Prosit™: A Novel Bioelectrical Dressing

Bio-electric Wound Healing

- FDA Approved
- Proven Broad-Spectrum Antimicrobial Efficacy
- Bacteriocidal, Fungicidal
- Proven Antiviral Efficacy
- Clinical Advantages:
  - Enhanced wound healing clinically compared to other commercial products
  - Decreased pain and inflammation

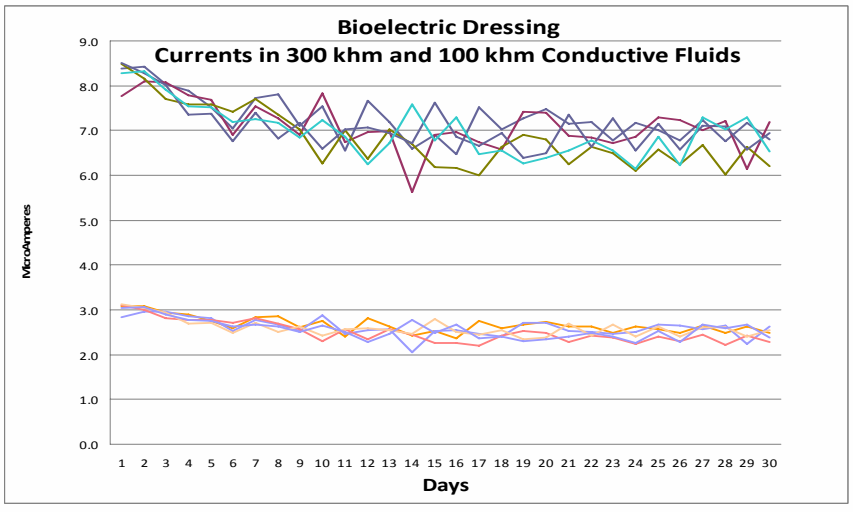
CMB™ Antimicrobial Dressing with PROSIT™ is FDA cleared for professional use as an antimicrobial barrier for partial and full-thickness wounds. PROSIT™ may be used for light to moderately exudating partial and full thickness wounds, including decubitus ulcers, venous ulcers, diabetic ulcers, first and second degree burns, surgical incisions, and graft donor/ recipient sites.

BACKGROUND

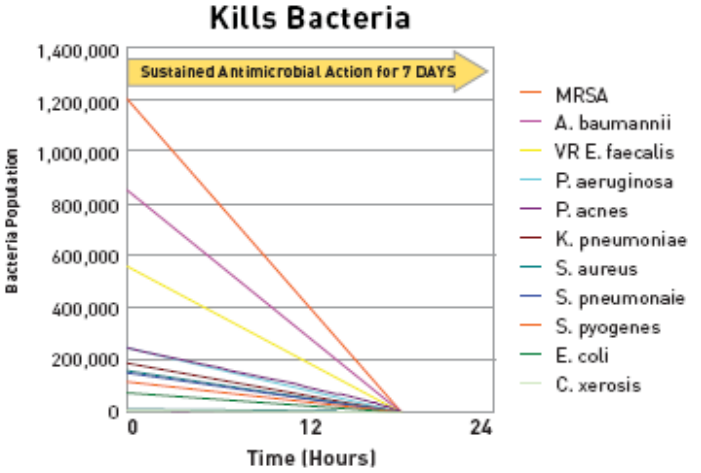
Prosit™ generates a sustained electrical microcurrent on the surface of the device. This technology is based upon a unique biophysical mechanism of action resulting from low levels of electrical energy produced passively within the dressing. **Prosit™** passed all tests for cytotoxicity, USP Systemic Injection, pyrogenicity, delayed hypersensitivity, and skin irritation. It is self-contained, wireless, and can be cut to fit. No external power supply is required. The device is activated in the presence of a conductive fluid, which may come from wound exudate or exogenous fluids including saline. When activated, biocompatible microcells spontaneously produce a sustained predetermined microcurrent similar to the microcurrent that occurs at areas of skin injury in normal hosts. This physiologic local microcurrent may be necessary for the initiation of wound healing and the transport of cells to the healing wound margins. In vivo studies indicated that **Prosit™** demonstrated faster wound healing with decreased pain. Additionally, antimicrobial action is enhanced since microbes are electrically charged and are attracted to the cathodes in the wound dressing. Subsequently, there is a cidal reaction to bacteria, fungi, mold and viruses. In the presence of fluids, the dressing can be used with an appropriate secondary barrier to help maintain a moist wound healing environment. **Prosit™** currently may be left in place over a wound for up to 3 days.

