Journal of Biomechanics 46 (2013) 116–121

Contents lists available at SciVerse ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

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

Geoffrey T. Desmoulin [a,b,c,*], William S. Enns-Bray [b,c,d], Carol R. Hewitt [d], Christopher J. Hunter [b,d,e]

[a] Optima Health Solutions International Corporation, Vancouver, BC, (KKT International), Canada
[b] Department of Mechanical and Manufacturing Engineering, Schulich School of Engineering, University of Calgary, Calgary, AB, Canada
[c] GTD Engineering Inc., Vancouver, BC, Canada
[d] McCaig Institute for Bone and Joint Health, University of Calgary, Calgary, AB, Canada
[e] Centre for Bioengineering Research and Education, University of Calgary, Calgary, AB, Canada

ARTICLE INFO

Article history:
Accepted 21 October 2012

Keywords:
Vibration
Bioreactors
Intervertebral discs
Mechanotransduction
Tissue mechanics
Biomedical engineering

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

* Corresponding author at: Department of Mechanical and Manufacturing Engineering, University of Calgary, 2500 University Drive NW, Calgary, AB, Canada T2N 1N4. Tel.: +1 604 842 4831; fax: +1 403 282 8406.
E-mail address: gtdesmoulin@gtdengineering.com (G.T. Desmoulin).

0021-9290/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jbiomech.2012.10.018

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.

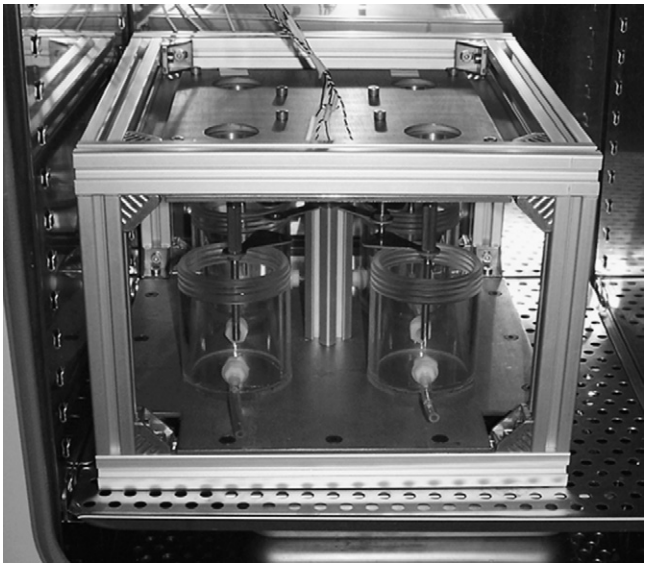

Fig. 1. Vibration loading platform with all four bioreactors inside a standard incubator to ensure in-vivo temperature and CO^2 levels.

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

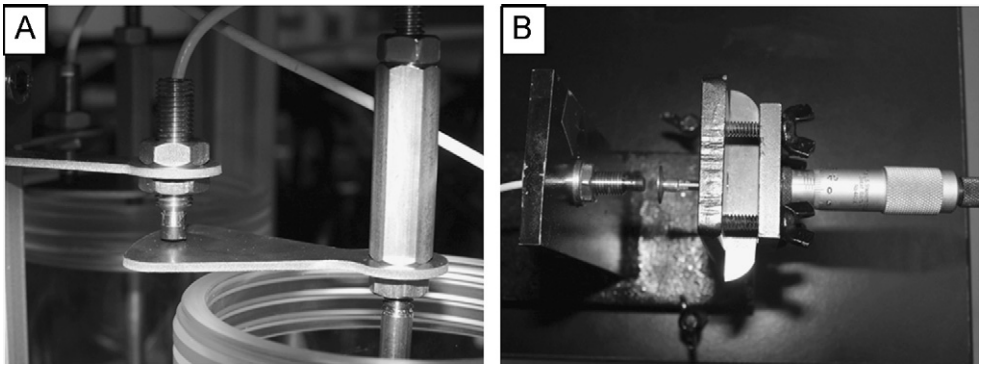

Fig. 2. Proximity probe measures displacement of steel projection attached to the pushrod entering the top of the bioreactor (A) using calibration data from proximity calibration device (B).

