PATENT ASSIGNMENT COVER SHEET

Electronic Version v1.1
Stylesheet Version v1.2

EPAS ID: PAT2656229

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT OF TWO-THIRDS INTEREST

CONVEYING PARTY DATA

Name	Execution Date
UNIVERSITY OF CALGARY	06/18/2009

RECEIVING PARTY DATA

Name:	OPTIMA HEALTH SOLUTIONS INTERNATIONAL CORPORATION
Street Address:	#308 - 828 WEST 8TH AVE
City:	VANCOUVER
State/Country:	CANADA
Postal Code:	V5Z 1E2

PROPERTY NUMBERS Total: 1

Property Type	Number
Application Number:	13433820

OP $40.00 13433820

CORRESPONDENCE DATA

Fax Number: (604)688-6445
Phone: (604)688-6442
Email: alin@cameronip.com
Correspondence will be sent via US Mail when the email attempt is unsuccessful.
Correspondent Name: CAMERON IP
Address Line 1: 1401 - 1166 ALBERNI STREET
Address Line 4: VANCOUVER, CANADA V6E 3Z3

ATTORNEY DOCKET NUMBER:	5389P04US
NAME OF SUBMITTER:	ALICE LIN
Signature:	/Alice Lin/
Date:	12/19/2013

Total Attachments: 23

source=Assignment_UofC#page1.tif
source=Assignment_UofC#page2.tif
source=Assignment_UofC#page3.tif
source=Assignment_UofC#page4.tif
source=Assignment_UofC#page5.tif
source=Assignment_UofC#page6.tif
source=Assignment_UofC#page7.tif
source=Assignment_UofC#page8.tif
source=Assignment_UofC#page9.tif
source=Assignment_UofC#page10.tif
source=Assignment_UofC#page11.tif
source=Assignment_UofC#page12.tif
source=Assignment_UofC#page13.tif
source=Assignment_UofC#page14.tif
source=Assignment_UofC#page15.tif
source=Assignment_UofC#page16.tif
source=Assignment_UofC#page17.tif
source=Assignment_UofC#page18.tif
source=Assignment_UofC#page19.tif
source=Assignment_UofC#page20.tif
source=Assignment_UofC#page21.tif
source=Assignment_UofC#page22.tif
source=Assignment_UofC#page23.tif

THE
UNIVERSITY
OF
CALGARY

Research Services

EXECUTION COPY

RESEARCH AGREEMENT

BETWEEN:

Optima Health Solutions International Corporation
Head Office
#308 – 828 West 8th Ave
Vancouver, BC – Canada V5Z 1E2(the "Sponsor")

- and -

THE GOVERNORS OF THE UNIVERSITY OF CALGARY
2500 University Drive N.W.
Calgary, Alberta, Canada
T2N 1N4
(the "University")

RECITALS:

The University intends to perform a research project entitled "Optimal Mechanics for Biosynthesis (Design, Build, Validate & Experiment)" under the direction and supervision of Dr. Christopher Hunter, of the University's Department of Mechanical and Manufacturing Engineering, Schulich School of Engineering;

The Sponsor has expressed an interest in making a financial contribution to the cost of the research project and receiving access to results emerging from the research project; and

The University desires to undertake the research project in cooperation with the Sponsor in accordance with the terms and conditions of this Agreement.

THE SPONSOR AND THE UNIVERSITY AGREE:

1. TERM

This Agreement will be effective from June1-2009 to Dec 31-2010, inclusive (the "Term").

2. PROJECT AND REPORTING

2.1 The University agrees to perform the research project described in the Workplan attached in Schedule "A" (the "Project").

2.2 The Project shall be carried out under the direction and supervision of Dr. Christopher Hunter (the "Principal Investigator"), who shall have responsibility for the scientific and technical conduct of the work.

2.3 The Principal Investigator will provide the Sponsor with interim reports on the Project as may be outlined in Schedule "A", and a final report within 60 days of the completion of the Project (the "Reports"). The Reports will be deemed to be accepted thirty (30) days after delivery unless the Sponsor gives written notice to the contrary.

