

Ex Vivo Optimization and In Vivo Measurement of Nucleus Pulposus Oxygen, Glucose, and Lactate in a Goat Model of Disc Degeneration

Karthikeyan Rajagopal^{1,2}, Thomas P. Schaer^{1,2}, Kyle D. Meadows³, Madeline Boyes¹, Rachel Hilliard¹, John C. O'Donnell¹, George R. Dodge¹, Dmitriy Petrov¹, Dawn M. Elliott³, Robert L. Mauck^{1,2}, Lachlan J. Smith^{1,2}, Neil R. Malhotra¹
¹University of Pennsylvania and ²CMC VA Medical Center, Philadelphia, USA, and ³University of Delaware, Newark, USA
karthikeyan.rajagopal@pennmedicine.upenn.edu

Disclosures: Karthikeyan Rajagopal (N); Thomas P. Schaer (5-PSI; ReGelTec, Peptilogics, Acuitive Technologies, PAX Therapeutics, OrimTech, SINTX Technologies, OsteoCentric Technologies, DePuy Synthes, Alcyone Therapeutics, Camber Spine, Heraeus); Kyle D. Meadows (N); Madeline Boyes (N); Rachel Hilliard (N); John C. O'Donnell (N); George R. Dodge (4-MechanoTherapeutics); Dmitriy Petrov (N); Dawn M. Elliott (9-ORS Board of Directors); Robert L. Mauck (5-4Web Medical, 4-MechanoTherapeutics; 8-JOR Spine); Lachlan J. Smith (8-JOR Spine, Connective Tissue Res; 9-ORS Spine Section); Neil R. Malhotra (N).

INTRODUCTION: Chronic low back pain is a leading contributor to disability and imposes a substantial socioeconomic burden worldwide [1]. A primary cause of this pain is intervertebral disc degeneration. Although the precise pathophysiological mechanisms driving disc degeneration remain elusive, early indicators include reduced vascular supply, consequent alterations within the microenvironment, and the disappearance of notochordal cells [2]. Emerging treatments such as mesenchymal stem cells (MSCs) to regenerate the central nucleus pulposus (NP) have shown promise; however, the degenerate disc microenvironment negatively impacts MSC survival and therapeutic efficacy [2]. Quantification of this microenvironment, including changes in nutrient availability as a function of degeneration severity, is therefore critical for development of more effective MSC-based treatments. Previous studies using contrast-enhanced imaging and computational modelling have predicted reduced glucose and oxygen levels with degeneration [4]; however, direct experimental evidence is lacking, partly due to lack of appropriate *in vivo* models and sensor limitations. Here we adapt Licox oxygen probes and microdialysis catheters, widely used for brain tissue monitoring, for *in situ* measurement of NP tissue oxygen, and glucose and lactate, respectively. To accomplish this, measurement parameters were first optimized in an *ex vivo* bovine caudal disc model and then applied *in vivo* to investigate changes in NP oxygen, glucose and lactate concentrations in a preclinical goat model of disc degeneration [5].

METHODS: Ex Vivo Optimization: Bovine tails purchased from a commercial vendor were utilized to optimize *in situ* oxygen, glucose and lactate measurements in the NP (Fig 1A). A 16G needle was used to place a Licox oxygen probe (Integra) or microdialysis catheter (M Dialysis) into the center of the NP of caudal discs (C1-C5). Oxygen levels (n=4 discs) were monitored for 90 mins. For glucose and lactate measurements, perfusion fluid was pumped through microdialysis catheters (10 mm, 20 kDa) at one of 2 flow rates: 0.3 μ L/min and 1 μ L/min (n=3 discs each). Microdialysates were collected at 30-min intervals for 150 mins. Glucose and lactate contents in microdialysates were assessed using two independent assays: the ISCUSflex Microdialysis Analyzer (M Dialysis) and single analyte high-sensitivity (HS) detection kits (Abcam and Sigma). **In Vivo Assessment of NP Oxygen, Glucose, and Lactate:** With IACUC approval, under general anesthesia and using an open, left, retroperitoneal, transspaoatic approach, a custom 16G spinal needle under fluoroscopy was used to position Licox probes and/or microdialysis catheters in the lumbar disc NPs (L1-L5) of 3 large frame goats. Oxygen was monitored for 30-45 mins (n=6-7 discs), and microdialysates were collected at a flow rate of 0.3 μ L/min for 150 mins (n=5-6 discs). Disc degeneration was then induced by injecting chondroitinase ABC (ChABC, 2U in 200 μ L) into the NP. After 12 weeks, oxygen and microdialysis measurements were repeated at the same levels in a second surgery. Glucose and lactate were assessed using HS kits. Differences in oxygen, and glucose and lactate levels in healthy vs degenerate discs were determined using Mann-Whitney tests (p<0.05 significant). To confirm degeneration, *in vivo* magnetic resonance imaging (MRI) was performed at baseline and 12 weeks. **Postmortem Histology and MicroCT:** Following euthanasia at 12 weeks, isolated spine segments were fixed in formalin and microCT used to image the vertebral endplates of degenerate and adjacent healthy discs. Samples were then decalcified and processed into paraffin, and midsagittal sections stained with Alcian blue and picrosirius red for assessment of disc condition.

