Effect of Hypoxia on 2D and 3D Culture of Human MSCs Used for Tissue Engineering of Whole Intervertebral Discs

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Disclosures: Katherine D. Hudson (N), Lawrence J. Bonassar (N)

INTRODUCTION: Several studies have examined the effects of hypoxic conditions during either cell expansion or three dimensional (3D) culture [1-4]. While some studies suggest that hypoxia limits differentiation [1,2], others show that hypoxia can enhance chondrogenesis and the production of extracellular matrix (ECM) proteins [3,4]. Very few studies have looked at the effects of hypoxia on the maturation of MSCs in different types of scaffold materials and none have addressed the role of hypoxia in the development of composite TE constructs. This study aims to determine the effects of both cell expansion in hypoxic conditions as well as the subsequent maturation of 3D TE-IVDs in hypoxic (5%) vs normoxic (21%) conditions.

METHODS: *Cell Culture:* Human MSCs (RoosterBio, Inc) at population doubling level (PDL) 7-9 were cultured in hBM-MSC High Performance Media (RoosterBio, Inc) to PDL 14-15 in either normoxic (21% O_2) or hypoxic (5% O_2) conditions before being harvested for TE-IVD manufacture.
TE-IVD Manufacture (Figure 1): To create the inner TE-NP, 3% alginate was seeded with hMSCs ($25x10^6$ cells/ml) and injected into custom molds (Figure1 A, B). Each TE-NP was placed in the well of a 24-well plate and 410 µl of type I collagen gel (4 mg/ml) seeded with hMSCs ($1x10^6$ cells/ml) was pipetted around the TE-NP to create the TE-AF (Figure 1C). The constructs were cultured for 2 weeks in DMEM media with 10% FBS, 2.5% HEPES, and 1% antibiotics in either normoxic (21%) or hypoxic (5%) conditions (Figure 2). Four groups were cultured in total.
Analysis (Figure 3): The discs underwent stress-relaxation tests in unconfined compression. The equilibrium and instantaneous moduli, and effective hydraulic permeability were determined by fitting the data to a poroelastic model. The AF and NP regions were separated and biochemical analysis was performed using a Hoechst assay for DNA content, a modified DMMB assay for glycosaminoglycan (GAG) content, and hydroxyproline assay for collagen content. All biochemistry values were normalized to dry weight. All data was analyzed using a three-way ANOVA with post-hoc Holm-Sidak tests. A p-value of <0.05 was considered significant.

RESULTS: *Mechanical Properties:* Hypoxic expansion (5% O_2) led to significantly higher equilibrium and significantly lower hydraulic permeability over all (Figure 4). Specifically, hypoxic expansion led to a 48-139% increase in the equilibrium moduli. Normoxic expansion (21% O_2) followed by hypoxic 3D culture led to a 38% decrease in equilibrium modulus and a 145% increase in hydraulic permeability. Mechanical properties did not change from two to four weeks.
Biochemical Properties: Expansion conditions had no significant effect on GAG content in the TE-AF region, although subsequent 3D culture in normoxia led to a significant increase in GAG content (Figure 5A). Expansion in hypoxia followed by 3D culture in hypoxia led, to a 46-54% decrease in GAG content. Expansion in hypoxia (5%) significantly increased the GAG content in the TE-NP region (Figure 5B). Subsequent 3D culturing the TE-IVDs in hypoxia further increased the GAG content in the TE-NP region compared to normoxic 3D culture.
Histology: An intermediate/gradient region was seen to develop between the TE-AF and TE-NP regions in all groups at 2 and 4 weeks.

DISCUSSION: These results indicate that expansion of human MSCs under hypoxic (5% O_2) conditions increased production of GAGs in TE-IVDs during subsequent 3D culture. Further increases were seen when 3D constructs were continued in hypoxic (5%) conditions. The increased deposition of GAGs in the TE-NP was paired with similar increases in the compressive moduli and decreases in the hydraulic permeability of the composite TE-IVDs. This transitional region evident in histological images more closely resembles native IVDs than previous discs made with primary disc cells. The effects of expansion under hypoxic or normoxic conditions continues to be evident even after 4 weeks of culture.

SIGNIFICANCE: Expansion of human MSCs in hypoxic conditions is an effective means of accelerating the maturation of composite TE-IVDs *in vitro* and represents a significant step towards efficient production of a clinically relevant TE-IVD replacement.

REFERENCES: [1] Cicione+, *Stem Cells International*, 2014 [2] Domm+, *Osteoarthritis and Cartilage*, 2002 [3] Adesida+, *Stem Cell Resarch & Therapy*, 2012 [4] Grayson+, *Biochem & Biophys R. Comm*., 2007.