2.4 If the Principal Investigator is unable to continue the Project, the University will notify the Sponsor in writing. In the event that a mutually acceptable replacement cannot be agreed upon by the parties within fourteen (14) days, either party may elect to terminate this Agreement in accordance with section 10.2 below.

3. FINANCIAL CONTRIBUTION

3.1 The Sponsor will pay to the University the fixed sum of $17,952.00 in Canadian funds for the completion of the Project (the "Financial Contribution"). Payments will be made in accordance with the following schedule:

1/3 of the total amount due ($5984.00) will be paid upon execution of this Agreement and issuance of an invoice by the University;

1/3 of the total amount due ($5984.00) will be paid when Research Results from Project #1 are received, preferably no later than Sept 1-2009, by the Sponsor and issuance of an invoice by the University;

1/3 of the total amount due ($5984) will be paid when the Research Results from Project #2 are received, preferably no later than Nov 30-2010, by the Sponsor and issuance of an invoice by the University;

3.2 The Sponsor will also provide in-kind support to the Project in the following form: the Sponsor will be responsible for paying Geoff Desmoulin's salary during the Term. Geoff Desmoulin will perform tasks indicated in Schedule A but will also be able to work on other projects during Term and may take leave of absence if necessary and resume without hindrance to desired project timelines. .

3.2 Payment will be due within thirty (30) days of issuance of an invoice by the University and shall be delivered to the following address:

Research Accounting
University of Calgary
2500 University Dr. N.W.

Calgary, AB
T2N 1N4

3.4 The University will permit the use of University premises, facilities, and services for the Project in accordance with the applicable policies and priorities of the University. Where the University would normally charge for the direct costs of services, such costs are acceptable charges against the Financial Contribution.

3.5 Within 3 months of being requested to do so in writing, the University will provide to the Sponsor a financial statement documenting the expenditure of the Financial Contribution for the Term .

3.6 Any unspent portion of the Financial Contribution remaining upon completion of the Project may be used in support of other research activities as determined by the University and the Principal Investigator, unless this Agreement is terminated in accordance with section 10.

4. EQUIPMENT

4.1 The University will retain title to any equipment purchased with funds provided by the Sponsor under this Agreement.

4.2 As part of the Project, the University will be building a device, which is descried in Schedule "A", under Project #2 (the "Device"). Title to the Device will belong to the University, however, ownership of the Device's design and other Research Results, as defined in Section 6.1, will be governed by the terms of Section 6 of this Agreement.

4.3 The University shall not sell or lease the Device to a commercial entity other than the Sponsor.

4.4 For the Term and 3 years afterward, the Sponsor may use the Device at mutually agreeable times, as arranged with the University's technical and scientific contact identified in Section 14. At the end of this 3-year use period, the Sponsor shall have the right to purchase the Device. The purchase price will be negotiated in good faith between the parties, but is not intended to exceed the materials cost for the construction of the Device. The Sponsor must notify the University at least 60 days prior to the expiry of the 3-year use period if it intends to purchase the Device. If no notice of intent to purchase is provided to the University within this time-frame, and the 3-year use period has expired, the University shall have no further obligation to the Sponsor with respect to the Device.

5. CONFIDENTIAL INFORMATION

5.1 The University and the Sponsor agree to keep confidential and not disclose to others information designated as confidential and supplied by them for the purpose of developing the Research ("Confidential Information"). The parties agree to advise and notify the other as to

which information disclosed, if any, constitutes Confidential Information. All written materials disclosed will have this clearly marked on them, while any oral disclosures will be followed by a written memorandum outlining the information disclosed and its confidential nature within ten (10) days of disclosure.

5.2 The obligations in respect to Confidential Information shall not apply to information that:

(a) is already known to the party to which it is disclosed;

(b) is or becomes part of the public domain without breach of this Agreement;

(c) is obtained from third parties which have no obligation to keep confidential to the contracting parties;

(d) is required to be disclosed by law; or

(e) is approved for publication in accordance with section 7.1 or is otherwise released from confidentiality by the party originally disclosing the Confidential Information.