RESULTS: Ex Vivo Optimization: In bovine discs, oxygen measurements equilibrated after ~45 mins, recording levels of 1.5 ± 1.6 mmHg after 90 mins (Fig 1B). Glucose and lactate levels measured using microdialysis reached equilibration after 30 mins (Fig 1C and D). Both glucose and lactate levels were higher in samples collected at 0.3 μ L/min compared to 1 μ L/min after 150 mins (p<0.05 for lactate). Measurements were approximately equivalent for both detection methods. **In Vivo Assessment of NP Oxygen, Glucose, and Lactate:** MRI confirmed that discs injected with ChABC exhibited progressive degeneration. Following equilibration, median baseline oxygen in healthy discs was 50.0 mmHg. In degenerate discs, median oxygen was significantly higher at 108.0 mmHg (Fig 2A). With respect to glucose and lactate, following equilibration, median baseline levels in healthy discs were 0.063 and 0.069 mmol/L, respectively. In degenerate discs at 12 weeks, median glucose and lactate levels were 0.104 and 0.883 mmol/L, respectively, with lactate significantly higher than baseline (Figs 2B and C). MicroCT revealed increased vertebral end plate porosity in all degenerate discs compared to healthy discs (Fig 3A). Histology demonstrated moderate to severe degeneration in ChABC-injected discs (Fig 3B).

DISCUSSION: Here we successfully optimized techniques, including probe placement, equilibration time, flow rate and detection method, for the *in situ* measurement of oxygen, glucose, and lactate within the disc NP, and applied these techniques to evaluate changes with disc degeneration in a preclinical large animal model. Notably, for glucose and lactate, collection flow rate impacted results, suggesting that lower flow rates are necessary for accurate measurements due to the low levels of these metabolites in the NP. Interestingly, while increased lactate with degeneration was expected, contrary to expectations [6] increased oxygen levels were also measured. Potential explanations could be reduced disc height (reducing nutrient diffusion distances), increased cell death (reducing overall metabolite consumption) or increased vertebral end plate porosity (enabling more ready diffusion of nutrients from the adjacent bone) in degenerate discs. Ongoing work is aimed at elucidating the microstructural and cellular contributions to altered metabolites using this model. Our ultimate goal is to leverage these insights in order to modify the disc microenvironment to enhance the efficacy of therapeutic stem cells.

SIGNIFICANCE: Physiologically accurate characterization of the degenerate disc metabolic microenvironment is essential for developing effective cell-based disc regeneration strategies.

REFERENCES: [1] Vos+ Lancet 2015; [2] Smith+ Dis Model Mech 2011; [3] Urban+ Arthritis Res Ther 2003; [4] Urban+ Spine 2004; [5] Gullbrand+ Osteoarthritis Cartilage 2017; [6] Huang+ Nat Rev Rheumatol 2014.

ACKNOWLEDGEMENTS: Funding from the National Institutes of Health (R01 AR077435) and the Department of Veterans Affairs (I01 RX001321).

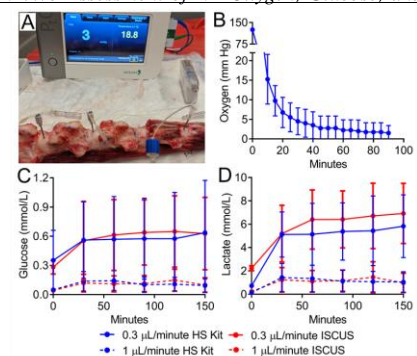


Figure 1. Experimental set up for *ex vivo* measurements. **A.** Oxygen levels in the NP over time. **C.** Glucose and **D.** Lactate levels over time measured using two different flow rates and analysis methods. N=3-4 discs; mean \pm SD.

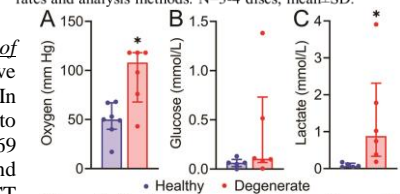


Figure 2. In *in vivo* measurement of: **A.** Oxygen **B.** Glucose and **C.** Lactate in the NPs of healthy and degenerate goat discs. N=5-7; *p<0.05; median/IQR.

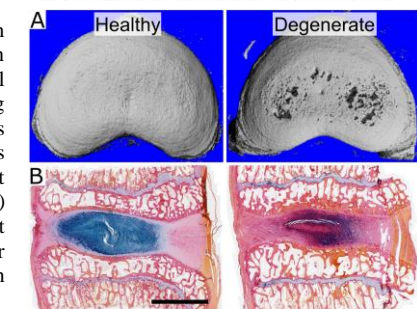


Figure 3. **A.** Representative axial microCT images showing increased porosity of a vertebral end plate adjacent to a degenerate disc. **B.** Representative histology of healthy and degenerate goat discs. Mid sagittal; Alcian blue/picrosirius red stain; scale=5mm.