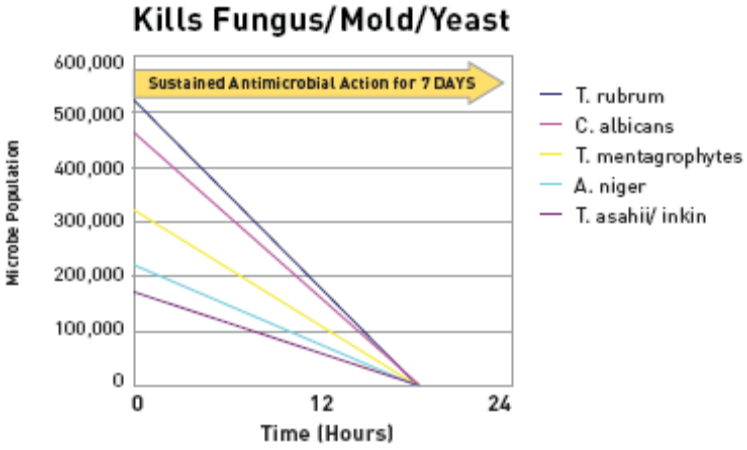
← A bioelectrical dressing generates a sustained electrical microcurrent simulating physiologic current of injury which is necessary to induce, enhance, and accelerate wound healing. It is activated when moistened.

Electric stimulation as a means for wound healing has been recognized as safe and effective in many studies and has been used successfully to reduce risk of infection, decrease pain and inflammation, and enhance wound healing. Endogenous electrical activity is normally present between cells in mammalian skin. With skin injury, physiologic electrical activity helps cells migrate throughout the wound site. This electrical signal is essential to the cascade of reactions and processes required to achieve wound healing. Studies show cells are transported along electrical current lines which are generated in wounds via a process called galvanotaxsis. Fibroblasts, keratinocytes, neutrophils, and mast cells migrate in the presence of low level micro-currents. The addition of external micro-voltage significantly enhances this process. In addition, pathogenic cells such as bacteria, fungi and viruses also have electrical activity. Numerous studies have shown that external electric current is lethal to electrically charged microbes, while providing a beneficial stimulus to wound healing in the host. The use of bioelectric dressings augments this antimicrobial process in addition to facilitating and enhancing wound healing.



Antimicrobial activity is enhanced by synergistic activity of the bioelectric environment and the element of silver used in the delivery system. Silver is a potent antimicrobial that on a cellular level attacks multiple sites within the microbial cell to inactivate critical physiological functions from cell-wall synthesis and membrane transport to protein function and DNA/RNA synthesis & translation; all essential for the viability of the pathogen. The development of resistance to silver in a bioelectric environment would be exceptionally rare because an organism would have to undergo multiple mutations of essential cellular functions, within a single generation. Prosit™ demonstrates broad spectrum antimicrobial coverage and is is cidal to bacteria, fungi and viruses.





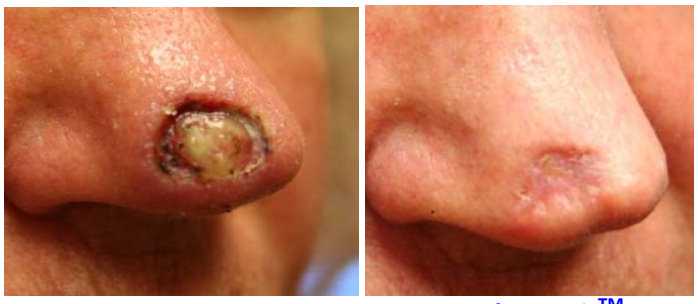
IN-VIVO STUDIES

Two porcine studies were conducted by the University of Miami's Miller School of Medicine in 2007 to evaluate the effects of the PROSIT™ on deep partial and full thickness wounds. Histological and chemical analysis were performed. PROSIT™ was found to significantly increase the rate of wound epithelialization ($p < 0.001$). Other histological findings noted with the use of the device were increased epithelial thickness, decreased crust formation, and decreased white blood cell infiltration of the wounds. PROSIT™ reduced levels of interleukin 1-alpha in the early phases of healing. Reduced interleukin 1-α levels are correlated with diminished pain and inflammation.

30 patients underwent cosmetic laser resurfacing of their face. 14 patients were treated with standard dressings. 16 were treated with a PROSIT™ mask after the procedure. All patients receiving the PROSIT™ mask reported a reduction in pain on the Visual Analog Pain Scale. 100% of the standard dressing population required narcotic analgesics after the procedure. In contrast, in the PROSIT™ group, 78% required no medication after the procedure. The remaining 22% took over the counter Nonsteroidal anti-inflammatories. The PROSIT™ group healed 40% sooner than the control group.

