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Development and validation of a bioreactor system for dynamic loading and mechanical characterization of whole human intervertebral discs in organ culture

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ABSTRACT

Intervertebral disc (IVD) degeneration is a common cause of back pain, and attempts to develop therapies are frustrated by lack of model systems that mimic the human condition. Human IVD organ culture models can address this gap, yet current models are limited since vertebral endplates are removed to maintain cell viability, physiological loading is not applied, and mechanical behaviors are not measured. This study aimed to (i) establish a method for isolating human IVDs from autopsy with intact vertebral endplates, and (ii) develop and validate an organ culture loading system for human or bovine IVDs. Human IVDs with intact endplates were isolated from cadavers within 48 h of death and cultured for up to 21 days. IVDs remained viable with ~80% cell viability in nucleus and annulus regions. A dynamic loading system was designed and built with the capacity to culture 9 bovine or 6 human IVDs simultaneously while applying simulated physiologic loads (maximum force: 4 kN) and measuring IVD mechanical behaviors. The loading system accurately applied dynamic loading regimes (RMS error <2.5 N and total harmonic distortion $<2.45\%$), and precisely evaluated mechanical behavior of rubber and bovine IVDs. Bovine IVDs maintained their mechanical behavior and retained $>85\%$ viable cells throughout the 3 week culture period. This organ culture loading system can closely mimic physiological conditions and be used to investigate response of living human and bovine IVDs to mechanical and chemical challenges and to screen therapeutic repair techniques.

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1. Introduction

Low back pain is the leading cause of disability worldwide with huge socioeconomic costs that total more than $100 billion annually in the United States alone (Katz, 2006; Vos et al., 2012). Intervertebral disc (IVD) degeneration is considered one of the key factors related to the development of low back pain (Andersson, 1999; Samartzis et al., 2013). Multiple attempts to treat IVD degeneration, such as injectable biologics or implants, have shown promise in animal models but have limited clinical success. Lack of an appropriate model for human disc disease remains a major obstacle for developing more effective treatments. Therefore, the overall goal of this study is to develop and validate a system in which human and large animal IVDs can be obtained, placed into culture, and maintained under physiological conditions. Utilizing such a system to screen potential therapies for IVD degeneration would accelerate the translation of repair therapies from "bench to bedside".

Organ culture is a technique whereby whole IVDs are removed from the adjoining vertebrae and cultured 'ex-vivo' for varying durations. IVD organ culture models retain the natural complexities (slow transport phenomenon, cell/matrix interactions) of the native microenvironment while allowing high control over the mechanical and chemical boundary conditions. The use of human IVDs provides a clinically relevant model that incorporates both age and disease related changes that accumulate over a lifetime. Prior studies have demonstrated the feasibility of whole human IVD organ culture; however, such models require removal of the vertebral endplates in order to prevent cell death (Gawri et al., 2011). The removal of endplates limits the scope of therapies that can be evaluated as it prevents the application of load and assessment of mechanical function. Multiple large animal models have shown that it is possible to retain the vertebral endplates and still maintain disc viability (Gantenbein et al., 2006; Junger et al., 2009). Therefore we investigated the feasibility of isolating human IVDs with intact vertebral endplates from autopsy, using techniques modified from large animal organ culture systems.

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The human spine normally experiences loads that are both large in magnitude and dynamic in nature during activities of daily life. The compressive forces that the IVD experiences have been estimated to be up to ~2500 N during dynamic lifting (Marras et al., 2001) and intradiscal pressures approach 1 MPa during normal daily activities such as climbing stairs (0.5–0.7 MPa) or jogging (0.35–0.95 MPa) (Wilke et al., 2003). The forces that the IVD experiences are expected to be higher under more extreme conditions. These high loads that develop during different activities emphasize the high magnitude of forces that are required in order to simulate physiologic conditions. Dynamic loading is also a critical component of any physiologic model as it plays an important role (i) in stimulating biosynthesis (Chan et al., 2011; Korecki et al., 2008; MacLean et al., 2004) and (ii) promoting molecular transport within the IVD (Huang and Gu, 2008; Urban et al., 2004). Therefore, culture systems must be able to dynamically apply high forces in order to simulate 'physiologic' loading that arises during normal activities. Multiple bioreactor systems have been designed for whole IVD organ culture (Chan et al., 2013; Haglund et al., 2011; Junger et al., 2009; Paul et al., 2012); however none have demonstrated the ability to apply the large forces that are required to simulate physiologic stresses commonly experienced by human IVDs.

For many therapies, the primary measure of success is the restoration of IVD height and mechanical function. Mechanical behaviors are also known to be sensitive to different types of injuries (Iatridis et al., 2013). Therefore, the assessment of mechanical function can provide an important tool to evaluate and compare repair strategies. Many emerging therapies aim to repair the IVDs structure using tissue engineering or biomaterial strategies which can involve biodegradable materials. Such materials require ongoing measurements of mechanical performance throughout the repair process as they integrate/interact with the native tissue. It is often challenging, or impossible, to assess how these treatments affect the mechanical behaviors of IVDs over time in in-vivo systems, or other model systems that retain the in-situ environment. Of the bioreactor systems available, only one has assessed mechanical properties during culture (Paul et al., 2012) and none are able to simulate the loads typically experienced by human IVDs. Therefore the development of a loading system that can assess changes in the IVDs mechanical function is a research priority.

