HBO$_2$ Therapy
Cell Signaling &
Mechanisms of Action

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“I have no relevant financial relationships with commercial interests to disclose.”
TWO MAIN EFFECTS

**BBBLE REDUCTION**

Air-saturated plasma

**HYPEROXYGENATION**

Oxygenated plasma
# OXYGEN LEVELS (mean ± SD)

| Insp $O_2$ ATA | $PaO_2$ mmHg | Tissue $pO_2$
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<td>0.21</td>
<td>90± 9</td>
<td><strong>Normal</strong> 41± 10</td>
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<td>1.0</td>
<td>625 ± 23</td>
<td>76 ± 45</td>
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Important to consider $O_2$ tension for HBO$_2$ mechanisms
HBO$_2$ Therapy: Oxidative stress in moderation.

Reactive O$_2$ species (ROS)
Reactive Nitrogen species (RNS)

INFECTION
Host Target
INFLAMMATION
ENDOTHELIUM-I/R

STEM CELLS
WOUND HEALING

IMMEDIATE LATE
ANTIBACTERIAL EFFECTS:

DIRECT – O₂ IS TOXIC TO ALL ORGANISMS!
Sensitivity varies (part. pressure and time-dependence)
BUT ……

Most clinical anaerobic isolates survive 8 hrs in 160 mmHg O₂
May be bacteriostatic on many facultative and strict anaerobes. (J Clin Microbiol 1:161, 1975)

INHIBITION OF TOXIN SYNTHESIS – Tissue pO₂ at 3 ATA O₂ ~ 330 mmHg. This part. pressure sufficient to inhibit Clostridium sp. Synthesis of the lethal α toxin.
(Ann NY Acad Sci 117: 688, 1965)
Augmented PMN bacterial killing

J Infect Dis 142: 915,’80

2 ATA O₂: Infected bone pO₂ = 104 mmHg
Normal bone pO₂ = 321 mmHg

Infected bone, Ambient air

23 mmHg O₂

Normal bone, Ambient air

45 mmHg O₂

109 mmHg O₂

150 mmHg O₂

Myeloperoxidase/
NADPH oxidase
Nitric oxide synthase
(O₂⁻, H₂O₂, HOCl, Fe/·OH,
·NO – intracellular sp. like
Mycobacteria, Leishmania)

Health Risk of Biofilms-Hypoxia and HBOT

Modification of target oxygenation …

DEFINITION:
Aggregate of microorganisms embedded within a self-produced matrix of extracellular polymeric substances. A thin, slimy film of bacteria that adheres to a surface.

Biofilms impede healing and obstruct disinfection

Although they do not block antibiotic uptake, the environment renders them anti-bacterial due to hypoxia. … organisms ‘dormant’, not killed by drugs or PMN.

In model systems, HBOT markedly effective at killing

But, clinical data to mirror lab investigations limited …
Health Risk of Biofilms - Hypoxia

Hypoxia arising from concerted $O_2$ consumption by neutrophils and microorganisms.


$O_2$ depletion in biofilms associated with chronic wounds.

Health Risk of Biofilms – Impaired Healing

Time Course Study of Delayed Wound Healing in a Biofilm-Challenged Diabetic Mouse Model.


Contribution of Stress Responses to Antibiotic Tolerance in Pseudomonas aeruginosa Biofilms.


Gel-Entrapped S. aureus as Models of Biofilm Infection Exhibit Growth in Dense Aggregates, Oxygen Limitation, Antibiotic Tolerance, and Heterogeneous Gene Expression

Efficacy of hyperbaric oxygen therapy in bacterial biofilm eradication. N.E. Sanford, et al. J Wound Care 27; Suppl 1: S20, 2018

Organisms reductions in a 74 yo pt.

**HBOT …**

- Sensitizes Anoxic *Pseudomonas aeruginosa* Biofilm to Ciprofloxacin

- Augments tobramycin efficacy in experimental *S. aureus* endocarditis

- Reinforces ciprofloxacin bactericidal effect on *P. aeruginosa* biofilm.

### Efficacy of hyperbaric oxygen therapy in bacterial biofilm eradication.

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N=11

\[67.01\% \text{ reduction}\]
HBO$_2$ Therapy: Oxidative stress in moderation.