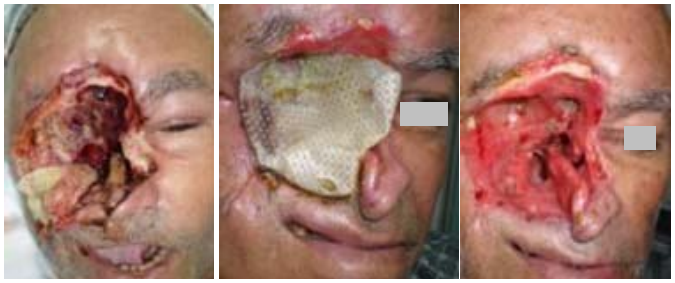
CASE STUDIES

Basal Cell Carcinoma: Surgically treated by curettage & electrodessication. Secondary healing instead of graft



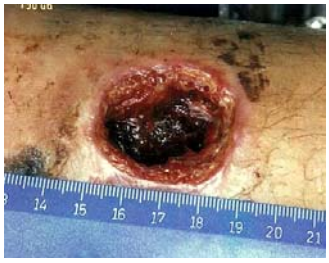
BEFORE **4 weeks Prosit™**

Squamous Cell Carcinoma: San José, Costa Rica



BEFORE Treatment 24 days **Prosit™**

Grade III Open Tibia-Fibula Fracture: Prevented muscle graft & skin graft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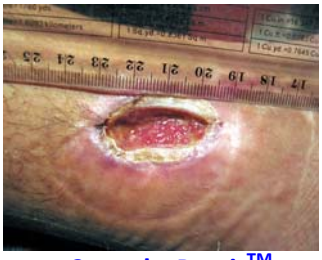
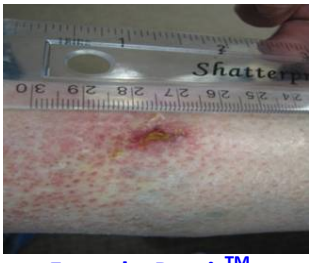
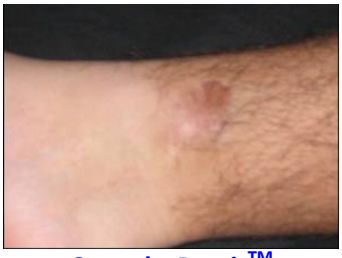
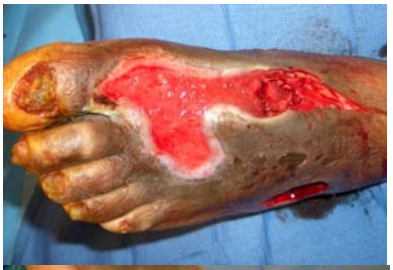
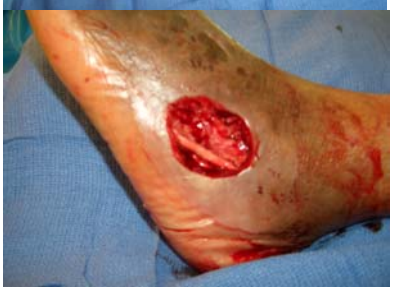

3 weeks Prosit™


5 weeks Prosit™


8 weeks Prosit™

Refractory Wound: Pre-Amputation. Failed Wound VAC, hyperbaric O2. Note exposed tendon & bone.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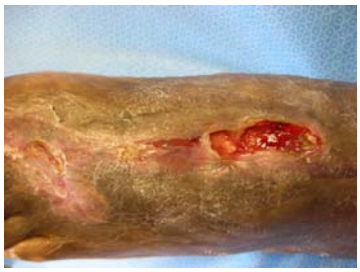
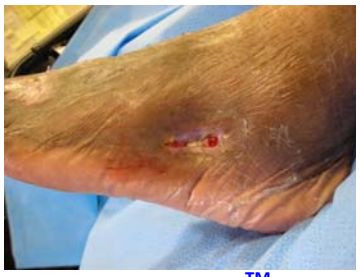
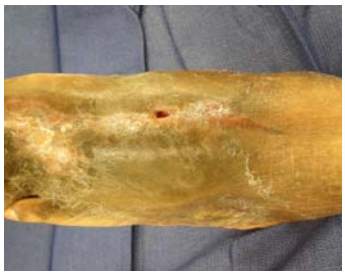
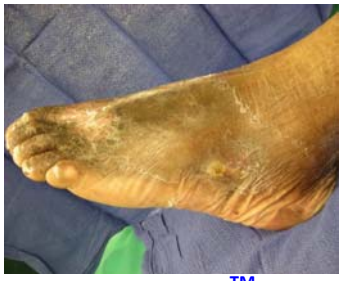
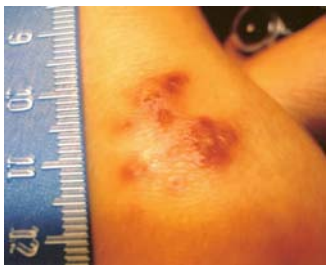