5.3 The parties agree not to use the Confidential Information for any purpose other than the purposes set forth in this Agreement for a period of five (5) years from the effective date of this Agreement.

6. OWNERSHIP AND USE OF RESEARCH RESULTS

6.1 "Research Results" means all data and information created in the performance of the Project and includes, but is not limited to, substances, processes, formulations, technical information, data, reports, photographs, drawings, plans, specifications, models, prototypes, inventions, patterns, samples, software designs, computer programs, databases or know-how, whether or not protected by patent, copyright, industrial design. "Research Results" shall not include: (i) proprietary confidential information of the Sponsor which is disclosed to the University to facilitate the Project; (ii) restatements of previously existing information by either the Sponsor or the University; or (iii) nonpublic methods, techniques, processes or computer codes utilized by the University for the conduct of the Project.

6.2 All Research Results will be owned by the Sponsor.

6.3 The Sponsor agrees to receive all Research Results, whether or not marked as such, as Confidential Information in accordance with section 4 of this Agreement. Research Results shall not be considered Sponsor Confidential Information for the purposes of Section 7.1.

6.4 Subject to 6.3, Sponsor hereby grants the University an irrevocable, world-wide, royalty-free, perpetual, non-exclusive right to use the Research Results in all its forms for internal business purposes, without the right to sublicense.

7. PUBLICATION

7.1 The University may, consistent with academic standards, publish or present the Research Results, provided such publication or presentation does not disclose Confidential Information of the Sponsor without the prior written consent of the Sponsor. The University will submit to the Sponsor a copy of the proposed publication (including abstracts, or presentation to a journal, editor, meeting, seminar or other third party) resulting from the Research Project at least sixty (60) days prior to submission of such publication for publication. The Sponsor may review the publication and request the removal of its proprietary Confidential Information. If no response is received from the Sponsor within sixty (60) days of the date the publication was submitted thereto, it may be conclusively presumed that the publication may proceed without delay.

7.2 If the Sponsor determines during its review of a proposed publication that such proposed publication contains patentable subject matter that requires protection, the Sponsor may request the delay of the publication for a period of time not to exceed six (6) months for the purpose of allowing the pursuit of such protection.

7.3 It is the intention of the parties that, in the event that any Research Results are deemed patentable, the Principal Investigator and other personnel working on the Project, including but not limited to Geoffrey Desmoulin, will be listed as non-controlling, co-inventors on any resulting patent applications where appropriate, giving due consideration to the laws and regulations governing patents in the jurisdiction where the application is submitted.

7.4 Notwithstanding section 6.2, the copyright in any graduate thesis prepared by a University student and containing the Research Results will vest in that student.

7.5 The participation of the Sponsor, the University, and the Principal Investigator shall be acknowledged in any publication unless written notice to the contrary is given.

7.6 Entities publishing work after the Term as a direct result of the use of the Device must acknowledge Optima Health Solutions International Corporation as "the "Sponsor" who helped design, build, and validate the device."

7.7 Authorship in any publication shall be determined by standard academic practice in the discipline. The author list and order of each planned publication shall be determined prior to the start of each project so that the work necessary to earn those spots is clearly outlined, understood and agreed upon by all potential authors. Authorship in an academic thesis will be governed by University policy.

8. NO WARRANTY

The University makes no warranties or conditions, either express or implied, regarding the Research Results. The University specifically disclaims any implied warranties or conditions of non-infringement or merchantability or fitness for a particular purpose. The Sponsor

acknowledges that the Project is of an experimental and exploratory nature, that no particular results can be guaranteed, and that the Sponsor has been advised by the University to undertake its own due diligence with respect to all matters arising from this Agreement.

