Hyperoxia-Driven Modulation of Tendon Healing: Insights from *In Vitro* and *In Vivo* Models

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INTRODUCTION: Tendon injuries remain a major clinical challenge in musculoskeletal health, as repair is often characterized by excessive scar formation, disorganized collagen, and reduced mechanical strength. This impaired healing is thought to arise, in part, from the inherently avascular nature of tendon tissue. Hyperbaric oxygen therapy (HBOT), a non-invasive treatment that delivers 100% oxygen under elevated atmospheric pressure, has demonstrated promise in reducing inflammation and enhancing vascularization in other tissues (1,2,3). However, its potential application in tendon repair remains largely unexplored. We hypothesized that HBOT would mitigate the inflammation-driven damage and promote improved tendon healing in both *in vitro* and *in vivo* models.

METHODS: For the *in vitro* studies, primary murine tenocytes were isolated from the Achilles tendons of four 14–16-week-old C57BL/6 mice, based on established protocols. Tendons were digested using collagenase, and cells were plated at a density of 10,000 cells/mL. At ~80–90% confluence, IL-1β (0.05 ng/μL) was added to all wells. After 24 hours a 0.50 mm scratch was introduced in half of the wells to simulate injury. Plates were then placed in a hyperbaric oxygen chamber (Reimers Systems Inc.) and exposed to 100% oxygen at 2.5 atmosphere absolute (ATA) for 60 minutes, while controls were placed adjacent to the chamber to ensure equivalent environmental exposure outside of the incubator. Following treatment, all wells were returned to standard culture conditions. Scratch assay wells were imaged every 6 hours for up to 24 hours to assess cell migration and closure. At 24 hours, all wells (with and without scratch) were analyzed for cell viability and proliferation via MTS assay, extracellular matrix (ECM) deposition via picrosirius red staining, and cytotoxicity via live/dead staining. Statistical significance was determined using unpaired t-tests with a p-value threshold of 0.05. For the *in vivo* studies, aged male C57BL/6 mice (20 months, n=8; NIH NIA aged rodent colony) underwent partial Achilles tenotomy on postoperative Day 0. Beginning on Day 1, animals received daily HBOT sessions consisting of 100% oxygen at 2.5 ATA for 60 minutes daily for 5 days. Control animals were injured but did not receive HBOT treatment. On postoperative Day 7, mice were sacrificed and both hindlimbs were harvested for histological evaluation. Tissue was fixed, sectioned, and stained with hematoxylin and eosin (H&E), picrosirius red/Alcian blue, and Masson's trichrome, and slides were imaged on a Keyence microscope. Data reported here reflect only male mice, with ongoing work including parallel analyses in age-matched females.

RESULTS SECTION: *In vitro*, IL-1β-treated cells exposed to HBOT demonstrated significantly reduced scratch closure compared to the controls. However, the addition of HBOT did not alter metabolic activity, proliferation, or collagen deposition with HBOT (Figure 1). *In vivo* histological analysis demonstrated increased cellularity and scar tissue in the operative limb compared to the contralateral control limb (Figure 2). Complete rejoining of the tendon tissue following partial tear was observed in all four HBOT-treated animals, compared to only three of four in the control group. Furthermore, Alcian blue staining was reduced in the HBOT-treated group compared to the controls (Figure 3).

