



# TOPICAL OXYGEN ALTERS ANGIOGENESIS-RELATED GROWTH FACTOR EXPRESSION IN CHRONIC DIABETIC FOOT ULCERS

G.F. Scott, R.E. Reeves, Department of Cell Biology and Genetics  
University of North Texas Health Science Center, Fort Worth, Texas

## ABSTRACT

**OBJECTIVE:** Following tissue vascular and oxygen delivery disruption, normal wound healing is a complex process requiring restoration of supplies of oxygen and other nutrients through blood vessel regeneration, or angiogenesis. Angiogenesis is stimulated by synergistic interactions of growth factors and cytokines secreted by damaged cells in wound tissues exhibiting hypoxia, high lactate levels and inflammation. Chronic wounds, often complications of diabetes, are characterized by chronic hypoxia, inflammation, nerve damage, and reduced growth factor secretion that is insufficient to support healing. To test growth factor sensitivity to reversed wound hypoxia, we administered pure oxygen topically [Topical HyperOxia Therapy (THOT)] and used molecular probes to measure growth factors in wound fluid secretions that were previously found to regulate angiogenesis and improve delayed healing.

**METHODS:** Subjects (3) were referred by physicians of diabetic patients whose planar foot wounds failed to respond to standard wound care treatments for a minimum four weeks. By multiplex ELISA assays of growth factor cytokines, we quantified pg/mg levels of total proteins detectable in fluids collected twice weekly from wounds after exposure to topical oxygen delivered in 0-50 mm Hg pulses at to above normal atmospheric pressure (760-810 mm Hg) during 90 minute treatments four days per week over a five week protocol.

**RESULTS:** Our initial data show increased expression in angiogenesis-related growth factors (FGF2, HB-EGF, HGF, KGF, VEGF) in wound fluid from chronic diabetic foot ulcers using the Topical Oxygen Chamber (Advanced Hyperbaric Technologies, Inc., Farmingdale, NJ). The most crucial angiogenic factor, VEGF, was altered by 3- to 20-fold increases using the described protocol, while FGF2, a VEGF enhancer, was increased from 5- to 76-fold. NT-3, a neurotrophic growth factor, was significantly quantified in wound fluids for the first time. This NT-3 detection at levels increased over 11-fold represents a novel discovery indicating a role for nerve growth in angiogenesis.

**CONCLUSIONS:** These data show evidence of a molecular mechanism for the scientific basis of topical oxygen-modulated growth factor expression in chronic diabetic wounds, previously unresponsive to standard wound care. We conclude that topically applied oxygen (THOT) alters angiogenesis-related growth factor expression in wound fluids from chronic diabetic foot ulcers in a manner consistent with revascularization and renewed healing.

**KEY WORDS:** angiogenesis, chronic wound, cytokines, diabetic foot ulcer, growth factor, hypoxia, inflammation, lactate, oxygen, wound fluid.

## ANGIOGENIC GROWTH FACTORS IN "HEALING" WOUNDS

Growth Factor MW(kD)	Structure	Source	Biologic Effect	Family
HB-EGF 6	Monomer	Macrophages	Mitogenic for keratinocytes, fibroblasts	EGF
PDGF 28-35	Dimer, A& B chains	Platelets, endothelial cells, macrophages, fibroblasts	Mitogenic for vascular smooth muscle cells, fibroblasts	PDGF
VEGF \$45	Homodimer VEGF-A angiogenic-isoform	Platelets, macrophages, fibroblasts, keratinocytes, (lactate and oxidant-induced)	Mitogenic specifically for endothelial cells in angiogenesis, vasodilation/permeability	PDGF
FGF 2 18	Monomer	Keratinocytes, fibroblasts macrophages	Mitogenic for fibroblasts, ECM collagen synthesis	FGF
KGF(FGF 7) 28	Monomer FGF2Ib receptor	Dermal and granulation fibroblasts, keratinocytes	Mitogenic specifically for keratinocytes	FGF
HGF(SF) 80	Heterodimer c-met receptor	Mesenchymal fibroblasts and smooth muscle cells	Mitogenic for endothelial and epithelial cells in tube vessels, enhances VEGF angiogenesis	

## OXYGEN IN TISSUES AND WOUNDS

- All nucleated cells use O<sub>2</sub> energy metabolism
- Cornea, epidermis to papillary dermis use topical O<sub>2</sub>
- From blood, O<sub>2</sub> diffusion through membranes is "concentration" dependent, arterial pO<sub>2</sub> limited
- In wounds, vessels disrupted, so lack O<sub>2</sub> gradient
- Wound ischemic hypoxia impairs O<sub>2</sub>-ase enzymes
  - Cytochrome O-ase for ATP generation, so lactate prevails
  - Prolyl hydroxylase for collagen synthesis, angiogenesis
  - Phagocytic O-ase for bacteria killing and signaling
- Highest priority to restore O<sub>2</sub>, thus angiogenesis
- Enforced O<sub>2</sub> concentration (THOT) increases diffusion distance
- Renewed O<sub>2</sub> gradient can activate repair molecules

## WOUND FLUID COLLECTION

- At the end of each 90 minute treatment, fluids from the wound bed will be absorbed onto a cotton swab by wiping to collect maximum fluid exudates' volume. Trimmed swabs containing wound fluids will be solubilized in 0.1 M Phosphate Buffered Solution (PBS), fractionated by centrifugation and stored at -20°C for subsequent assay using the customized multiplex enzyme-linked immunosorbent assay (ELISA).
- Following the final weekly oxygen administration, wound fluids are again collected and stored as described above for subsequent simultaneous immunoassay of all 10 samples at the end of 5 weeks. Wounds will be digitally photographed for evaluation after treatment on day one and day four of each week's treatments.