9. INDEMNITIES

9.1 The University will indemnify and save harmless the Sponsor, its officers, directors, employees and agents from and against all losses, claims, damages, actions, causes of action, costs and expenses that the Sponsor may sustain, incur, or be put to at any time, either before or after the expiration or termination of this Agreement, where the same are based upon, arise out of or occur, directly or indirectly, by reason of any act or omission of the University or its Board of Governors, directors, officers, employees, faculty, contractors, students or agents pursuant to this Agreement.

9.2 The Sponsor will indemnify and save harmless the University, its Board of Governors, directors, officers, employees, faculty, contractors, students or agents from and against all losses, claims, damages, actions, causes of action, costs and expenses that the Sponsor may sustain, incur, or be put to at any time, either before or after the expiration or termination of this Agreement, where the same are based upon, arise out of or occur, directly or indirectly, by reason of any act or omission of the Sponsor, or of any officer, director, employee or agent of the Sponsor pursuant to this Agreement.

9.3 Neither party will be liable to the other party for special, indirect, consequential or punitive damages resulting from or arising out of this Agreement or the Research Project including, without limitation, loss of use, loss of profit, or business interruptions, however the same may be caused and regardless of the another party's sole or concurrent negligence.

9.4 In no event will the University be liable to the Sponsor, its officers, employees and agents for claim in either contract or tort arising out of this Agreement or the Research Project resulting in damages exceeding the Financial Contribution paid by the Sponsor to the University.

10. TERMINATION

10.1 If either party:

(a) commits a material breach of any term or condition of this Agreement that is not capable of remedy; or

(b) commits a curable breach of any material term of this Agreement and fails to cure the breach within thirty (30) days after receipt of a notice of the breach or is not consistently and diligently endeavouring to remedy such breach if it is not capable of remedy within thirty (30) days,

the other party may terminate this Agreement immediately upon notice. Any such termination is without prejudice to or limitation of any other right or remedies of any terminating party including the right to collect sums due to it at the time of such termination.

10.2 This Agreement may be terminated for any reason by either party by giving sixty (60) days written notice to the other party. In the event of termination, the University shall take all necessary steps to effect the orderly termination of the Project. The University shall be reimbursed for all costs incurred, or bound to be incurred, through the effective date of termination including the cost of any final reporting.

10.3 For greater certainty, in the event of any termination of this Agreement, rights provided in Section 6.2 and Section 6.4 shall apply to any Research Results generated to the date of termination and the University shall provide to the Sponsor any Research Results generated to the date of termination.

11. ASSIGNMENT

11.1 This Agreement shall not be assigned or otherwise transferred by any Party, in whole or in part, by operation of law or otherwise, without the prior written consent of the other Party, except in the case of a sale or other transfer of all or substantially all of a Party's assets or equity, whether by sale of assets or stock or by merger or other reorganization (including consolidation, acquisition, amalgamation, or the like), provided that the assignee or transferee of such Party's assets or equity has agreed in writing to be bound by all the terms and conditions of this Agreement. No assignment or transfer of this Agreement or of any rights under this Agreement shall relieve the assigning Party of any of its obligations or liabilities under this Agreement.

11.2 Subject to the limitations set forth herein, this Agreement shall inure to the benefit of and be binding upon the Parties, and their successors and permitted assigns.

12. SURVIVAL OF PROVISIONS

The following provisions shall survive the expiration or sooner termination of this Agreement: section 5 (Confidential Information), section 6 (Ownership and Use of Research Results), section 7 (Publication), section 8 (No Warranty), section 9 (Indemnities), section 11 (Assignment), section 13 (Freedom of Information and Protection of Privacy Act (Alberta)) and section 16 (General Conditions).

13. FREEDOM OF INFORMATION AND PROTECTION OF PRIVACY ACT (ALBERTA)

The Sponsor and the University acknowledge that this Agreement and the relationship between the Sponsor and the University will be subject to the provisions of *The Freedom of Information and Protection of Privacy Act* (Alberta).