DISCUSSION: Contrary to prior *in vitro* studies reporting increased metabolic activity with HBOT (4), our inflammatory model showed reduced scratch closure without changes in viability, proliferation, or collagen deposition. Rather than indicating a lack of efficacy, these results suggest that HBOT may modulate cellular responses to inflammatory stress without impairing viability, offering mechanistic insight into the tissue-level changes observed *in vivo*. One possibility is that hyperoxia under inflammatory conditions temporarily alters migration pathways, while preserving cell health and matrix synthesis, setting the stage for more organized remodeling. Previous studies have reported that hyperoxia can exacerbate oxidative stress under pro-inflammatory conditions, which could impair cell migration despite preserved viability and metabolic activity. Additional mechanistic studies, such as assessing oxidative stress markers or inflammatory cytokine expression, may clarify these findings. In contrast, the more complex *in vivo* environment—integrating vascular, immune, and mechanical inputs—appeared to harness the pro-regenerative effects of HBOT, leading to qualitative improvements in tendon healing. Histological analysis revealed that HBOT-treated limbs showed reduced inflammatory cell infiltration (H&E staining) and decreased Alcian blue staining, indicating reduced proteoglycan accumulation, both consistent with a shift toward a more regenerative matrix phenotype. These changes are consistent with a shift away from a tendinopathic phenotype, which is typically characterized by proteoglycan-rich, disorganized ECM, toward a more organized, collagen I–rich matrix that supports tensile strength and functional recovery (5–7). Moreover, complete tendon rejoining was observed in all HBOT-treated animals, compared to partial healing in the control group, further supporting a potential therapeutic benefit. These preliminary findings suggest that while HBOT may not enhance migration in a simplified inflammatory model, its effects *in vivo* likely rely on a synergistic interaction with systemic cues, enabling a more organized and collagen I–rich repair response. Ongoing work will expand on these observations by incorporating molecular analyses, including quantitative assessment of tendon healing markers (COL1A1, COL3A1, VEGF) and oxygen-responsive genes (HIF-1α). Additionally, the inclusion of female animals will determine potential sex-specific differences in tendon biology and HBOT responsiveness, an important consideration for translational relevance.

SIGNIFICANCE/CLINICAL RELEVANCE: Effective strategies to improve tendon healing remain limited, as repair is often compromised by scar formation and subsequent re-rupture. HBOT offers a non-invasive, clinically translational approach with the potential to enhance outcomes in tendon repair.

REFERENCES: [1]Buckley *StatPearls* 2025; [2]Tal *Frontiers in Human Neuroscience* 2017; [3]Oyaizu *Scientific Reports* 2018; [4]Limberg *ORS Abstract* 2025; [5]Chatterjee *JOR* 2023; [6]Roberts *Br J Dermatol* 1994; [7]Kuran *Acta Orthop Traumatol Turc* 2012.

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IMAGES AND TABLES:

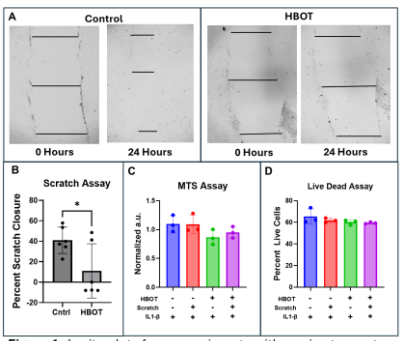

Figure 1: *In vitro* data from experiments with murine tenocytes with IL-1β treatment with and without HBOT.

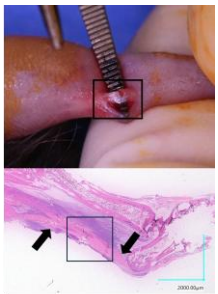

Figure 2: Intraoperative photo of the partial Achilles tenotomy (top) and an H&E histological image showing the tear in a control animal. Black arrows indicate the Achilles tendon in the histology image.

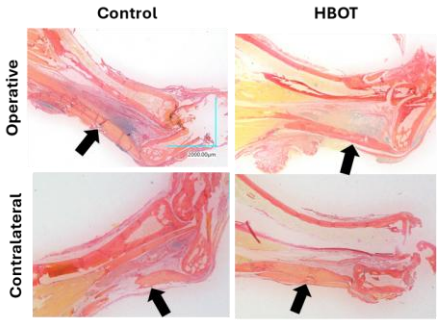

Figure 3: Representative images of picrosirius red and Alcian blue staining on operative and contralateral limbs for both control and HBOT groups. Black arrows indicate the Achilles tendon.