## METHODS AND DESIGN

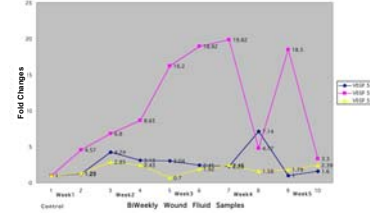
### CRITERIA FOR INCLUSION/EXCLUSION OF SUBJECTS

- Inclusion criteria:**
  - Adult participants, over age 21, both genders, any races
  - Type 2 diabetes diagnosis
  - Palpable posterior tibial pulse
  - Open wound on foot
  - Lack of response in 4 weeks of Standard Wound Care (SWC) prior to oxygen treatment, i.e. non-healing, chronic ulcer
- Exclusion criteria:**
  - Ulcer depth to bone or tendon through deep fascia
  - Active osteomyelitis
  - Tissue-threatening gangrene
  - Uncontrolled diabetes
  - Untreated sepsis

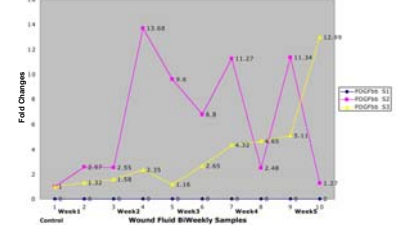
## ELISA GROWTH FACTOR DETECTION AND QUANTIFICATION

- At end of five weeks of treatments, all 10 samples are thawed and simultaneously analyzed using the Pierce SearchLight chemiluminescent Human Angiogenesis Array.
- This ELISA is specifically customized for the measurement of natural and recombinant human PDGF-BB, KGF, HGF, TGFβ, FGF-2, VEGF, and HB-EGF angiogenesis growth factors, as well as MMP-9, a metalloproteinase which breaks down tissue matrix, and Angiopoietin-2 (an anti-angiogenic control factor) or NT-3, a neurotrophic growth factor.
- Evaluation of growth factor expression results will determine up-regulation and down-regulation modulation trends due to topical oxygen delivery over five weeks of treatment.

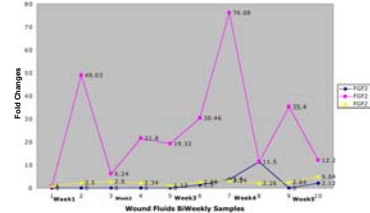
THOT Effects on VEGF in Chronic Diabetic Wounds in Subjects 1, 2, & 3



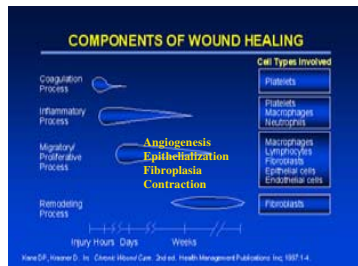
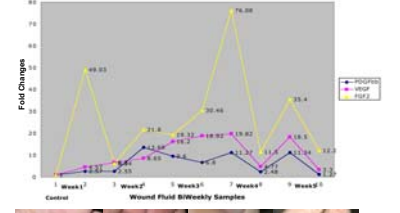
THOT Effects on PDGF-BB in Chronic Diabetic Wound Fluid of Subjects 1, 2 & 3



THOT Effects on FGF2 in Chronic Diabetic Wounds in Subjects 1, 2, & 3



THOT Effects on PDGF, VEGF, & FGF2 in Subjects 2 Male age 59



## REVIEW OF RESULTS OF TOPICAL HYPERBARIC OXYGEN TREATMENTS

- Ang 2 (Angiotensin 2) antiangiogenic factor not expressed, so successful control for THOT effects
- Angiogenic Growth Factors
  - VEGF 2.85 to 19.82 fold increases
  - FGF-2 5.04 to 76.08 fold increases
- All Growth Factors detected (PDGF-bb, HBEGF, HGF, KGF, TGFβ) exhibit increases & consistent expression validating method
- Matrix MetalloProteinase
  - MMP-9 reduction or minimal increase
- Nerve Growth Factors (novel discovery in wound fluid)
  - NT-3 11.45 fold increase

## CONCLUSION

- In spite of small study group, results are very dramatic at the molecular level of *critical* cellular responses using cytokine growth factor molecular probes.
- In light of *minimal risk* (esp. compared to systemic HBO), THOT can provide *enormous potential benefits* in improving "active wound therapy" to correct the balance of microcirculatory factors to "normally regulate wound repair" and thus save limbs!