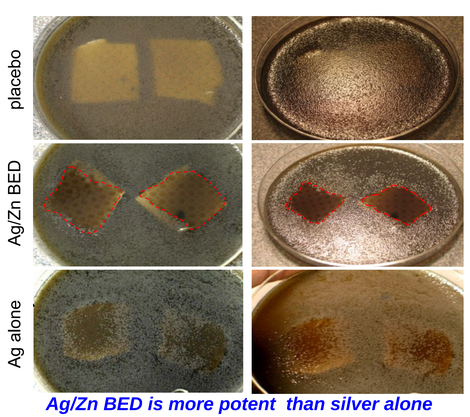
E) Ag/Zn BED has bacteriostatic activity

Ag/Zn BED may disrupt electrical communication in biofilm



Paul Stoodley, ..., Patrick DeMeo, Curr Orthop Pract. 2011 November





Ag/Zn BED is more potent than silver alone

F) Clinical outcome

Case #1 is a gentleman with lung cancer invading the apex as well a cervical spine. He had a Pancoast tumor removed and then radiated. The radiation wound involved skin soft tissue, muscle and spinal processes from C3-T2. He underwent surgical debridement including removal of all the spinal processes. The wound was initially treated with BED, wound VAC and hyperbaric oxygen. The wound VAC and the product were removed daily for the 2 hour treatment; but the actual total change with every 5 days. Wound healed completely in a total of 90 days.

Case #2 is a woman with pyoderma based on untreated rheumatoid arthritis. She was debrided only in clinic and the BED was applied directly to the wounds and covered with a moist saline dressing as well as a light Tubigrip. The product remained in place for 7 days and only the saline was changed daily. She also began treatment for her rheumatoid arthritis with methotrexate. Time to almost healed was less than 60 days. ***Images: courtesy Dr. Richard Schlanger, OSU Comprehensive Wound Center.***

Disclosure: Supported in part by a research contract from Vomaris Innovations Inc. to The Ohio State University