5 weeks Prosit™



7 weeks Prosit™

Herpes Zoster: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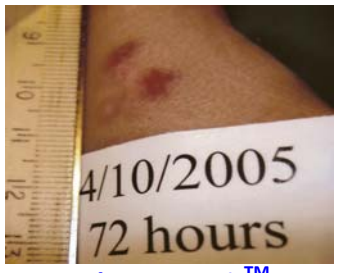
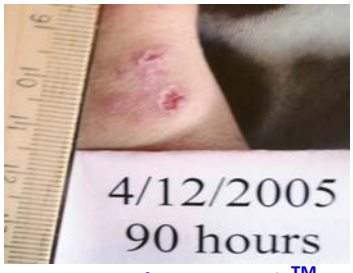
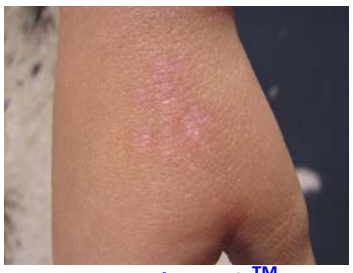


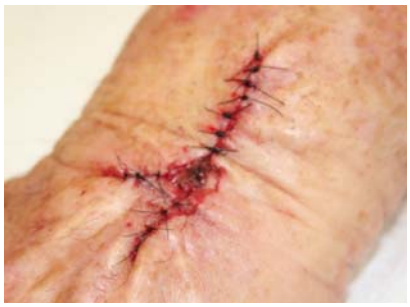
72 hours Prosit™



90 hours Prosit™


1 week Prosit™

Surgery Site: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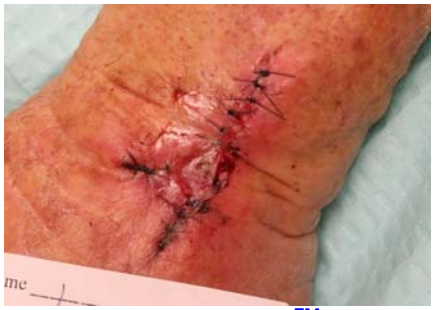
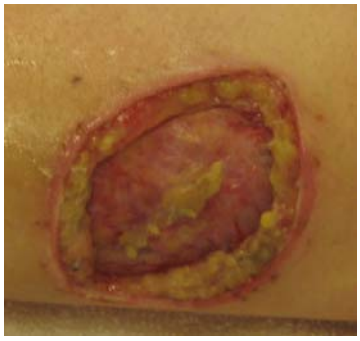

4 days Prosit™

Forearm Burn Site: Excised burn complicated with S. aureus infection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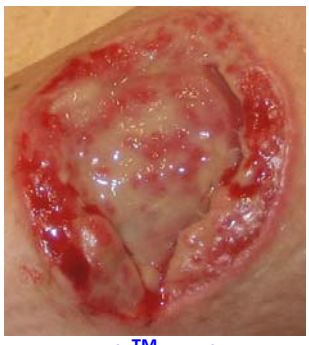
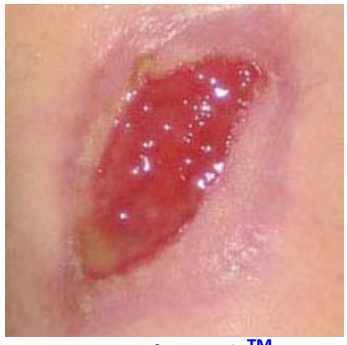
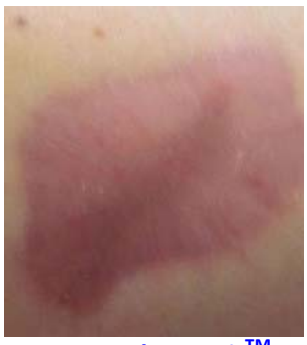
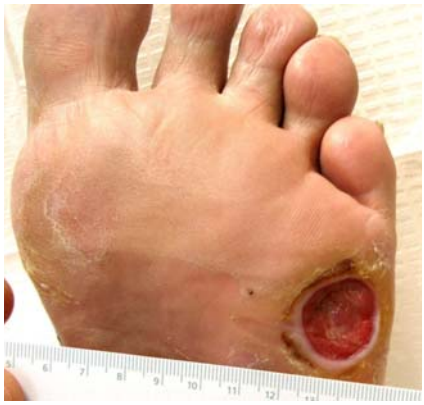

