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Multi-unit sustained vibration loading platform for biological tissues: Design, validation and experimentation

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ABSTRACT

The relationships between mechanical inputs and resulting biological tissue structure, composition, and metabolism are critical to detailing the nuances of tissue mechanobiology in both healthy and injured tissues. Developing a model system to test the mechanobiology of tissues ex-vivo is a complex task, as controlling chemical and mechanical boundary layers in-vitro are difficult to replicate. A novel multi-unit vibration loading platform for intervertebral discs was designed and validated with both independent electronic data and experimental loading of 6 bovine intervertebral discs (IVDs) and an equal number of unloaded controls. Sustained vibration was applied using closed-loop positional control of pushrods within four independent bioreactors with circulating phosphate buffered saline. The bioreactors were designed to be modular with removable components allowing for easy cleaning and replacement. The loading regime was chosen to maximize target mRNA expression as reported in previous research. Aggrecan, decorin, and versican mRNA all reported statistically significant increases above control levels. Biglycan, collagen type I and II showed no significant difference from the control group. Further study is required to determine the resulting effect of increased mRNA expressions on long-term disc health. However these results indicate that this research is past the proof of concept stage, supporting future studies of mechanobiology utilizing this new device. The next stage in developing this novel loading platform should consider modifying the tissue grips to explore the effects of different directional loading on different gene expression, and also loading different types of tissues.

1. Introduction

Degeneration of intervertebral discs (IVDs) is a common cause of lower back pain, which is a significant health problem in today's society. The relationship between mechanical inputs and IVD structure, composition, and metabolism are critical to detailing the nuances of disc mechanobiology in healthy, diseased, or injured IVDs. Developing a model system to test the mechanobiology of IVDs ex-vivo is a complex task, as the natural chemical and mechanical boundary layers are difficult to replicate in-vitro. Further complexity arises from structural differences of the annulus fibroses and the nucleus pulposes of the IVD. Bovine discs have been considered a prime candidate for IVD mechanobiology studies due to their large size and similar resting pressure (0.2–0.3 MPa), aspect ratio, composition, hydration, collagen profile, proteoglycan profile, and similar rate of proteoglycan synthesis to human discs (Demers et al., 2004; Oshima et al., 1993). Additionally, coccygeal bovine discs are inexpensive, can be quickly obtained, and are easier to extract than lumbar discs. An important difference between bovine and human discs is that a subpopulation of notochordal-like cells remains in the bovine disc (Gilson et al., 2010). Notochordal cells affect cell matrix production, which is an important factor in cell therapies aimed at increasing activity in the nucleus pulposes. Previous research has shown that different loading regimes (Korecki et al., 2007) and limited nutrition (Jünger et al., 2009) also have a significant effect on overall disc degeneration. Thus maintaining a controlled culture medium during IVD loading protocol is necessary to fully understand the mechanobiology of IVDs.

Previous biomechanical studies reported increased expressions of mRNA in healthy IVDs in response to mechanical vibrations (Desmoulin et al., 2010; 2011a). These experiments determined an optimal window for bovine IVDs of 16–80 Hz frequency, 40 N tare load, and 10 min duration of vibration, which maximized specific gene expression. The motivation for this research was to investigate an existing intervention known as

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the Khan Kinetic Treatment (KKT), which aims to accelerate soft tissue healing non-invasively in this manner. However, research studying the effects of vibrations upon IVD tissue is ongoing. Different studies have reported increased risk of disc degeneration (Jensen et al., 2008), no effect (Kumar et al., 1999), analgesic effect (Desmoulin et al., 2007), or even positive effects (Desmoulin et al., 2010) all due to applied vibrations. Thus, the effect of different vibration parameters on cell and tissue scale processes warrants further investigation (Hill et al., 2009).

The testing apparatus used to show increased mRNA expression (Desmoulin et al., 2010, 2011a) was a simplified bioreactor that did not consider the benefits of circulating culture medium, and was controlled with an open loop loading protocol. For this study, a fully automated device was designed to improve accuracy and efficiency of experiments by loading four discs simultaneously, in constantly circulating culture medium, while capable of different loading regimes, and a higher degree of numerical accuracy with closed loop control. Improving experimental controls such as temperature, air quality and culture medium were also added to preserve the cellularity of ex vivo tissue, in order to produce what we believe to be more accurate results. The system was designed to maximize messenger RNA expression within tissues treated with sustained axial vibration loading. The efficacy of the system was validated by comparing independent experimental data to previous studies showing vibration loading 16-80 Hz positively affects mRNA expression in bovine nucleus pulposi (Desmoulin et al., 2010, 2011a). This paper summarizes the design, validation, and experimental results, technical advantages, and research limitations of this novel vibration loading platform.