HBO$_2$

- Reactive O$_2$ species (ROS)
- Reactive Nitrogen species (RNS)

- INFECTION
- INFLAMMATION
- ENDOTHELIELUM-I/R
- STEM CELLS
- WOUND HEALING

IMMEDIATE

LATE
INFLAMMATORY CYTOKINE SUPPRESSION

HBO$_2$ inhibits leukocyte pro-inflammatory cytokine production 
(NF-κB, IL-1β, IL-6, TNFα)


Possible link to wound healing, TBI, shock, DCS, vascular & lung injury, spinal cord injury, stroke, diabetic wounds

**Hyperbaric oxygen therapy** ....

**Decreases TNFα**

Hyperbaric oxygen preconditioning attenuates neuroinflammation after *intracerebral hemorrhage* in rats by regulating microglia characteristics.  

**Decreases NF-κB**

Hyperbaric oxygen reduces inflammatory responses in acute *pancreatitis* by inhibiting NF-kappaB activation.  

**Decreased IL-6, TNFα, increased VEGF and EGF**

Effects of alpha lipoic acid and its R+ enantiomer supplemented to hyperbaric oxygen therapy on interleukin-6, TNF-α and EGF production in *chronic leg wound healing*  
**Hyperbaric oxygen therapy ....**

Decreased IL-1β, TNFα, astrocyte activation

The analgesic effect of early hyperbaric oxygen treatment in chronic constriction injury rats and its influence on nNOS and iNOS expression and inflammatory factor production

Ding, Y., et al. Molecular Pain 14: 1, 2018 also – Oncotarget 9: 7513, 2018

Decreased PMN influx, IL-1β, TNFα, lower NF-κB activation

Hyperbaric Oxygenation Ameliorates Indomethacin-Induced Enteropathy in Rats by Modulating TNF-α and IL-1β Production


Hyperbaric Oxygen Therapy in Rats Attenuates Ischemia-reperfusion Testicular Injury Through Blockade of Oxidative Stress, Suppression of Inflammation, and Reduction of Nitric Oxide Formation.

Zhang, Y. et al. Urology 82: e9, 2013
Hyperbaric oxygen therapy ....

Inhibits TLR2 mediated NF-κB, decreased IL-1β, TNFα

Protective effects of hyperbaric oxygen treatment against spinal cord injury in rats via toll-like receptor 2/nuclear factor-κB signaling

Inhibits TLR4 mediated NF-κB, decreased IL-1β, IL-6, TNFα

Hyperbaric Oxygen Alleviates Secondary Brain Injury After Trauma Through Inhibition of TLR4/NF-kB Signaling Pathway

Inhibits TLR4 mediated NF-κB, increases mTOR pathway

Hyperbaric oxygen protects against myocardial reperfusion injury via the inhibition of inflammation and the modulation of autophagy
Hyperbaric oxygen therapy ….

Increased Sirt1 and Nrf2/antioxidant defense pathway

Nrf2/antioxidant defense pathway is involved in the neuroprotective effects of Sirt1 against focal cerebral ischemia in rats after hyperbaric oxygen preconditioning


Sirt1 Mediates Improvement in Cognitive Defects Induced by Focal Cerebral Ischemia Following Hyperbaric Oxygen Preconditioning in Rats


Sirt1 is a class III histone deacetylase that has been linked to antioxidant ‘pre-conditioning’, neuronal protection and plasticity
ANTI-INFLAMMATORY HBOT EFFECT

IMPAIRED NLRP3 INFLAMMASOME

Neutrophils

ASC

NALP3

Merged

STIM + HBO₂

+ STIMULATION

5 um
HBO₂ Therapy: Oxidative stress in moderation.

HBO₂

- Reactive O₂ species (ROS)
- Reactive Nitrogen species (RNS)

INFECTION
INFLAMMATION

ENDOTHELIELUM-
I/R

WOUND HEALING
STEM CELLS

IMMEDIATE
LATE
“REPERFUSION” INJURY AND HBO$_2$:

CO-mediated brain injury -
Toxicol. Appl. Pharm. 123: 248,’93
Brain ischemia-reperfusion injury -
Undersea & Hyperb. Med 27: 185,’01

Hepatic reperfusion-
Hepato-Gastroent. 46: 1798,’99
Decompression sickness -
Aviat. Space Env. Med. 73:565,’02

ENDOTHELIELUM CHANGES

PMN ADHERENCE
*VIA SELECTINS*

PMN ACTIVATION
\(\beta_2\) INTEGRINS

MICROVASCULAR DAMAGE

TISSUE INJURY
Neutrophil activation is complex, multiple pathways but two main categories:

- ‘OUTSIDE-IN’ activation as with ischemia-reperfusion (HBO$_2$ inhibits this one)
- ‘INSIDE OUT’ activation via G-proteins as with immune surveillance

References:
1. J. Biol. Chem. 283: 10822,’08
2. J. Biol. Chem. 286: 32854,’11
3. J. Biol. Chem. 287: 30346,’12
HBO$_2$ inhibits human PMN $\beta_2$ integrin fxn.