Table 1
Software validation results.

Output freq (Hz)	cDAQ output (V)	Labview freq (Hz)	Picoscope freq (Hz)	Labview amp (mV)	Picoscope amp (mV)	%Difference
25	0.75	25	25	480	456.5	5.2
25	1	25	25	650	616.5	5.4
25	1.25	25	25	833	802.5	3.8
50	0.5	50	50	321	306.5	4.7
50	0.75	50	50	491	479	2.5
50	1	50	50	672	647.5	3.8
50	1.25	50	50	862	855.5	0.8
100	0.5	100	100	404	412.5	−2.1

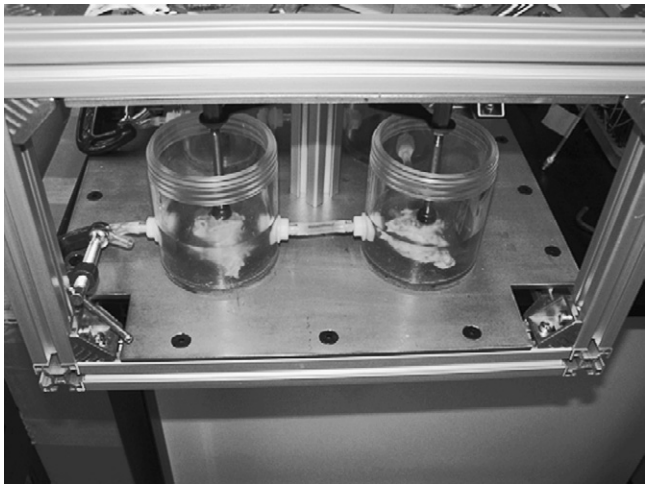

Fig. 3. IVDs within bioreactors, with PBS culture medium, ready for loading.

overshoot, while reaching 95% of desired amplitude in fewer than 10 s. The frequency and amplitude automatically changed at desired time intervals to allow automatic management of multiple loading schemes. The existing Khan Kinetic Treatment (KKT) uses higher frequency vibrations (> 16 Hz) to improve neck pain and mobility (Desmoulin et al., 2007, 2012). This loading scheme uses vibrations frequencies distinctly higher than loading experienced by during daily activities such as walking or running, which produce low frequency loads on the spine (< 10 Hz) (Schmidt et al., 2010). However other tissues may respond to a different window of vibration parameters, thus the device was designed to load tissue in a full 0–200 Hz range of frequencies for maximum capabilities.

2.3. Tissue

All procedures were approved by the University of Calgary's Animal Care Committee. Bovine tails from skeletally mature cattle were obtained from a local slaughterhouse within 6 h of death. The muscle, fat, fascia, and vertebrae remained intact when shipping, though the abattoir removed the skin. Prior to testing, the experimenter removed all musculature and fascia and two thin slices of the connecting proximal and distal vertebrae remained on either side of each dissected disc to provide grip to the push-rods and holders within the bioreactors (Fig. 3).

2.4. Experimental protocol

To demonstrate the devices efficacy, identical tissue preparation, displacement, frequency, loading durations, and reverse transcription polymerase chain reaction (Rt-PCR) were used as in the study by Desmoulin et al. (2011). IVD samples were compressed on average by 0.6 mm peak-to-peak compression at 16 Hz for the first 5 min, and 65 Hz for the next 5 min. Control discs were dissected and suspended in the bioreactors without loading for the same duration of time, and then snap-frozen identically to loaded discs, in a randomized order to eliminate time-dependencies. All bovine samples used in this experiment were acquired, extracted, loaded, dissected, and frozen in one day, to minimize morphological changes and time-dependencies in IVD tissue after death. All conditions were run on a minimum of 6 separate discs, from at least six different tails. The goal of this loading regime was not to simulate normal loading, but instead to yield maximum expression of target mRNA to provide supporting