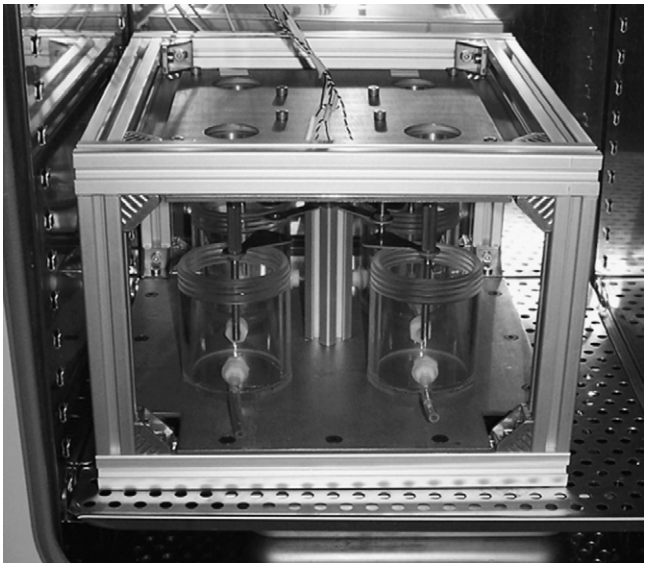

Fig. 1. Vibration loading platform with all four bioreactors inside a standard incubator to ensure in-vivo temperature and CO^2 levels.

2. Materials and methods

2.1. Bioreactor device design overview and validation

The novel test device used a quadruple bioreactor system that circulates culture medium between the four chambers, each of which contains a vibrating pushrod that loads the discs. The 1.5 L of phosphate buffered saline medium was circulated at ~0.05 L/min using tubing that connects all four chambers and a pump (Master Flex ♯HV-07575-10, Cole-Palmer, Montreal), insuring equal quality of culture medium surrounding each sample. The device was designed to fit within a standard cell culture incubator ($470 \times 450 \times 470\ mm^3$) (Fig. 1), and the medium circulation rate, constant temperature (37 C), and constant environmental CO_2 levels (5%) within the incubator were chosen to best represent in-vivo conditions (Kofoed and Levander, 1987). The bioreactors were designed as modular, removable components allowing for easy separation from the device for cleaning and replacement. The stainless steel frame and polymethylpentene containers were autoclavable, non absorbing, and chemically inert to the tissue samples and culture medium.

2.2. Controller design

The push rods loading the IVDs were actuated by voice coils (40 N tare load prototype, Crowson Technology, LLC, Carpinteria, USA) capable of 10 N of dynamic loading. The mounted voice coils were powered by Linear Current Amplifier Modules (LCAM-1, Quanser, Markham, ON) controlled by a custom Labview program. The LCAM was powered by a 27 V source, and cooled by a 7.06 CFM fan (♯2412PS-12W-B30, NMB-MAT, China) to eliminate temperature fluctuation of the output. Vertical translation of the push rod was measured with eddy current proximity probes from a 10 mm Rotor kit (♯126376-01, Bently Nevada, USA), which measured small displacements of the pushrod at a high-speed sampling rate of 1000 Hz. The sensors measured displacement of steel projections attached to the pushrod, rather than the coils themselves to avoid electrical interference from the coil's magnetic field (Fig. 2A). Probes were individually calibrated by using a device that measured the analog output over a distance of 1–10 mm (Fig. 2B). Labview Full Development System (version 9.0.1, National Instruments, USA) was used to calculate the compression applied to IVDs by converting analog output from the probes into displacement using calibration data. The National Instruments Compact DAQ Input Module (NI 9215, National Instruments, USA) and Labview software were validated for software bias using a PC Oscilloscope (PicoScope 2203, Pico Technology, UK) and PicoScope software (version 5.19.1, Pico Technology, UK). Sensor feedback data was measured over a range of output frequencies and amplitudes for Labview and Picoscope software separately (Table 1). There was a maximum 5.4% difference in measured amplitude voltage (0.018 mm), and identical frequency readings between Labview and Picoscope software at maximum output.

The Labview proportional-integral-derivative control virtual interface (PID.vi) was used to create closed-loop PID control of the coil's amplitude. Feedback data from proximity sensors was used by the Single Tone Extractor VI to determine amplitude and frequency of a generated waveform that optimally fits the sampled data. The frequency remained in open loop control due to its high stability and on-screen display. The PID parameters were obtained experimentally to prevent any

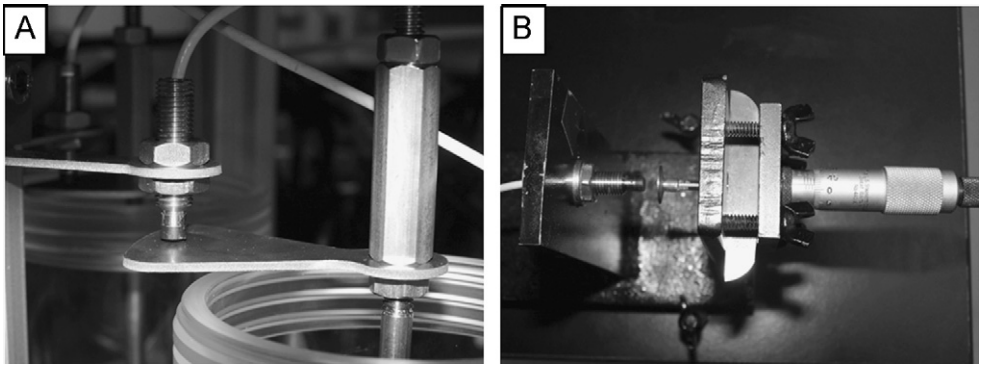

Fig. 2. Proximity probe measures displacement of steel projection attached to the pushrod entering the top of the bioreactor (A) using calibration data from proximity calibration device (B).