Prosit™ Begins


1 Month Prosit™


2 months Prosit™

Diabetic Lower Extremity Ulcer: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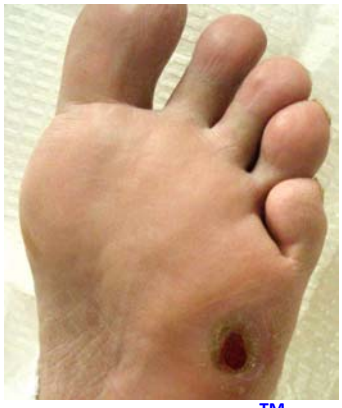
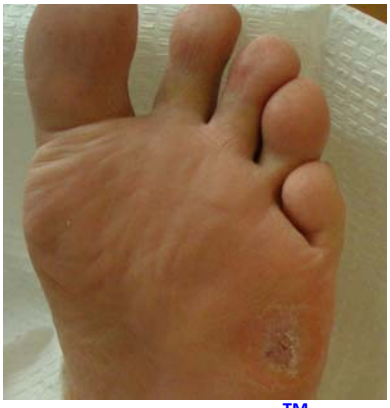
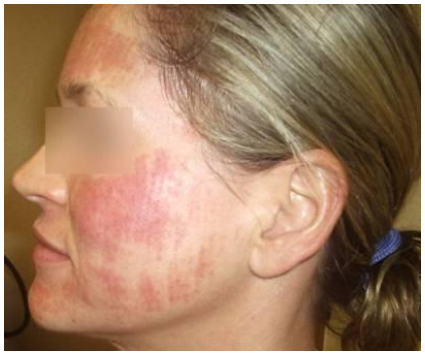
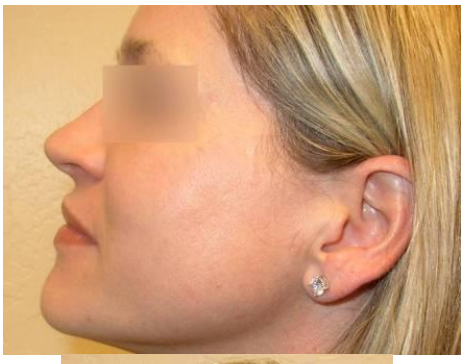
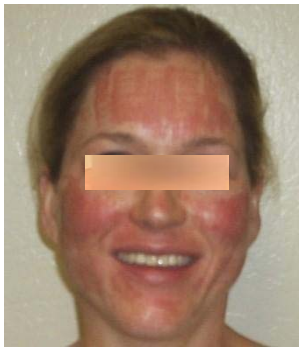

3 Months Prosit™


5 Months Prosit™

Cosmetic Laser Facial Resurfacing Burn:



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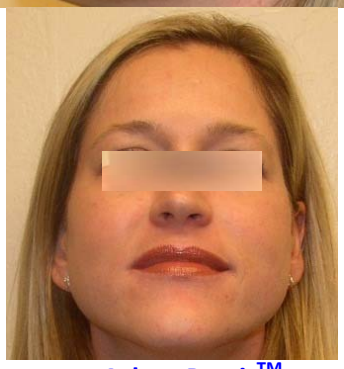
4 days Prosit™

LITERATURE REVIEW

A Nobel Prize went to two German scientists (Erwin Neher and Bert Sakmann) in 1991 for their work in detecting subtle electrical currents in all types of cell membranes throughout the body.
FDA Approved micro-current technology (approximately 7 microamperes RMS) is in use to stimulate bone fracture non-unions.
Vanable 1989 teaches that the epidermis of the skin acts as a battery and that the existence of wound currents has been recognized for more than 200 years. In early experiments, a wound was found to produce about 1 microampere of current when immersed in saline (Barker et al., 1982 and Jaffe and Vanable, 1984). Illingsworth and Barker reported in 1980 a measured current from 10 – 30 microamperes/sq cm leaving the stump surface of an accidentally amputated finger of a child.