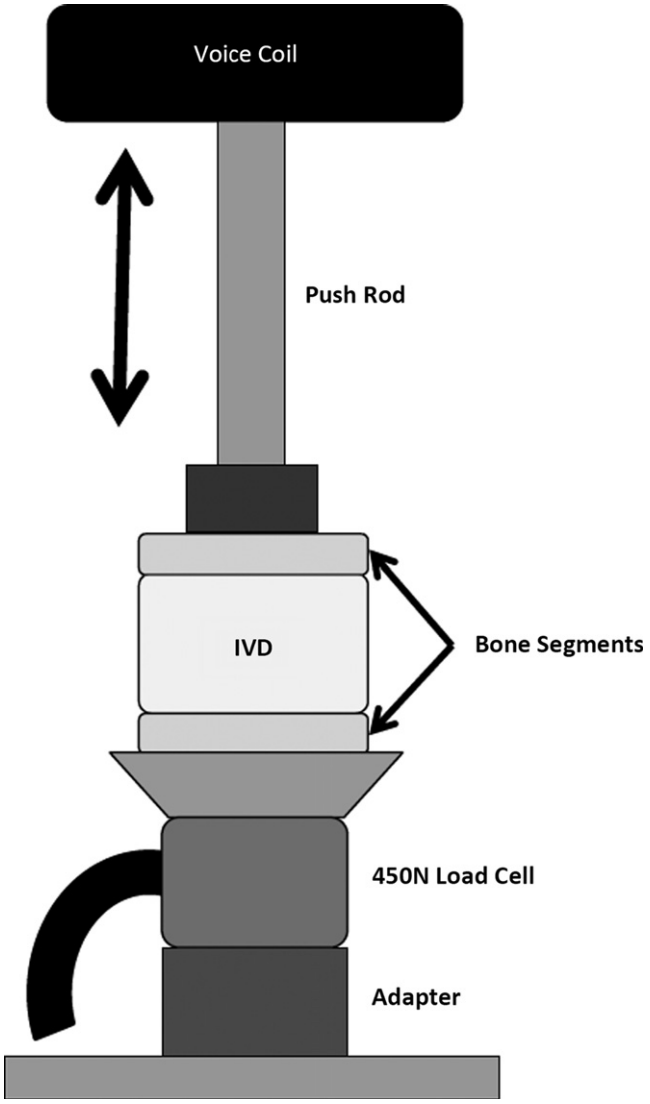

Fig. 4. Schematic of IVD compression, with load cell inserted to measure the loading profile.

evidence that optimal vibration parameters affect cellular activity in the tissue, which could possibly translate to future treatment. The effect of the experimental loading regime was compared to unloaded tissues held in the bioreactor under the same conditions.

The loading force of the device was measured without laboratory controls, and samples were tested immediately after dissection to compensate for the removed bioreactors and culture medium. A 450 N load cell (Electroforce 3200, Bose, USA) was inserted underneath the IVD sample and fixed to the base of the vibration platform with an adapter (Fig. 4). The forces generated by the voice coil were transmitted through the push rod and IVD, and were detected by the load cell at a sampling rate of 700 Hz. Three different bovine discs were tested with a mean

diameter of 25 mm. The average initial tare load for these samples was 50 ± 3.5 N, which is approximately 20% of the pressure experienced by human lumbar discs during relaxed standing (Wilke et al., 1999). Loading profiles were collected at maximum output from voice coils at low and high frequency output (Fig. 5). When loading frequency was increased, the peak to peak amplitude of the loading profile decreased from approximately 4 N to less than 1 N. Over time the discs exhibited stress relaxation under fixed compression from the push rods, resulting in an average final load of 41 ± 0.9 N, or an average decrease of approximately 9 N of compressive force. This stress relaxation was assumed to be present in all experimental discs, however no experimental control was established to prevent stress relaxation from occurring.