Labrouche, Thrombosis Res. 96:309,’99
Kalns, Immunol. Lett. 83:125,’02
PMN attached to fibrinogen-coated plates.

Mechanism: Modification of cytoskeletal actin, NOS-2 and MPO

Recent paper suggests NO from VEGF-activated endothelium

J. Biol. Chem. 283: 10822,’08
J. Biol. Chem. 286: 32854,’11
J. Biol. Chem. 287: 30346,’12
Multiple HBO effects on several cell types -

Reduces expression of glial fibrillary acidic protein, vimentin, and ICAM-1 (intercellular adhesion molecule-1) at gene and tissue levels.

Repetitive HBOT, prevents glial scarring and limits expression of inflammatory mediators (no functional assessments done, mechanisms unclear).
HBO_2 Therapy: Oxidative stress in moderation.

HBO_2

Reactive O_2 species (ROS)
Reactive Nitrogen species (RNS)

INFECTION
INFLAMMATION
ENDOTHELIELIUM-I/R

STEM CELLS

LOCAL CHANGES
Mobilization

WOUND HEALING

IMMEDIATE LATE
Hyperbaric oxygen reduces inflammation, oxygenates injured muscle, and regenerates skeletal muscle via macrophage and satellite cell activation. Oyaizu, T. et al. Sci Rep 8: 1288, 2018

HBO2 accelerated regeneration processes including satellite cell proliferation (resident stem cells) with improved muscle fiber regeneration and strength.

HBO2 accelerated local WBC transition from an inflammatory to anti-inflammatory response.

Rat contused muscle – HBO2 reduced hypoxia, maintained high oxygenation.

32 consecutive refractory UC patients: Largest English literature report, with documented mucosal healing after HBOT.

All patients manifested clinical improvement by the 40th HBOT (2.8 ATA, 60 min). 
No with persistent blood passage (p=0.002). Severity index significantly improved HBOT (z=−4.97, p<0.001).

Pre and post-treatment biopsies show increase in CD44 membranous staining.
Hyperbaric oxygen promotes osteogenic differentiation of bone marrow stromal cells by regulating Wnt3a/β-catenin signaling—An in vitro and in vivo study  

Bone formation is increased via osteogenic differentiation of bone marrow stromal cells regulated by Wnt3a/β-catenin signaling.

HBO₂ increased cell proliferation, Wnt3a production in differentiated bone stromal stem cells.  
...Increased osteogenesis

Dose dependent increase of bone morphogenetic protein & osterix.

In vivo rabbit model: HBO₂ increased translocated β-catenin that stimulated expression of target genes.

Conclusion: HBO₂ acted via Wnt3a secretion and signaling.
ONE MECHANISM FOR STEM CELL MOBILIZATION INVOLVES ‘NO SYNTHESIS

Cytokine → Receptor → NOS → ‘NO → MMP-9
Membrane-cKit (aka stem cell factor)
Soluble-cKit
Stem cells mobilized

HBO₂ ELEVATES ‘NO SYNTHESIS
J Neurobiol. 51: 85, 2002
Am J Physiol. 284: H1230, ‘03

HBO₂ MOBILIZES STEM CELLS
Am J Physiol. 290: H1378, ’06
Stem Cells 24: 2309, ’06
J Clin Invest 117:1249,’07
HUMAN STEM CELL MOBILIZATION BY HBO₂

Am. J. Physiology 290: H1378, 2006

% CD 34+ IN GATED CELL POPULATION

n=26

- PATIENTS PREVIOUSLY EXPOSED TO RADIATION -
Stem cell mobilization & post-traumatic wound healing


Sham HBO (n=22)  Daily HBO (n=97)

EPCs = CD34⁺/Sca-1 & CD34⁺/CXCR4

HBO EPCs increase 5.5 & 7.3x (No increase in sham group)

EPCs correlation with healing $r^2=0.84$ $p<0.01$
Increased circulating stem cells and better cognitive performance in traumatic brain injury subjects following hyperbaric oxygen therapy

