New Therapeutic Angiogenesis Biomarkers for Chronic Diabetic Foot Ulcers Treated with Transdermal Hyperoxia/Topical Wound Oxygen (TWO_2)

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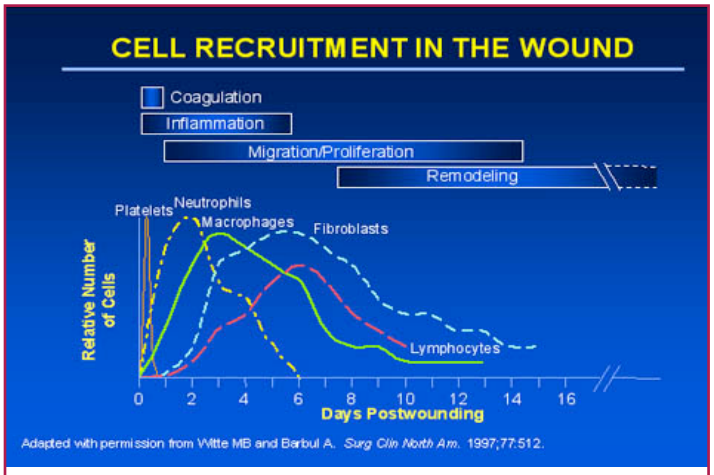


ANGIOGENESIS & HEALING = REQUIRES PROLIFERATION OF (1) CAPILLARY CELL WALLS (ENDOTHELIAL CELLS), (2) SUPPORTED BY COLLAGEN SCAFFOLDING (FIBROBLASTS), (3) COVERED BY EPITHELIUM (KERATINOCYTES)

Local Molecular & Cellular Abnormalities in a Chronic (non-healing) Diabetic Wound

- Growth factor and cytokine deficiencies
- Endothelial dysfunction
- Neuropathy: associated with endothelium dependent and independent dysfunction in diabetics predisposed to foot ulceration
- Arterial occlusive disease (PAD): associated with peripheral neuropathy, slower conduction velocity of sensory nerves, depression of autonomic responses
- Abnormalities in fibroblast function
- Abnormalities in extracellular matrix and decreased cellular infiltrate
- Decreased angiogenesis (thus sustained O_2 deprivation)

Oxygen in Tissues and Wounds

- All nucleated cells use O_2 energy metabolism (via mitochondria)
- Epidermis into papillary dermis use transdermal O_2
- From blood Hb, O_2 diffusion through membranes into is "concentration" dependent

In wounds, vessels disrupted, so lack O_2

- Wound ischemic hypoxia impairs O_2-ase enzymes
  - Cytochrome O2-ase for ATP generation, uses 8O% of O2 breathed
  - Prolyl hydroxylase for collagen synthesis, req. for angiogenesis
  - Phagocytic O2-ase for bacteria killing via 'respiratory burst'

Obvious rationale for supplemental O_2

Enforced O_2 concentration (TWO_2) increases diffusion distance

Renewed O_2 supply can activate repair molecules

Highest priority to restore O_2, thus angiogenesis required!!

Chronic Wound Evaluation

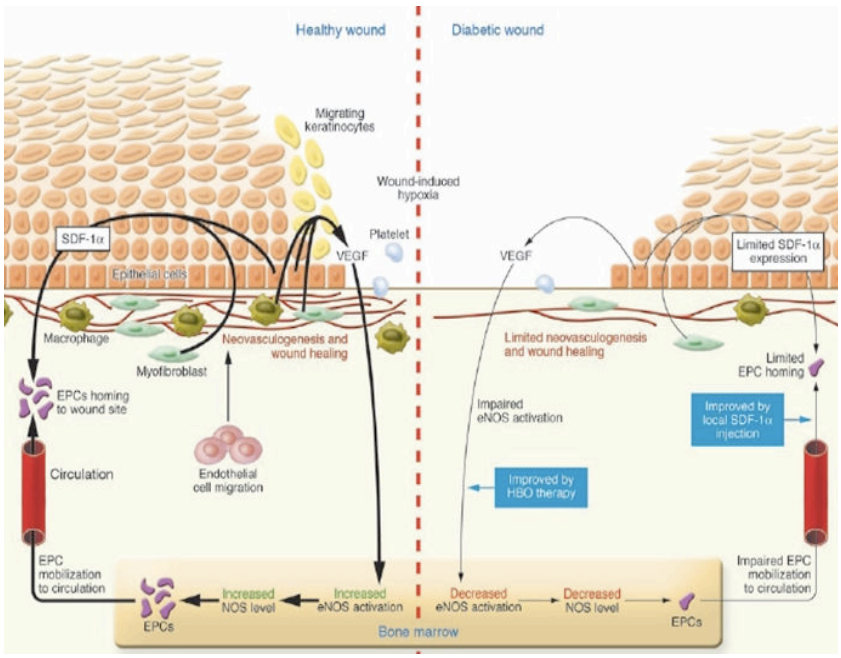
- Healing is "stalled" in chronic non-healers, typically hyper-inflammed, hyp-oxic.
- Angiogenesis, new capillary synthesis, is required for wound healing to restore blood flow (O_2 & nutrients in, waste & toxins out).
- Growth factors, secreted by platelets, neutrophils and macrophages, are required to induce angiogenesis.
- *Angiogenic biomarkers of new healing are needed:*
  - Endogenous growth factors, ie VEGF, FGF2
  - Functional neo-vessel surface marker, ie Integrin $\alpha v\beta 3$
  - Endothelial Progenitor Cell homing signal, ie SDF-1
  - Endothelial secreted vasodilator, ie Nitric Oxide

Does Oxygen Restore Healing in Chronic Wounds?