14. NOTICES

14.1 Any written communication from one Party to the other must be mailed, personally delivered, faxed, or electronically transmitted as follows:

University:

For contractual and administrative matters:

Senior Executive Director
Research Services and Research Accounting
University of Calgary
2500 University Drive, NW
Calgary, AB
T2N 1N4

Telephone: 403-220-6534
Fax: 403-289-0693

For technical and scientific matters:

Christopher Hunter
Department of Mechanical and Manufacturing Engineering
Schulich School of Engineering
University of Calgary
2500 University Drive, NW
Calgary, AB
T2N 1N4

Telephone: 403-220-8503
Email: chunter@ucalgary.ca

Sponsor

For contractual and administrative matters:

Contact name: Marni Adrian
308 – 828 West 8th Ave.
Vancouver, BC – Canada V5Z 1E2

Telephone: 604-266-5338
Fax: 604-267-0911

Email:madrian@khankinetic.com

For technical and scientific matters:

Contact name: Geoffrey Desmoulin
308 – 828 West 8th Ave.
Vancouver, BC – Canada V5Z 1E2

Telephone: 604-266-5338 ext 239
Fax: 604-267-0911
Email:gdesmoulin@gmail.com

14.2 Any written communication from either party will be deemed to have been received by the other party on the fifth business day after mailing; on the date of personal delivery if personally delivered, or on the date of transmission if faxed or sent by email.

14.3 Either party may, from time to time, notify the other party in writing of a change of address and, following the receipt of such notice, the new address will, for the purposes of section 14.1 of this Agreement, be deemed to be the mailing address of the party giving notice.

15. FORCE MAJEURE

Notwithstanding anything contained in this Agreement, neither party will be responsible to the other party for failure to perform any of its obligations set forth in this Agreement if such failure is occasioned by or results from the destruction or damage caused by fire, strike or lockout, civil commotion or disturbance, an act of God, a supervening illegality or any other act or cause that is beyond the reasonable control of such party.

16. GENERAL CONDITIONS

16.1 Nothing in this Agreement shall be construed as constituting either Party as the agent, employee or representative of the other Party; or creating a partnership or as imposing upon either Party any partnership duty, obligation or liability to the other Party.

16.2 The Schedule(s) to this Agreement are an integral part of this Agreement.

16.3 Each party will, at the reasonable request and at the expense of the other party, execute and deliver any further documents and do all such acts and things as may be required to carry out the intent and meaning of this Agreement.

16.4 This Agreement, including the Schedules, is the entire Agreement between the Sponsor and the University with respect to the Project and the Financial Contribution, and supersedes all previous agreements, negotiations and understandings.

16.5 No amendment to this Agreement is valid unless made in writing and signed by both parties.

16.6 No term or condition of this Agreement will be deemed to have been waived unless such waiver is in writing signed by the University and the Sponsor.

16.7 If any term, covenant or condition of this Agreement, or its application to any person or circumstance, to any extent, is invalid or unenforceable, the remainder of the Agreement or the application of such provision to persons or circumstances other than those to which it is held invalid or unenforceable shall not be affected and each remaining term, covenant or condition of this Agreement shall be separately valid and enforceable to the fullest extent permitted by law.

16.8 This Agreement will inure to the benefit of and be binding upon the parties, and their successors and permitted assigns.

16.9 This Agreement and any amendment may be executed in counterparts and all such counterparts taken together will constitute one and the same instrument. If this Agreement is executed in counterparts, no signatory will be bound until both the parties named below have duly executed a counterpart of this Agreement.

16.10 This Agreement will be governed by and interpreted in accordance with the laws of the Province of Alberta. The parties agree to the jurisdiction and venue of the Courts of Alberta for all disputes and litigation arising out of this Agreement.

[REMAINDER OF PAGE LEFT INTENTIONALLY BLANK]

The authorized representatives of the parties have executed this Agreement below.

SPONSOR

Name
Title
C.E.O.