2.5. Rt-PCR

After the treatment period, the discs were harvested from the vertebral sections laying on either side, divided into nucleus pulposus (NP) and annulus fibrosus (AF), flash-frozen in liquid nitrogen, and stored at −80 °C until extraction of total RNA. All discs were visually inspected at the time of RNA harvest and found to be approximately equal to a human Thompson Grade II disc (opaque fibrous nucleus, clear nuclear/annular demarcation, and distinct lamellae). Only the NP was analyzed for the current study; AF samples were stored for future testing, as pilot studies indicated minimal changes in the AF (data not shown). The frozen tissue was ground in Trizol reagent; full details of the protocol are provided elsewhere (Reno et al., 1997). Briefly, total RNA was isolated using the Trispin method and quantified using the Ribogreen assay (Invitrogen, USA). A sample containing 1 μg of RNA was reverse-transcribed using poly-T primers (Qiagen, Omniscript RT kit, USA). The resulting cDNA was probed with custom intron-spanning primers for aggrecan, biglycan, collagen type I, collagen type II, decorin, GAPDH, and versican (Table 2). Real-time Rt-PCR was performed using SYBR green chemistry (Bio-Rad, SYBR Green Premix, USA) on an iCycler IQ system (Bio-Rad, USA). Starting quantity was determined using the ddCt method, as calculated by the iCycler software. All data was normalized to GAPDH expression and then normalized to control sample set.

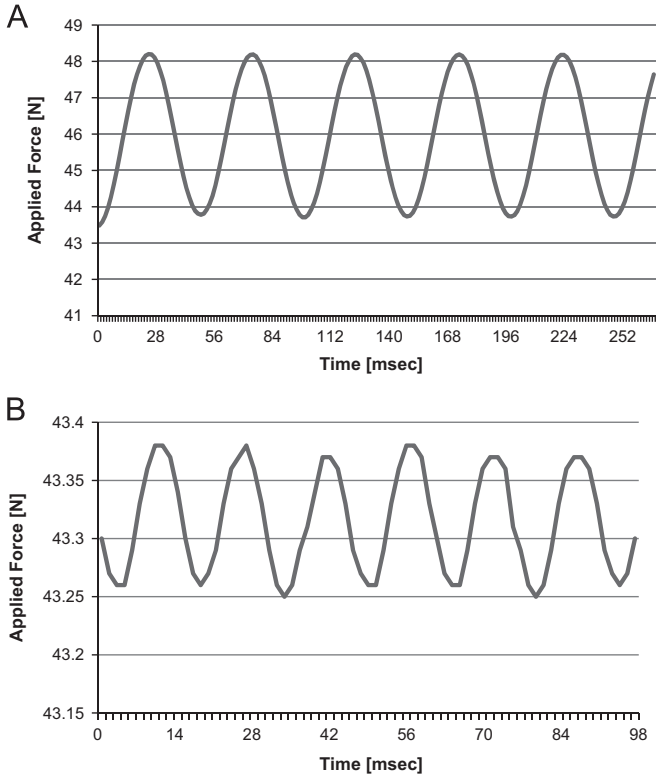

Fig. 5. Exemplary loading profile for maximum output at 20 Hz (A) and 65 Hz (B).

2.6. Data analysis

General Linear Model (GLM) analysis of Rt-PCR data showed substantial non-normalities within normal probability plots, therefore a Kruskal–Wallis nonparametric test was implemented. In order to conduct post-hoc analyses on the non-normal data, a Box-Cox analysis was performed. In all cases a transform of lambda=0.5 was found to be optimal. ANOVA was used to check significance, followed by a Tukey's post-hoc test. The original Kruskal–Wallis and the transformed ANOVA results were consistent in all cases, suggesting that the transform was effective in normalizing the data. The transform was only applied for the analysis, not the presentation of the data in the figures. Pairwise comparisons were considered significant at or below the $p=0.05$ value.

3. Results

3.1. Medium flow

A Computational Fluid Dynamic (CFD) test was done in SolidWorks Simulation to qualitatively investigate any stagnation points or high flow regions. The goal was not to simulate normal fluid flow in the body, merely to control the quality of fluid between bioreactors. Given the experimental flow of ~0.05 L/min and a density/viscosity similar to water, flows show no stagnations affecting the samples (Fig. 6).

3.2. Rt-PCR

Both the Kruskal–Wallis test and the ANOVA on transformed data indicated that there were significant differences between treatments for aggrecan, decorin, and versican ($p=0.045$, 0.044, and 0.008, respectively) with a strong trend for biglycan ($p=0.052$) but no significant differences for collagen type I or collagen type II ($p=0.49$ and 0.14 respectively) (Fig. 7).