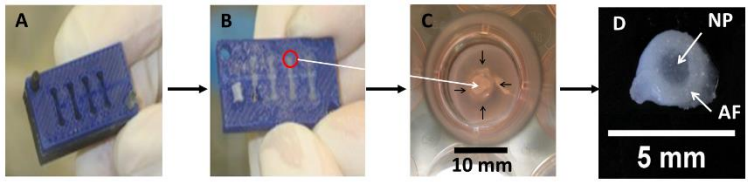

Figure 1: Tissue Engineered IVD fabrication process: (A) 3D printed mold for NPs, (B) 3% alginate w/ 25x10^6 MSCs (RoosterBio Inc.)/mL injected into mold, (C) NP surrounded by 4 mg/mL type 1 collagen w/ 1x10^6 MSCs/mL, arrows indicate contraction, (D) Final TE-IVD

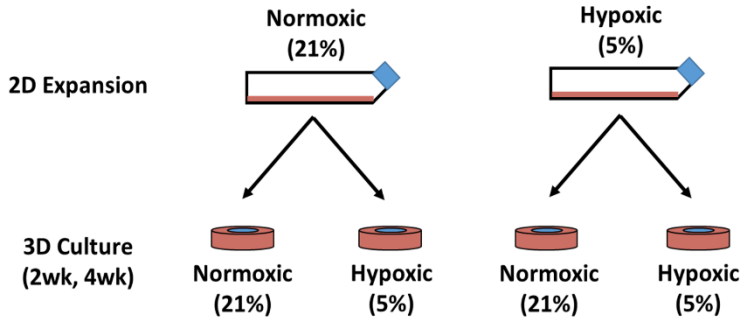

Figure 2: Experimental design. MSCs expanded in either normoxic (21%) or hypoxic (5%) before construction of 3D TE-IVDs (see Fig. 1). Discs either continued in the same pO_2, or switched to the other pO_2. Discs were harvested at 2 and 4 weeks

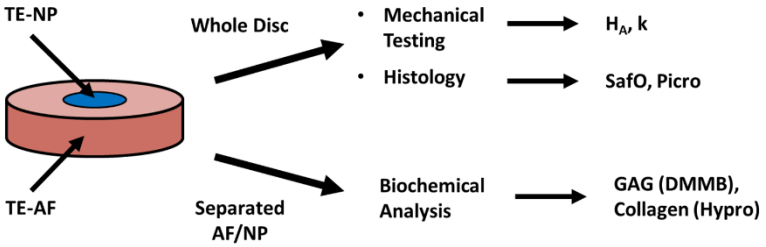

Figure 3: Construct analysis flowchart, statistical analysis was preformed using a two way ANOVA with a Tukey post-hoc, p values of <0.05 was considered significant

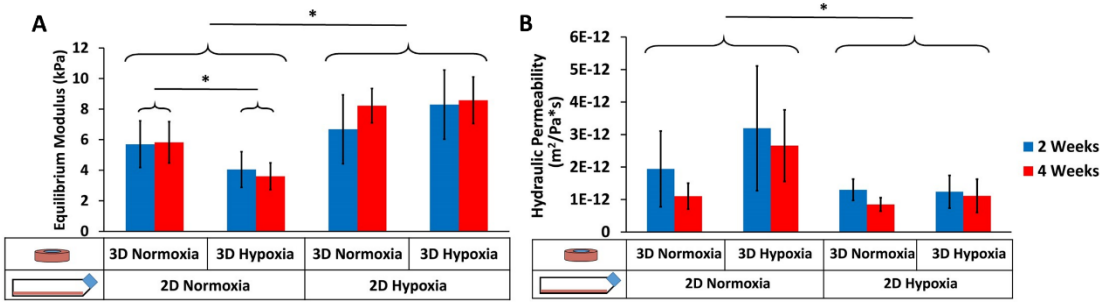

Figure 4: Mechanical properties: (A) Equilibrium modulus (kPa) for each culture group at 2 and 4 weeks, mean±SD, n=4-5, * indicates p<0.05 (B) Hydraulic permeability (m^2/Pa*s) at 2 and 4 weeks, mean±SD, n=4-5, * indicates p<0.05

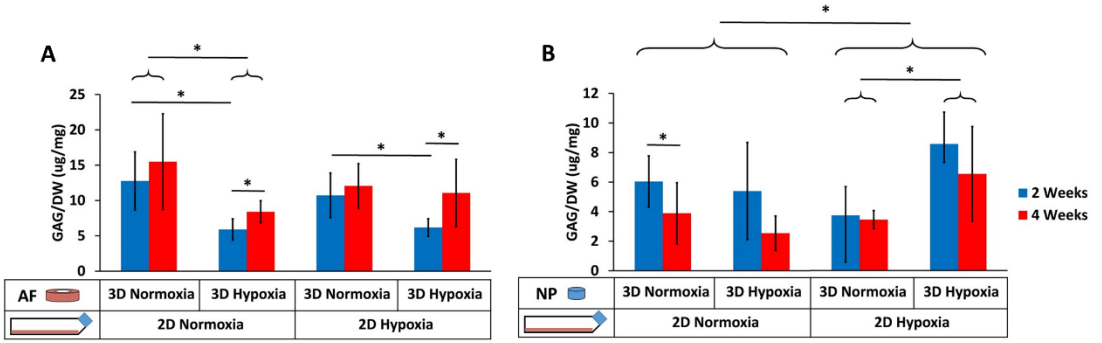

Figure 5: GAG content in AF or NP regions: (A) GAG content (ug/mg) in the AF at 2 and 4 weeks, mean±SD, n=4-5, * indicates p<0.05 (B) GAG content (ug/mg) in the NP at 2 and 4 weeks, mean±SD, n=4-5, * indicates p<0.05