- What Growth Factors stimulate new blood vessel formation? **VEGF & FGF2**
- What biomarker do new capillary endothelial cells express that measures functionality? **Integrin** $\alpha v\beta 3$
- What biomarker targets EPCs to injured ischemic tissue? **SDF-1**
- What O_2-sensitive molecules deficient in chronic wounds respond to TWO_2? **VEGF, FGF2, Integrin $\alpha v\beta 3$, SDF-1**

Treatments and Wound Fluid Collection

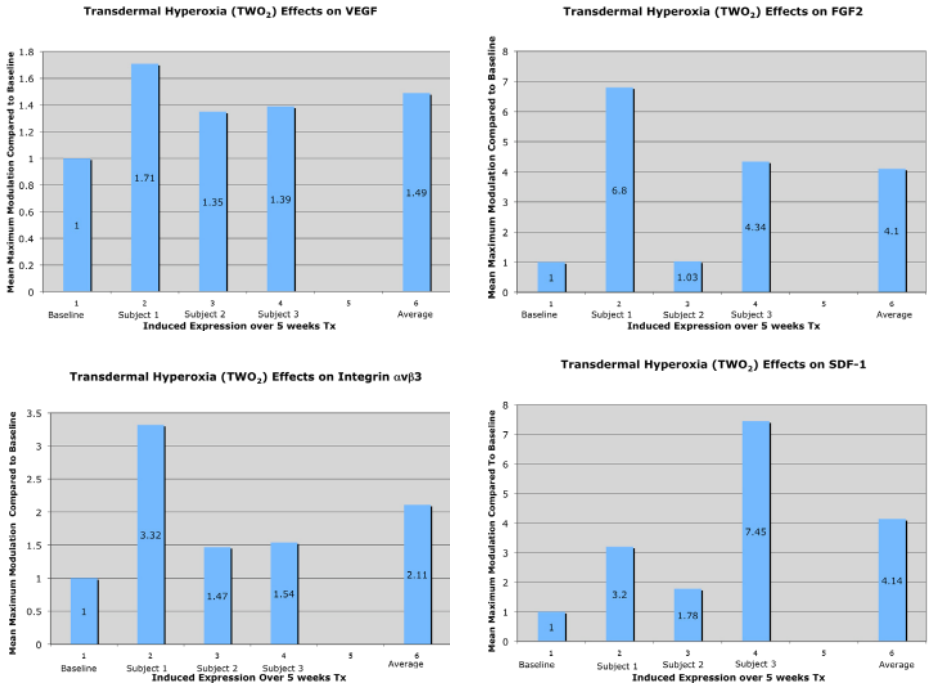
- Topical Wound Oxygen Treatments (TWO_2) were administered with medical grade oxygen (>95% pure) in a TOCE (Topical Oxygen Chamber for Extremities) for 4 consecutive days, 90 minutes per treatment for 5 weeks.
- Wounds were digitally photographed and wound fluids were collected after treatment on day one and day four of each week's treatments.
- Fluids from the wound bed were absorbed onto a cotton swab by wiping to collect maximum fluid exudates' volume. Trimmed swabs containing wound fluids were solubilized in 0.1 M Phosphate Buffered Solution (PBS), fractionated by centrifugation and stored at –20oC for subsequent assay
- Simultaneous quantification of analytes was performed using a customized multiplex enzyme-linked immunosorbent assay (ELISA) at end of 5 weeks of treatment. Total protein in samples was measured.
- Analyte concentration changes per unit of total protein standardized for sample volume variance.
- In current ongoing studies, baseline wound fluid samples are collected weekly for 2 weeks prior to treatment for treatment effect comparison.



Physiological relevance of "new" Therapeutic Angiogenic biomarkers

Summary of Results of Therapeutic Angiogenic BioMarkers During Transdermal Hyperoxia (TWO_2) Treatments

- Angiogenic Growth Factors
  - VEGF &FGF-2 increased significantly
- Integrin αVβ3 (only transiently expressed in new endothelial membrane) increases correspond to angiogenic growth factors' changes
  - confirms formation of new functional capillaries and O_2 re-supply
  - not previously quantified in human wound fluids
- SDF-1 targets BMEPCs (bone marrow-derived endothelial progenitor cells) to injury site (vasculogenesis augments angiogenesis)



Conclusions

- This physiologically relevant set of biomarkers quantify therapeutic angiogenesis indicating evidence of renewed activation of dormant cells in chronic wounds, and thus healing.
- These 'endogenous' angiogenic biomarkers as surrogate end-points of healing provide evidence allowing comparison of treatment benefits at far earlier timepoints than ultimate clinical endpoints, i.e. full wound closure.
- This mechanism of action analysis of wound responses to transdermal hyperoxia treatment (TWO_2) demonstrates efficacy that reduces costs while improving benefits to a larger number of patients.

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