The driving current on the epidermis is created by the transepithelial potential that the epidermis maintains across itself, resulting from the positively charged sodium NA+ channels between epithelial cells. The transepithelial potential may vary with position on the body and has been measured by Barker and Foulds in 1982, and White et al., 1983, to vary between 10 mV to almost 60 mV with no difference between sexes. Barker et al. (1982) found that an application of amiloride to a slit made in a cavy tarsal pad skin reduced the transepithelial potential at the slit to about one-half its original value, suggesting that mammalian skin has a Na+ dependent battery similar to that found in amphibians.

Barker et al., 1982 found that when tissue is wounded, an electrical leak is produced allowing current to flow out of the wound as long as the wound is not dry. Vanable observed that the highest potential occurs in the first 0.5 mm of skin bordering the wound and that there is a voltage gradient in the vicinity of the wound. Dry wounds have such a high resistivity that the current is blocked.

K.R. Robinson (1985) reports that the migration and/or orientation of cells can be controlled by imposing relatively modest electrical fields across them *in vivo*. Cells affected are epithelial cells, fibroblasts, macrophages, and leucocytes. In other studies, osteoclasts have been observed migrating towards the anode (Ferrier et al., 1986) while in other studies cells moved towards the cathode. When there is an elicited inflammatory response, the cells moved preferentially towards the cathode. Vanable suggests that the migration of cells may be affected by field strength and whether the macrophages are activated or not.

Nannmark et al. (1985) have reported that the presence of an electrical field can cause an increase in capillary permeability to macromolecules and leucocytes and an extravasation of white blood cells from the capillaries. Neither event was seen in non-stimulated controls. Silver also decreases wound edema allowing capillary proliferation to increase.

Vanable (1989) concludes that current of injury found to exist in the epidermis bordering wounds could, in theory, facilitate cell migration that must occur during healing. He also claims that only those events preepithelialization can be considered since the epithelialization sets up barriers to current flow.

Alvarez et al. (1983) placed a silver-impregnated nylon electrode into a wound bed in pig skin and applied 50 – 300 μA DC and observed a 29% faster wound healing than the controls. His findings also suggested that migration and/or proliferation of fibroblasts were influenced by the electric field.

Gram negative microbes have a net negative charge. Gram positive microbes have a lipopolysaccharide coating (LPS) which carries a negative charge. All microbes, therefore, are attracted to the anode containing silver. Silver then binds the sulfhydril groups and denatures the proteins which destroy the respiratory system of the microbe and other essential proteins within the microbe. Silver also kills viruses and fungi in a similar method by binding with sulfhydril groups and denaturing other proteins.

Cyclic AMP-dependent protein kinase A plays a role in the directed migration of human keratinocytes in a DC electric field.

Cell Motil Cytoskeleton. 2001 Dec; 50(4):207-17

Pullar CE, Isseroff RR, Nuccitelli R.

Department of Dermatology, University of California, Davis, CA 95616, USA.

Skin wound healing requires epithelial cell migration for reepithelialization, wound closure, and re-establishment of normal function. We believe that one of the earliest signals to initiate wound healing is the lateral electric field generated by the wound current. Normal human epidermal keratinocytes migrate towards the negative pole, representing the center of the wound, in direct currents of a physiological strength, 100 mV/mm. Virtually nothing is known about the signal transduction mechanisms used by these cells to sense the endogenous electric field. To elucidate possible protein kinase (PK) involvement in the process, PK inhibitors were utilized. Two important findings have been described. Firstly, addition of 50 nM KT5720, an inhibitor of PKA, resulted in a 53% percent reduction in the directional response of keratinocytes in the electric field, while not significantly affecting general cell motility. The reduction was dose-dependent; there was a gradual decrease in the directional response from 5 to 50 nM. Secondly, addition of 1 microM ML-7, a myosin light chain kinase inhibitor, resulted in an approximate 31% decrease in the distance the cells migrated without affecting directional migration. The PKC inhibitors GF109203X at 4 microM and H-7 at 20 microM and W-7, a CaM kinase inhibitor, did not significantly alter either directed migration or cell migration, although they all resulted in a slight reduction in directional migration. D-erythro-sphingosine at 15 microM, a PKC inhibitor, had virtually no effect on either migration distance or directed migration. These findings demonstrate that divergent kinase signaling pathways regulate general cell motility and sustained directional migration and highlight the complexity of the signal transduction mechanisms involved. The inhibitor studies described in this paper implicate a role for PKA in the regulation of the directional migratory response to applied electric fields, galvanotaxis. Copyright 2001 Wiley-Liss, Inc

Migration of human keratinocytes in electric fields requires growth factors and extracellular calcium.

