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 result in restoration of tissue integrity or function. Mechanisms of IVD degeneration are not fully understood, although aging, injury, genetics, nutrition, metabolism and/or mechanical stress are suspected to be primary culprits. One method for investigating potential mechanisms of IVD degeneration is *in vitro* culture models using cells, single tissues, or whole organ methods. Models using cells deprived of extracellular matrix commonly result in cell dedifferentiation and/or loss of cell viability (Haschtmann, 2006). In addition, cell culture models do not allow for use of important outcome measures such as biomechanics. While tissue culture of IVD alone allows for maintenance of cell differentiation and biomechanical testing, the biologic and biomechanical influences of endplate cartilage and bone are lost (Ariga, 2003). For these reasons, establishing a validated whole organ culture model of IVD is desirable. Ideally, this model would provide for long term maintenance of cell and tissue integrity, architecture, composition, and viability, allow for assessment of both health and disease, and employ outcome measures that address biologic and biomechanical aspects of IVD physiology and pathology. To our knowledge, few studies on IVD whole organ culture 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 whole organ culture of IVD with initial assessments of biological and biomechanical aspects of health and disease.

MATERIALS AND METHODS

Under ACUC approval, the tails were collected from 12 skeletally mature Sprague Dawley rats (n=12) after they were euthanized for reasons unrelated to this study. Soft tissue were dissected and removed from the caudal vertebrae under aseptic conditions. Explants consisting of cranial body half, endplate, IVD, endplate, and caudal body half were harvested using a saw (Fig.1). The IVD whole organ explants were randomly assigned to one of two groups: **Injured** or **Uninjured**. For the injured group, the posteriolateral annulus fibrosis of each explant was penetrated with a 20G needle to enter the center of the nucleus pulposus, which was then aspirated with a 1mL syringe to 0.5mL (n=20). The uninjured group received no treatment (n=22). (Fig.1) For both groups, the explants were cultured in a rotating wall vessel bioreactor (Synthecon, Inc.) at 50 rpm for 14 days in Dulbecco's modified Eagle's medium supplemented with sodium pyruvate, L-glutamine, ascorbic acid, MEM N-E Amino Acid solution, ITS, penicillin-streptomycin. Culture media were changed on days 1 and 7 of culture.

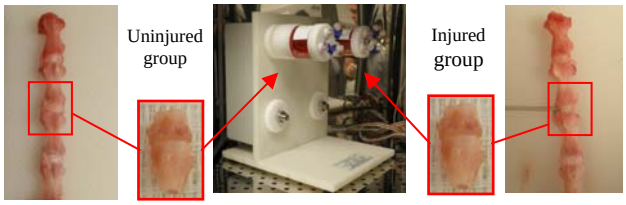

Fig. 1. Method of preparation, injury and culture of whole organ intervertebral disc explants

Cell viability in each IVD was examined by staining with calcein-AM and ethidium homodimer-1 (EthD-1) immediately after preparation (day 0), and at days 1, 7, and 14 of culture. IVD tissue was then fixed in 10% neutral buffered formalin for histologic processing. Sections were cut and stained with hematoxylin eosin (HE) and subjectively assessed by one pathologist for cell and tissue morphology and architecture. Biomechanical testing of IVD explants was performed day 0 (after injury) and day 14 by loading the tissue in axial compression at a rate of 0.001 mm/s. Stiffness of each IVD was determined from the linear regions of the force-displacement curves.

RESULTS

Annulus fibrosis (AF) and nucleus pulposus (NP) cells in the uninjured group maintained high viability through 14 days of culture. Viability of AF and NP cells was also high in the injured group at day 1 of culture. However, cell viability at the injury site in the AF and within the NP of the injured IVDs was markedly lower at days 7 and 14 of culture. (Fig 2) Based on subjective histologic assessment, uninjured IVDs maintained normal cell and tissue morphology and architecture through 14 days of culture, while injured IVDs showed areas of cell death corresponding to the loss of cell viability noted, as well as loss of extracellular matrix integrity and architecture by day 14 of culture (Fig 3).

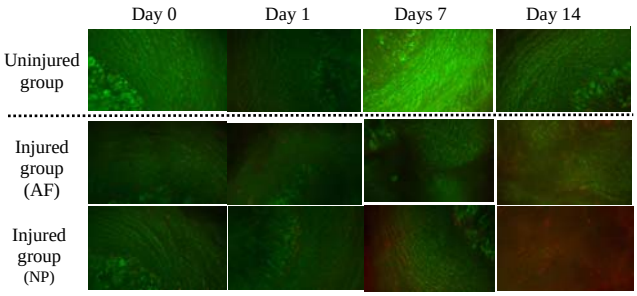

Fig. 2. Cell viability of the discs at 0, 1, 7 and 14 days of culture. Green and red showed live and dead cells, respectively.

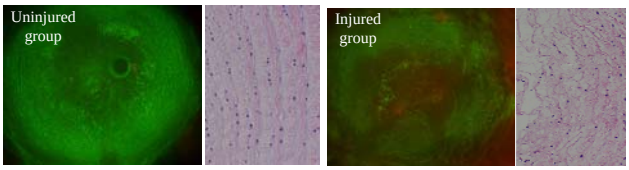

Fig. 3. Cell viability and histology of IVD explants at day 14 of culture

Stiffness values for uninjured IVDs were similar at days 0 and 14, while stiffness of injured IVDs was lower than the uninjured group at day 0 (immediately after injury) (Table). The stiffness of the injured group was approximately 2.5 times higher at day 14 than day 0.

Table. Stiffness of IVD explants at days 0 and 14 of culture

Group	Day 0	Day 14
Uninjured	80.8 ± 34.8 N/mm	89.2 ± 23.1 N/mm
Injured	52.9 ± 1.7 N/mm	130.8 ± 37.0 N/mm

DISCUSSION

The results of this study suggest that whole organ IVD explants can be successfully cultured in a rotating bioreactor to maintain cell viability and tissue architecture in both annulus fibrosis and nucleus pulposus for at least 14 days. In addition, the injury we used in this model produced pathologic changes in the annulus fibrosis and nucleus pulposus similar to those seen in degenerative IVD disease in people, including cell death, abnormal extracellular matrix, and increased stiffness. In light of previous studies that reported significant loss of cell viability using other IVD explants culture models (Gawri 2011), we suggest that the use of the rotating bioreactor is critical for providing nutrient supply to cultured IVD explants, thus maintaining viability, tissue composition and architecture, and material properties. We were able to perform multiple outcome measures on IVD explants to assess biologic and biomechanical aspects of IVD physiology and pathology.

SIGNIFICANCE

This novel model has high potential for providing important translational data for delineating disease mechanisms and developing therapeutic strategies for treatment of intervertebral disc disease in people.

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