Mechanisms of disc degeneration
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A whole organ culture model for intervertebral disc using rat tail explants in a rotating bioreactor
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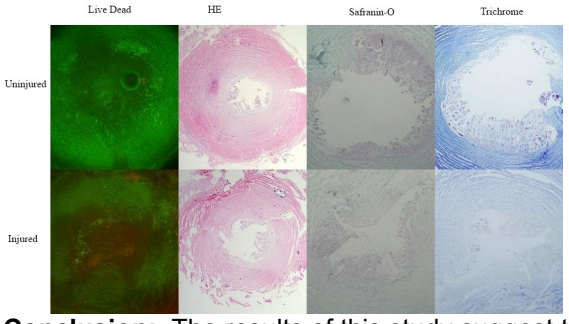
Introduction: Intervertebral disc (IVD) disorders resulting in pain and disability are extremely prevalent and current treatment options do not restore tissue integrity or function. Mechanisms of IVD degeneration are not fully understood. As such, in vitro culture models are being used to investigate these mechanisms. Models using cells deprived of extracellular matrix (ECM) result in loss of differentiation and viability (Haschtmann 2006) and limit the use of important outcome measures, such as biomechanics. While tissue culture of IVD explants allows for maintenance of cell morphology, preservation of ECM, and biomechanical testing, the biologic and biomechanical influences of endplate cartilage and bone are lost (Ariga 2003). For these reasons, our goal is to develop and validate a whole organ culture model. Ideally, this model will provide long-term maintenance of cell and tissue integrity, architecture, composition, viability, allow for clinically relevant assessment of IVD health and disease. To our knowledge, few studies on whole organ culture for the study of IVD disorders have been published and none have established a validated model addressing the optimal criteria outlined above. Therefore, the objective of this study was to investigate a novel methodology for IVD whole organ culture using biological and biomechanical assessments.
Materials and Methods: Under ACUC approval, tails were collected from mature Sprague Dawley rats (n=12) euthanatized for reasons unrelated to this study. Soft tissues were aseptically removed from the caudal vertebrae. Bone-disc-bone explants were prepared by sawing the proximal and distal vertebral bodies in half. Explants were randomly assigned to either the Injured group (n=20) in which the posterolateral annulus fibrosus (AF) was penetrated with a 20G needle to enter the nucleus pulposus (NP), which was aspirated with a 1 mL syringe to 0.5 mL, or the Uninjured group (n=22), which received no insult. Explants were cultured in a rotating bioreactor (Synthecon) at 50 rpm for 14 days in supplemented DMEM+ITS media. Culture media were changed on days 1 and 7. Cell viability in each IVD was determined using calcein-AM and ethidium homodimer-1 at day 0, 1, 7 and 14. IVDs were fixed and processed for histology and stained with hematoxylin eosin (HE), Safranin-O and Trichrome, and assessed by a pathologist, blinded to treatment, for cell and tissue morphology and architecture. Biomechanical testing of explants was performed at days 0 (after injury) and 14 by loading in axial compression at 0.001 mm/s. Stiffness was determined from the linear regions of the force-displacement curves.
Results: Uninjured AF and NP cells maintained high viability through day 14. Injured AF and NP cells showed high viability at day 1, but viability in injured AF and NP cells was notably lower at days 7 and 14. Histologically, uninjured IVDs maintained normal characteristics of cells and ECM through day 14. Uninjured IVDs retained more collagen in AF and proteoglycan in NP. Injured IVDs show histologic evidence of cell death corresponding to areas of loss of cell viability and loss of proteoglycan staining in NP and loss of collagen staining in AF. Stiffness for uninjured IVDs was similar at days 0 (80.8 ± 34.8 N/mm) and 14 (89.2 ± 23.1 N/mm), while stiffness of injured IVDs was lower than the uninjured group at day 0 immediately after injury (52.9 ± 1.7 N/mm) and then increased approximately 2.5 times by day 14 (130.8 ± 37.0 N/mm)
Image / Graph:

Figure (Day 14 of Culture) Representative Images Showing Cell Viability and Histologic Characteristics of IVDs on Day 14



Conclusion: The results of this study suggest that whole organ (bone-disc-bone) IVD explants can be cultured in a rotating bioreactor such that cell viability and tissue architecture in AF and NP are maintained for at least 14 days. The

injury used in this model produced pathologic changes in AF and NP similar to those seen in human IVD degeneration, including cell death, abnormal ECM, and increased stiffness over time. In light of previous studies that reported significant loss of cell viability using other culture models (Gawri 2011), we suggest that the use of the rotating bioreactor is critical for providing nutrient supply to IVD explants, thus preserving biologic and biomechanical properties. We were able to perform multiple outcome measures on explants to assess IVD physiology and pathology. Ongoing research in our laboratories is aimed at further optimizing this model to investigate mechanisms of disease and test therapeutic interventions.

References:

I confirm having declared any potential Conflict of Interest for ALL authors listed on this abstract: Yes

Disclosure of Interest: None Declared