J Invest Dermatol. 1998 Nov; 111(5):751-6.

Fang KS, Farboud B, Nuccitelli R, Isseroff RR.

Department of Dermatology, University of California, Davis 95616-8641, USA.

Currents that leak out of wounds generate electric fields lateral to the wound. These fields induce directional locomotion of human keratinocytes in vitro and may promote wound healing in vivo. We have examined the effects of growth factors and calcium, normally present in culture medium and the wound fluid, on the directional migration of human keratinocytes in culture. In electric fields of physiologic strength (100 mV per mm), keratinocytes migrated directionally towards the cathode at a rate of about 1 micron per min. This directional migration requires several growth factors. In the absence of these growth factors, the cell migration rate decreased but directionality was maintained. Epidermal growth factor alone restored cell migration rates at concentrations as low as 0.2 ng per ml. Insulin at 5-100 microg per ml or bovine pituitary extract at 0.2%-2% vol/vol also stimulated keratinocyte motility but was not sufficient to fully restore the migration rate. Keratinocyte migration in electric fields requires extracellular calcium. Changes in calcium concentrations from 3 microM to 3.3 mM did not significantly change keratinocyte migration rate nor directionality in electric fields; however, addition of the chelator ethyleneglycol-

bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid to migration medium reduced, and eventually abolished, keratinocyte motility. Our results show that (i) growth factors and extracellular calcium are required for electric field-induced directional migration of human keratinocytes, and (ii) keratinocytes migrate equally well in low and high calcium media.

Imposition of a physiologic DC electric field alters the migratory response of human keratinocytes on extracellular matrix molecules.

J Invest Dermatol. 1996 Apr; 106(4):642-6.

Sheridan DM, Isseroff RR, Nuccitelli R.

Department of Dermatology, University of California, Davis, USA.

Outwardly directed ionic currents have been measured leaving skin wounds in vivo. These currents generate physiologic electric fields of approximately 100 mV/mm, which may function to direct keratinocyte migration toward the healing wound. We investigated whether the substrate on which the keratinocyte migrates modulates the galvanotactic response to an electric migratory signal. Cultured human keratinocytes were plated on different matrices; types I and IV collagen, fibronectin, laminin, and tissue culture plastic. The effect of an applied direct current (DC) electric field on directional migration was monitored by time-lapse video microscopy over a 2-h period. Directionality was quantitated by calculating the cosine of the angle of migration in relation to anodal-cathodal orientation. Migration toward the negative pole was observed on all matrices as compared with controls (no applied field), which displayed random migration. No significant increase in directional response occurred when the field strength was increased by 100 mV/mm (physiologic levels) to 400 mV/mm. The degree of directionality and the average net cell translocation however, varied significantly with the substrate. The greatest cathodal migration in response to a DC electric field was observed with keratinocytes plated on types I and IV collagens and plastic. The directional migratory response was least on a laminin substrate, whereas cells on fibronectin demonstrated a response that was intermediate between those of collagen and laminin. These results suggest that physiologic ionic currents in concert with underlying matrix may influence the rate of reepithelialization of skin wounds.

Wound closure after split-thickness skin grafting is accelerated with the use of continuous direct anodal microcurrent applied to silver nylon wound contact dressings.

Huckfeldt R, **Flick AB**, **Mikkelson D**, **Lowe C**, **Finley PJ**.

St. John's Regional Health Center, Springfield, Missouri 65804, USA.

Wound healing after graft closure of excised burn wounds is a critical factor in the recovery process after thermal injury. Processes that speed time to stable wound closure should lead to improved outcomes, shorter lengths of hospital stays, and decreased complications. A randomized clinical trial to test the ability of continuous direct anodal microcurrent application to silver nylon wound contact dressings was designed. Time for wound closure after split-thickness skin grafting was observed. Thirty patients with full-thickness thermal burns were randomized into two groups. The control group received postoperative dressing care using moistened silver nylon fabric covered with gauze after tangential burn wound excision and split-thickness skin grafting. The study group received an identical protocol with the addition of continuous direct anodal microcurrent application. Time to 95% wound closure was measured using digital photography. The digital photographs were evaluated by a burn surgeon blinded to the patient's randomization. An independent t-test was used to analyze the data. The study group experienced a 36% reduction in time to wound closure (mean of 4.6 days) as compared to the control group (mean of 7.2

days). This was statistically significant at a P value of <.05. The use of continuous direct anodal microcurrent decreased time to wound closure after split-thickness skin grafting.

The rate of re-epithelialization across meshed skin grafts is increased with exposure to silver.

