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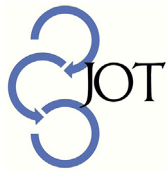

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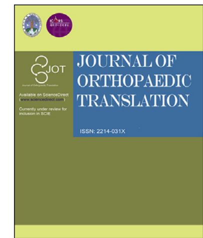

ORIGINAL ARTICLE

Development of a whole organ culture model for intervertebral disc disease

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Summary *Background/Objective:* Whole organ *in vitro* intervertebral disc models have been associated with poor maintenance of cell viability. No previous studies have used a rotating wall vessel bioreactor for intervertebral disc explants culture. The purpose of this study was to develop and validate an *in vitro* model for the assessment of biological and biomechanical measures of intervertebral disc health and disease.
Methods: To this end, endplate-intervertebral disc-endplate whole organ explants were harvested from the tails of rats. For the injured group, the annulus fibrosus was penetrated with a 20G needle to the nucleus pulposus and aspirated. Explants were cultured in a rotating wall vessel bioreactor for 14 days.
Results: Cell viability and histologic assessments were performed at Day 0, Day 1, Day 7, and Day 14. Compressive mechanical properties of the intervertebral disc were assessed at Day 0 and Day 14. In the annulus fibrosus and nucleus pulposus cells, the uninjured group maintained high viability through 14 days of culture, whereas cell viability in annulus fibrosus and nucleus pulposus of the injured intervertebral discs was markedly lower at Day 7 and Day 14. Histologically, the uninjured intervertebral discs maintained cell viability and tissue morphology and architecture through 14 days, whereas the injured intervertebral discs showed areas of cell death, loss of extracellular matrix integrity, and architecture by Day 14. Stiffness values for uninjured intervertebral discs were similar at Day 0 and Day 14, whereas the stiffness for the injured intervertebral discs was approximately 2.5 times greater at Day 14.
Conclusion: These results suggest that whole organ intervertebral discs explants can be successfully cultured in a rotating wall vessel bioreactor to maintain cell viability and tissue architecture in both annulus fibrosus and nucleus pulposus for at least 14 days. In addition, the

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injury used produced pathologic changes consistent with those seen in degenerative intervertebral disc disease in humans. This model will permit further study into potential future treatments and other mechanisms of addressing intervertebral disc disease.
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Introduction

Intervertebral disc (IVD) disorders (desiccation, degeneration, herniation, etc.) have been directly associated with low back pain and disability [1]. Current treatment options for IVD disorders do not restore normal tissue integrity or function. The direct medical and indirect costs of these conditions are unknown, but have been estimated in the range of $50 to $100 billion per annum, placing an economic burden on society [2–4]. Thus, the development of prevention strategies for disc degeneration and repair/regeneration options for pathologic tissues would seem to be of great value. Unfortunately, mechanisms of disc degeneration are not fully understood, although aging, injury, genetics, nutrition, metabolism, and mechanical stress are all suspected to be significant factors [4–6].

In vitro culture models provide a controlled method for investigating mechanisms of disc degeneration and can be performed using cells, single tissues, or whole organs. Models using cells alone allow for the control of certain variables and are typically less complex and expensive to employ than other options [5]. However, monolayer cell culture models deprived of extracellular matrix (ECM) commonly result in rapid cell dedifferentiation and/or loss of cell viability [7]. In addition, these cell-alone culture models call into question their validity for assessment of clinically relevant outcome measures such as biomechanics and morphological integrity. Tissue cultures of IVDs without the adjacent endplates allow for better maintenance of cell distribution and differentiation, ECM integrity, and material properties, but the biologic and biomechanical influences of endplate cartilage and vertebral bone are lost, and the nucleus pulposus (NP) is allowed to freely swell in culture [8,9]. Based on these limitations, establishing a valid whole organ culture model of IVD is desirable. Ideally, this model would provide long-term maintenance of cell and tissue integrity, architecture, composition, and cell viability; allowing for the assessment of biological and biomechanical aspects of disc physiology and pathology.

In the last decade, there has been growing interest in using organ culture models to investigate the effects of injury, degeneration, or repair [10,11]. Once validated, these *ex vivo* models could provide an excellent method for evaluating disease mechanisms and therapeutic strategies regarding the disc's biological and mechanical functions in a controlled culture environment. Early studies demonstrated that culturing the intervertebral disc *ex vivo* with the vertebral bodies attached resulted in a decrease in the amount and distribution of living cells due to limited nutrient diffusion [5,7,9]. Some models employ the removal of the endplates to maintain cell viability [7,12]; however, the endplate is crucial for constraining the NP, which has extreme swelling capabilities, and for maintaining the *in situ* nutrient diffusion pathways [13]. Additionally, many of these studies have been limited to histological and gene expression analyses, excluding the critical mechanical functions of the disc. Therefore, it is important for a validated organ culture model to maintain cell viability, biochemical, and mechanical properties.

Translational research using animal models for eventual clinical application of *in vitro* studies will be a necessary step for this work. Unfortunately, there is no ideal animal model for the study of human disc degeneration [14]. Small animal models are beneficial for studying genetic alterations and providing a cost effective option for mechanistic research. Large animal models allow for more clinically relevant interventions and outcomes measures, but are more costly and labour intensive. Previous animal model studies using needle puncture to damage the disc have been successful in causing reproducible degenerative-like changes over time, including a decrease in disc height, water content, and glycosaminoglycan content [15]. Developing an *in vitro* model based on these successful *in vivo* degeneration models would provide an efficient, cost-effective method for initial translational research prior to performing experiments requiring the use of animals.

The aims of this study were to: (1) develop a whole organ IVD culture model that would allow for maintenance of cell viability, ECM integrity, and mechanical function for a relevant time period; and (2) produce degenerative-like changes in the disc *in vitro*. To the authors' knowledge, there have been no reports on whole organ IVD culture using a rotating wall vessel (RWV) bioreactor. This study employed the use of a RWV bioreactor as a novel approach for development and initial validation of a whole – organ IVD culture system, with or without nucleotomy, for the study of biological and biomechanical aspects of disc health and disease. We hypothesized that: (1) cell viability, ECM integrity, and compressive material properties of discs could be maintained for 14 days in whole organ IVD culture using a RWV bioreactor at levels not significantly different than at time of harvest; and (2) that needle-puncture nucleotomy and NP aspiration would be associated with significant loss of cell viability, ECM composition and architecture, and compressive material properties over 14 days in whole organ IVD culture using a RWV bioreactor.

Materials and methods

Preparation of IVD explants

Under Animal Care and Use Committee approval, tails were collected from 12 skeletally mature Sprague-Dawley rats after they were euthanised for reasons unrelated to this study. The muscles and tendons were dissected and

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