4. Discussion

Our experimental multi-unit vibration loading platform successfully accomplished its intended purpose by achieving similar increases in target mRNA expression in IVDs due to applied vibration loading. The efficacy of the system was validated using similar loading parameters previously as reported in literature, and by demonstrating a similar biological response in IVD tissue (Desmoulin et al., 2010, 2011). The ability to load four tissue samples independently was highly advantageous; the accelerated experimental method allowed the loading of more discs in less time. This is an important factor when testing tissues taken ex-vivo, as maximum cellularity must be maintained in order to demonstrate the biological effect of mechanical loading. The loading platform also improved the in-situ testing environment

Table 2
PCR primers and thermocycler settings.

Gene	Forward primer	Reverse primer	Annealing temperature
GAPDH	GGC GTG AAC CAC GAG AAG TAT AA	CCC TCC ACG ATG CCA AAG T	60
Aggrecan	GAG TGG AAC GAT GTC CCA TGT	GCA TTG ATC TCG TAT CGG TCC	50
Biglycan	GCT CCT CCA GGT GGT CTA TC	GCT GAT GCC GTT GTA GTA GG	50
Collagen I	AAG AAC CCA GCT CGC ACA TG	GGT TAG GGT CAA TCC AGT AGT AAC CA	50
Collagen II	GCA TTG CCT ACC TGG ACG AA	CGT TGG AGC CCT GGA TGA	50
Decorin	TGA CTT TAT GCT GGA AGA TGA G	TGG ACA ACT CGC AGA TGG	50
Versican	GAG AGT GTC GGT GCC TAC	GTC CTG TGT GTC TTC AAT CC	50

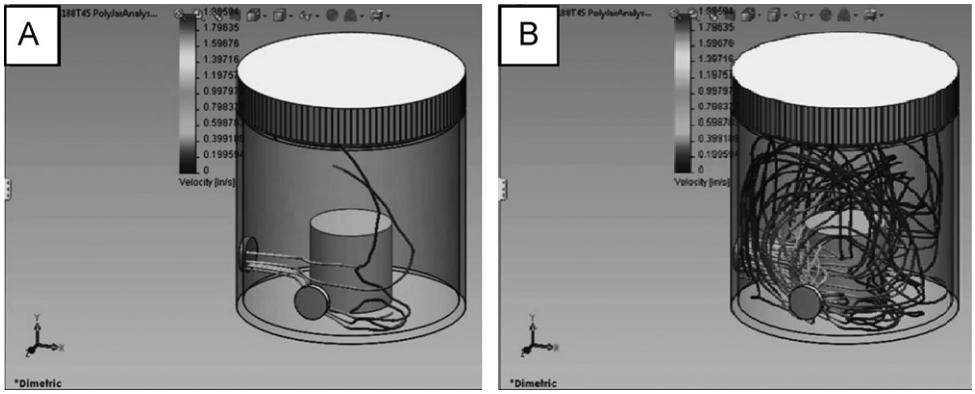

Fig. 6. Computational Fluid Dynamic results within bioreactors at the start of flow (A) and after 1 min (B).

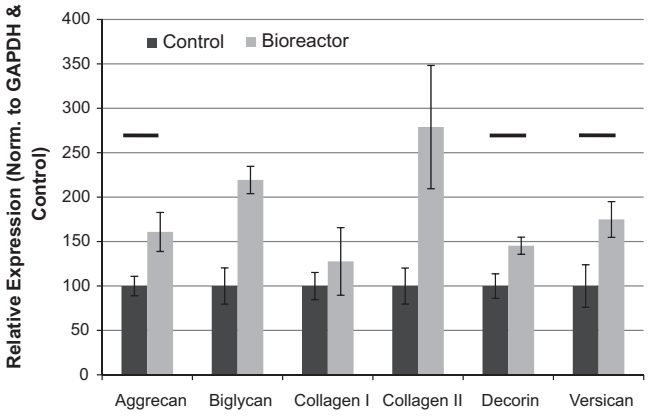

Fig. 7. Gene expression levels normalized to GAPDH levels and to control values (black bars represent significant difference $p < 0.05$).

by controlling temperature and humidity in the incubator, and by equalizing medium quality through constant circulation. The fully automated Labview control program also included a built-in timer allowing for more complicated loading regimes, which enabled the seamless switch of vibration parameters at specific time points during testing. The goal of these additions was to enhance experimental controls and preserve tissue quality by accelerating the testing procedure.