Burns. 2002 May; 28(3):264-6.
Demling RH, Leslie DeSanti MD.

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The objective in this study was to determine whether exposure to pure silver increases the rate of reepithelialization across a partial thickness wound. A meshed skin graft, placed on an excised burn wound was used as a healing model. Methods: The rate of meshed skin graft epithelial closure on an exposed burn using a moist healing environment was shown. A moistened silver delivery system (Acticoat) was compared with a standard xeroform and eight ply gauze dressing continually moistened with a 0.01% neomycin and polymyxin solution (NP). Twenty burn patients with deep burns of over 15% of TBS were excised and grafted with 2:1 meshed grafts. One graft area was treated with the antibiotic solution and another with the silver delivery. The meshed graft was performed within 3 days of injury. Results: No infections were noted and quantitative swab cultures gave less than 10(2) bacteria in all cases at wound closure. At day 7, reepithelialization was complete with silver and 55% closed with NP solution. Wound closure was complete in the NP solution group at day 10. Silver increased reepithelialization rate by over 40%, a significant increase. Graft take was over 95% in both groups. Conclusion: Silver released in a moist wound surface environment significantly increases the rate of reepithelialization compared to a standard antibiotic solution.

Exposure to extremely low frequency magnetic fields affects insulin-secreting cells

Bioelectromagnetics. 2008 Feb; 29(2):118-24.
Sakuri T, Yoshimoto M, Koyama S, Miyakoshi J..

A study was conducted on “hamster-derived insulin-secreting cell line.” The cell line was exposed to extremely low frequency magnetic field (ELFMF) conditions. More specifically, the exposure unit used was a sinusoidal magnetic field at a frequency of 60Hz, 5mT. After the cell lines were exposed, the survival and function of the cells were assessed. In “5 days in the absence of glucose increased cell number, exposure for 2 days in the absence of glucose and for 5 days with 100 mg/dl glucose increased the insulin secretion to the culture medium, and exposure for 2 and 5 days with 40 and 100 mg/dl glucose increased intracellular insulin concentration in HIT-T15 cells.” Poignantly, exposure to ELFMF under apoptotic conditions (natural degradation of cells) may lead to new therapeutic concepts for diabetic treatments.

Combined Magnetic Fields Increase Insulin-like Growth Factor-II in TE- 85 Human Osteosarcoma Bone Cell Cultures.

Endocrine Society. 1995; 136, 3100-3106.
Fitzsimmons RJ, Ryaby JT, Mohan S, Magee FP and Baylink DJ.

In vitro exposure to low-energy, combined magnetic fields (CMF) increased the release of insulin-like growth factor (IGF)-II from human TE-85 osteosarcoma cells. Short-term CMF exposure of only 10 min increased IGF-II levels in conditioned medium 1 h post CMF exposure. IGF-II levels were measured with a radioreceptor assay using H-35 cells that contain abundant IGF-II but not IGF-I receptors. This assay also uses a recently validated BioGel P-10 acid gel filtration method to remove IGF binding protein before quantitation of either IGF-I or IGF- II. In addition to an increase in IGF-II levels, DNA synthesis, as an index of cell proliferation, was increased during the 24-h period post CMF exposure. A monoclonal antibody against IGF-II blocked the increase in cell proliferation following CMF exposure, whereas a control monoclonal antibody against osteocalcin did not attenuate the mitogenic action of CMF exposure. The effect of CMF exposure to increase both cell proliferation and IGF-II was cell-density dependent with greater stimulation by CMF observed at lower densities. Together, these data are consistent with the hypothesis that CMF exposure stimulates release/production of IGF-II from bone cells and that increased IGF-II then promotes an increase in cell proliferation.

Low-amplitude, low-frequency electric field-stimulated bone cell proliferation may in part be mediated by increased IGF-II release.

Journal of Cellular Physiology. 2005: 150(1): 84-89
Fitzsimmons RJ, Strong DD, Mohan S, Baylink DJ.

The article above discusses low amplitude, low frequency electrical field incorporated in an in vitro study. The study showed that the electrical field stimulated human bone production is directly related with increasing IGF-II mRNA accumulation and IGF-II secretion.

Electric field effects on insulin chain-B conformation.

J Phys Chem. B. 2005; 109(47):22641-8.
Budi A, Legge FS, Treutlein, Yarosky I.

This article tests the insulin chain-B under both oscillating and static electrical fields. The application of both electrical fields has stabilized the chain. Moreover, there shows to be a restriction on “flexibility that is crucial for insulin’s biological activity.”