The objectives of this study were to (1) verify the feasibility of using whole human IVDs with intact vertebral endplates for organ culture experiments and (2) to develop and validate a dynamic loading system capable of loading human and large animal IVDs which can also assess the mechanical properties of the IVD throughout culture. The design goals for this bioreactor system were that the system must be able to (i) maintain viable cultures, (ii) fit within an incubator, (iii) apply simulated physiologic loads on human or bovine IVDs (Table 1), and (iv) assess mechanical behaviors and IVD height throughout culture. The validation of the mechanical performance of the dynamic loading organ culture system was performed using bovine caudal IVDs since they are readily available and approach the size of human IVDs.

Table 1
Loading system force requirements for bovine and human IVDs.

	Average force required for given stress				
	0.2 MPa	0.6 MPa	0.8 MPa	1 MPa	2 MPa
Bovine caudal[a]	101 *N*	303 *N*	403 *N*	504 *N*	1008 *N*
Human lumbar[b]	366 *N*	1097 *N*	1462 *N*	1828 *N*	3655 *N*

[a] Bovine average area (504 mm^2) $N=23$ IVDs.
[b] Human average area (1828 mm^2) $N=14$ IVDs.

2. Methods

2.1. Organ culture set-up and culture conditions

Human and bovine IVDs were subject to similar cleaning and culturing processes, although additional safety precautions were taken for human specimens. Whole IVD organs (human and bovine) were isolated with intact vertebral endplates using a histologic band saw (Exakt 310, Exakt, Norderstedt, Germany), as previously described (Gantenbein et al., 2006; Illien-Junger et al., 2013). Following isolation, endplates were cleaned with a wound debridement system (Pulsavac®, Zimmer, Warsaw, IN) to remove potential blood clots. IVDs were then briefly rinsed (~10–15 s) with 70% ethanol and soaked in washing solution (3% penicillin/streptomycin and 1.5% fungizone in PBS) for 10 min. IVDs were then placed into culture at 37 °C and 5% CO_2. Culture media consisted of high glucose Dulbecco's Modified Eagle Medium, 10% fetal bovine serum, 50 μg/mL ascorbic acid, 1% penicillin/streptomycin, 0.5% fungizone (Fisher-Scientific, Waltham, MA), and 1:500 primocin (Invivogen, San Diego, CA). All reagents were obtained from Invitrogen (Carlsbad, CA) unless otherwise noted.

2.1.1. Human organ culture

Permission was obtained for use of cadaveric material prior to specimen procurement. All spinal segments were graded via a previously established radiographic scoring system (Anterior–Posterior x-rays; Fig. 1A) which correlates with the Thompson morphologic grading scheme (Benneker et al., 2005; Thompson et al., 1990; Wilke et al., 2006). Highly degenerated IVDs (Thompson grade 5) were excluded from culturing, as these are not relevant to biologic treatments. To ensure that the spinal segment obtained from autopsy services was viable prior to the organ culture experiment, an excess piece of IVD tissue was checked for cell viability. Excess tissue consisted of either an adjacent IVD not used for organ culture or partial IVD tissue remaining at the superior or inferior end of the spinal segment. IVD tissue was placed into media containing thiazolyl blue tetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, MO) at 1 mg/mL and incubated during the extraction process (Walter et al., 2011). If the tissue was not visibly blue (viable) by the end of the extraction process the cultures were not continued.

Four human lumbar IVD organs were isolated from three human spinal segments within 48 h after death (Table 2). Isolated IVDs were placed into a custom human organ culture chamber and cultured for 7, 14 or 21 days (Table 2) under either (i) diurnal loading (0.1 MPa/0.2 MPa) with weights changed every 12 h or (ii) static loading (0.2 MPa). The human organ culture chambers were designed and built to be identical in function to previously described bovine caudal IVD chambers (Lee et al., 2006; Oshima et al., 1993) but large enough to accommodate human lumbar IVDs up to 88 mm in diameter (Fig. 1B). The lower chamber has an inner diameter of 94 mm, height of 70 mm, and were fabricated from polyetherimide (Ultem®, Advanced Industrial, Cornelius, NC) which is autoclavable, easy to machine, and translucent for viewing media levels. Ultem® has a compressive modulus (ASTM D695) of ~3.3 GPa which enabled mechanical loading with minimal deflections. Chambers also contain porous metal platens (Mott Corporation, Farmington, CT) that served as a permeable interface between the chamber and the vertebral endplates of the IVD organ culture. The culture medium was changed every three to four days.

2.1.2. Bovine organ culture (system validation)

Twelve bovine caudal IVDs were harvested from six bovine tails obtained from a local abattoir (Green Village Packing Co., Green Village, NJ). Bovine culture chambers were newly fabricated from Ultem® and are nearly identical to those previously described (Lee et al., 2006; Oshima et al., 1993) with an inner diameter of 47 mm and height of 90 mm. IVDs were cultured for either 6 ($N=6$) or 20 days ($N=6$) under simulated physiologic loading modified from a previous study (Junger et al., 2009). Loading consisted of diurnal loading (8 h: 0.2 MPa/16 h: 0.6 MPa) with 2 five-hour bouts of 'exercise' (0.6 ± 0.2 MPa @ 0.1 Hz) during the daytime cycle. All cultures were held at 0.2 MPa static compression until the start of the simulated physiologic loading. In order to have uniform durations of dynamic loading, the simulated physiologic loading began at 9AM on the day following organ culture set up which involved slight differences in timing of dissection, isolation, and preparation of each IVD. The culture medium was changed on days 4, 6, 10, 14, and 18.

2.2. Viability

Tissue viability was assessed on both human and bovine IVDs as previously described (Walter et al., 2011). Briefly, tissue was double stained with MTT to stain viable cells, and 4′,6-diamidino-2-phenylindole (DAPI, Roche Diagnostics, Germany) to stain cell nuclei. Three 10 μm thick sections were taken from each section and photographed at 10× or 20×. Percent viability (Dual stained cells

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