Sabrina Shandley, E. George Wolf, Christine M. Schubert-Kabban, Laura M. Baugh, Michael F. Richards, Jennifer Prye, Helen M. Arizpe, John Kalns

UHM 44: 257, 2017

N= 13 sham, 15 HBO

Trend for increase in SPCs with HBO, not seen with sham (NS). Significant improvement on cognitive measures with SPCs

Together these results support the hypothesis that stem cell mobilization may be required for cognitive improvement.
O₂ Pressure Matters for SPCs Mobilization
20 consecutive patients, tx for radionecrosis prophylaxis

* p<0.05 2-tailed test
HBO$_2$ Changes Stem Cell Growth Potential

Monocytes (100,000/plate) colonies counted 14 days later.
ANTIOXIDANT RESPONSE/
THIOREDOXIN SYS’SIS
(Trx can be a transcription factor)

Diabetic Wound Cells

HIF-1 & CD133 DUAL positive

n=7, * p<0.05

Trx1 & CD133 dual positive

Start STANDARD TX 19 + 2 d ~16 days Start 17 + 2 days + HBO2 TX

Prospective study of 25 patients with T2DM received intra-pancreatic stem cells and peri-infusion HBO₂

Improved metabolic control, reduced insulin need over time.
WOUND HEALING MECHANISMS

Those with diabetic complications
Likely applicable to radiation injury

1. Improved ischemic tissue $O_2$ gradient stimulates macrophage recruitment.

2. HBO$_2$ improves wound growth factor synthesis (esp. VEGF).

3. HBO$_2$ stimulates vasculogenic stem cell mobilization & function.
HBO₂ Therapy: Oxidative stress in moderation.

HBO₂

Reactive O₂ species (ROS)
Reactive Nitrogen species (RNS)

INFECTION
INFLAMMATION
ENDOTHELIUM-
I/R

STEM CELLS

‘Fuel’ Cell Responses

WOUND HEALING

IMMEDIATE LATE
O$_2$ tension of irradiated field
Shallow, ~ 10-20 mmHg

O$_2$ tension under HBO$_2$
Steep, ~ 230 mmHg gradient

...thought critical for Mφ activation/growth factor synthesis

From Marx, Problem Wounds (Davis & Hunt, eds. 1988)
HBO$_2$ temporarily improves oxygenation, transient vasoconstriction (sometimes) may diminish edema


Components in experimental compartment syndrome tx.

HBO$_2$ increases VEGF in experimental wounds (Arch Surg 135: 1293, 2000)

HBO$_2$ increases basic fibroblast growth factor & transforming growth factor β1 in human dermal fibroblasts. (Arch Facial Plast Surg 6: 31, 2004)

HBO$_2$ increases angiopontin-2 in human umbilical vein endothelial cells (BBRC 296: 710, 2002)

HBO$_2$ up-regulates platelet derived growth factor receptor in experimental wounds (Undersea. Hyperb Med 25: 211,’98)
Hyperbaric oxygen therapy - chronic wounds
Cochrane Database Syst Rev: 2015
P. Kranke, M.H. Bennett, M. Martyn-StJames, A. Schanabel, S.E. Debus, S. Weibel

Twelve trials (531 patients) enrolling DFU, pooled data showed increase healing (mean difference 33%, RR 2.35) at 6 weeks but not 1 year.

Effect on amputation rate not significant.

...the trials had various flaws in design and/or reporting ... More trials are needed.
... HBO₂ in wound healing: EVIDENCE PROVIDES SUPPORT BUT…

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<td>Ma, 2013</td>
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SUMMARY: HBO$_2$ Mechanisms

1. Improved O$_2$ delivery, bubble reduction, tissue O$_2$ gradient.

2. E$_h$, anti-bacterial, improved WBC ‘cidal fxn.

3. Anti-inflammatory / ↓cytokine sys’sis

4. Impair neutrophil β$_2$ integrin adhesion.

5. Increased local stem cell responses/ cell signaling growth responses.

6. Increased stem cell mobilization.

7. Increased wound growth factor synthesis.
WHERE TO FROM HERE?

More basic work:

Clinical Trials:

Incorporate mechanistic Issues in design

“What’s the opposite of ‘Eureka’?”

?Int’l collaboration

Bio-samples collection/sharing
Thank you .... Questions??

Baltimore – Inner Harbor