Displacement feedback was used to control the amplitude of vibrations, however understanding the mechanobiology demonstrated here could be improved by imposing an equivalent pressure on disc with controlled mechanical loads (Beckstein et al., 2008). Another limitation of our new device was the grip used to fix the IVD and transmit force from the push rod. Instead of a compression spring to clamp the disc, a rigid rod compressed the disc that made consistent initial compression difficult. Future work and modifications to this device will likely include incorporated load cells and redesigned tissue grips to address these issues. The experiment could be further improved by adding the ability to change the angle at which the tissue is fixed and loaded. For IVDs this would allow the comparison of gene expression in response to axial compression versus transverse shear loading. A new modular grip design would also enable other types of tissues to be loaded within the bioreactors.

To demonstrate the utility of the system, this study has presented pilot data that is consistent with scientific literature. Several mRNA expressions were increased within the cells of IVD tissue in response to applied mechanical vibrations, supporting that gene expression is responsive to mechanotransduction. Aggrecan, decorin, and versican all reported a statistically significant increase above control levels. Collagen types I and II showed no significant difference compared to the control group. Biglycan also exhibited a strong trend that suggest it is also affected by the applied vibrations. These results are largely consistent with previous studies, where aggrecan, decorin, and versican expression were also increased in response to vibration loading (Desmoulin et al., 2011). This independent experimental data was able to demonstrate similar effects in bovine IVD tissue using a completely new device, thereby achieving our design goals.

Aggrecan is a very large proteoglycan (>2800 kDa) with a primarily mechanical function in the tissue matrix (Benjamin and Ralphs, 2004; Rufai et al., 1995). The absence of aggrecan has been shown to be critical in disc health. Analysis of excised painful IVDs has shown the presence of nerves and blood vessels within the usually aneural and avascular tissues of the IVD, which has been suggested as a result of altered aggrecan biology (Freemont et al., 2009). Collagen type II is the dominant collagen in the nucleus, however changes in Collagen type II during disk degeneration remains unclear (Benjamin and Ralphs, 2004; Rufai et al., 1995; Freemont et al., 2009), and may not mirror the loss of proteoglycans. Versican is also a large proteoglycan (1000 kDa) similar to aggrecan, but with a less clear function. It is involved with cell adhesion and cell signaling (Rufai et al., 1995). However a decrease in versican expression occurs at various stages of disk degeneration (Freemont et al., 2009) in the same way as aggrecan.

5. Conclusion

A new fully automatic vibration loading platform was designed and constructed with four independent bioreactors, incubator compatible dimensions, and circulating culture medium. By using previously reported vibration parameters, the device achieved positive influence of mRNA expression, thus demonstrating its efficacy. The evidence provided by this study supports the use of this device for vibration loading experiments of tissues perhaps outside the realm of IVDs. Current data remains unable to determine whether gene expression changes translate into altered protein expression, and further research is still required to determine how these increased mRNA expressions actually affect long term disc health. Nevertheless, the use of sustained vibrations to manipulate gene expression has moved past the proof of concept stage and warrants further investigation. Future work on IVDs could investigate directional vibration loading other than axial loading to explore the effect of directional loading on different mRNA expression. This methodology should also be expanded beyond IVDs, and optimal vibration parameters should be investigated in other tissues as well.

Conflict of interest statement

All the contributors in this project, all of whom are titled as authors, received their regular hourly rate from their respective funding agencies while working on this project. No contributor in this project owns shares in the sponsor company, nor did any party receive any bonuses for working on the project. Thus there is no conflict of interest between contributing parties and the outcome of this project.

Acknowledgments

CJH is an Alberta Heritage Foundation for Medical Research Scholar. GTD is the President and Senior Engineer for the consulting entity GTD Engineering and is also sponsored in part by the National Research Council of Canada's Industrial Research Assistance Program. This work was supported under a research contract from Optima Health Solutions International Corp. (KKT International).

References

Beckstein, J.C., Sen, S., Schaer, T.P., Vresilovic, E.J., Elliott, D.M., 2008. Comparison of animal discs used in disc research to human lumbar disc: Axial compression mechanics and glycosaminoglycan content. Spine 33 (6), E166–E173.

Benjamin, M., Ralphs, J.R., 2004. Biology of fibrocartilage cells. International Review of Cytology 233, 1–45.

Demers, C.N., Antoniou, J., Mwale, F., 2004. Value and limitations of using the bovine tail as a model for the human lumbar spine. Spine (Phila Pa 1976) 29, 2793–2799.

Desmoulin, G.T., Yasin, N.I., Chen, D.W., 2007. Spinal Mechanisms of Pain Control. Clinical Journal of Pain 23 (7), 576–585.

Desmoulin, G.T., Reno, C.R., Hunter, C.J., 2010. Free axial vibrations at 0 to 200 Hz positively affect extracellular matrix messenger ribonucleic acid expression in bovine nucleus pulposi. Spine 35 (15), 1437–1444.

Desmoulin, G.T., Reno, C.R., Hunter, C.J., 2011. Disc strain and resulting positive mRNA expression from application of a non-invasive treatment. Spine 34, 222–226.

Desmoulin, G.T., Szostek, J.S., Khan, A.H., Al-Ameri, O.S., Hunter, C.J., Bogduk, N., 2012. Spinal intervention efficacy on correcting cervical vertebral axes of rotation and the resulting improvements in pain, disability, and psychsocial measures. Journal of Musculoskeletal Pain 20 (1), 31–40.

Freemont, A., 2009. The cellular pathobiology of the degenerate intervertebral disc and discogenic back pain. Rheumatology 48, 5–10.

Gilson, A., Dreger, M., Urban, J.P., 2010. Differential expression level of cytokeratin 8 in cells of the bovine nucleus pulposus complicates the search for specific intervertebral disc cell markers. Arthritis Research and Therapy 12, R24.

Hill, E.T., Desmoulin, G.T., Hunter, C.J., 2009. Is vibration truly an injurious stimulus in the human spine? Journal of Biomechanics 42 (16), 2631–2635.

Jensen, A., Kaerlev, L., Tuchsen, F., Hannerz, H., Dahl, S., Nielsen, P.S., Olsen, J., 2008. Locomotor diseases among male long-haul truck drivers and other professional drivers. International Archives of Occupational and Environmental Health 81 (7), 821–827.

Jünger, S., Gantenbein-Ritter, B., Lezuo, P., Alini, M., Ferguson, S.J., Ito, K., 2009. Effect of limited nutrition on in situ intervertebral disc cells under simulated-physiological loading. Spine (Phila Pa 1976) 34 (12), 1264–1271.

Kofoed, H., Levander, B., 1987. Respiratory gas pressures in the spine. measurements in goats. Acta Orthopaedica Scandinavica 58 (4), 415–418.

Korecki, C.L., Jeffrey, J.M., Iatridis, J.C., 2007. Characterization of an in vitro intervertebral disc organ culture system. European Spine Journal 16, 1029–1037.

Kumar, A., Varghese, M., Mohan, D., Mahajan, P., Gulati, P., Kale, S., 1999. Effect of whole-body vibration on the low back. A study of tractor-driving farmers in north India,. Spine 24 (23), 2506–2515.

Oshima, H., Ishihara, H., Urban, J.P., Tsuji, H., 1993. The use of coccygeal discs to study intervertebral disc metabolism. Journal of Orthopaedic Research 11, 332–338.

Reno, C., Marchuk, L., Sciore, P., Frank, C.B., Hart, D.A., 1997. Rapid isolation of total RNA from small samples of hypocellular, dense connective tissues. Biotechniques 22 (6), 1082–1086.

Rufai, A., Benjamin, M., Ralphs, J.R., 1995. The development of fibrocartilage in the rat intervertebral disc. Anatomy and Embryology (Berlin) 192 (1), 53–62.

Schmidt, H., Shirazi-Adl, A., Galbusera, F., Wilke, H.J., 2010. Response analysis of the lumbar spine during regular daily activities–a finite element analysis. Journal of Biomechanics 43 (10), 1849–1856.

Wilke, H.J., Neef, P., Caimi, M., Hoogland, T., Claes Lutz, E., 1999. New in vivo measurement of pressures in the intervertebral disc in daily life. Spine. 24 (8), 755–762.