27 May 09.
Date

THE GOVERNORS OF THE UNIVERSITY OF CALGARY

Glenn McMurray
Senior Executive Director, Research Services
and Research Accounting

June 10/09
Date

SCHEDULE "A"

Statement of Work

PROJECT #1 - KKT Disk Biosynthesis (Project Date: June1 to Sept1, 2009)

Introduction/Purpose: This project aims to implement the known frequencies to cause intervertebral disk (IVD) biosynthesis in axial free vibration (Desmoulin et al. submitted, Spine) and monitor imparted mechanics to the disk in the hopes that we may publish a paper showing KKT efficacy in this area. Since, a large percentage of our patients present with disk degeneration.

Imparted Mechanics at Disk Level (Start Date: June 1 – 2009)

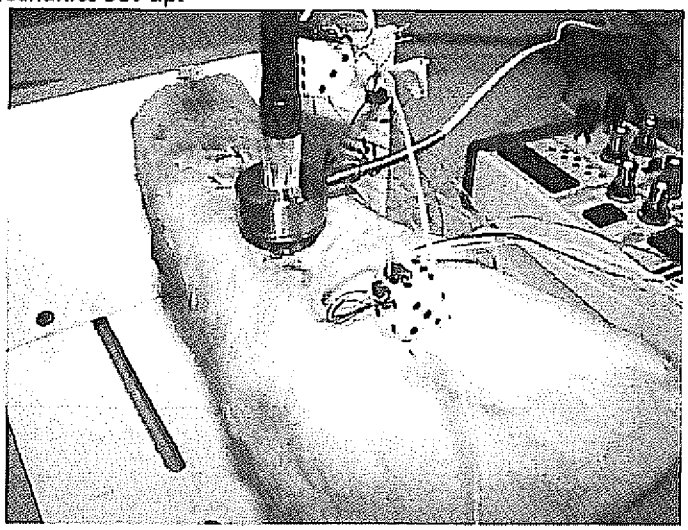
Justification: It is important to understand what causes the biosynthesis so providing a link between biosynthesis results and imparted mechanics directly is critical. Therefore, we plan to track the imparted mechanics at the disk level.

Aim: To measure the imparted mechanics of KKT at the disk level in ox tails so that we may link the mechanics to any biosynthesis should we find any.

Technical Objectives:

1. *KKT stylus frequency modification:* Modify KKT control program (firm ware and hardware) to administer vibration at a preselected ideal window (Desmoulin et al. submitted, Spine) known to cause biosynthesis in free axial vibrations.
2. *Imparted Mechanics Recording:* Apply KKT treatment with modified control program to 10 isolated bovine tail segments and track stylus force and vertebral acceleration to determine disk strain in three dimensions (Figure 1).

Figure 1 Imparted Mechanics Set-up.



Plan of Work: Datrend Systems Inc. will modify the hex file code to include three different files for testing – 1) 16Hz for 0.7sec and 50-80Hz for 0.7sec; 2) 16Hz for 0.7sec and 50-104Hz for 0.7sec; 3) 16Hz

for 20sec while ensuring that the magnitude of vibration stays the same at the lower frequencies by swapping out a resistor at the microcontroller output.
Geoff Desmoulin will carry out the experiments using a KKT unit with a modified control program at the University of Calgary under the supervision of Dr. Christopher Hunter. A 100lbs load cell (seen in Fig. 1) will measure the force of the stylus tip and two ADXL accelerometers will be attached to adjacent vertebrae. Knowing the original accelerometer orientation three dimensional disk strain can be obtained.
Technical Difficulties: There is low risk for both technical objectives.
Description of Work to Date: This experiment has been performed previously (Table 1). However, we will be repeating the experiments using a KKT unit with a modified control program. Therefore, we expect differences.
Table 1 Imparted Mechanics Results using 50-104Hz frequency sweep. Need to repeat with modified control program.

Clinical Emulation (50-104Hz)						
Intensity	Force on Vertabrae (N) - Ave	SD	Acceleration of Vertabrae (g) - Ave	SD	Relative Strain of Disk (%) Z-Ave	Z-SD
20%	7.8	1.9	1.30	0.20	3.60	1.7
50%	10.3	1.9	2.19	0.62	5.20	1.9
80%	14.6	1.0	4.67	1.09	6.90	1.9

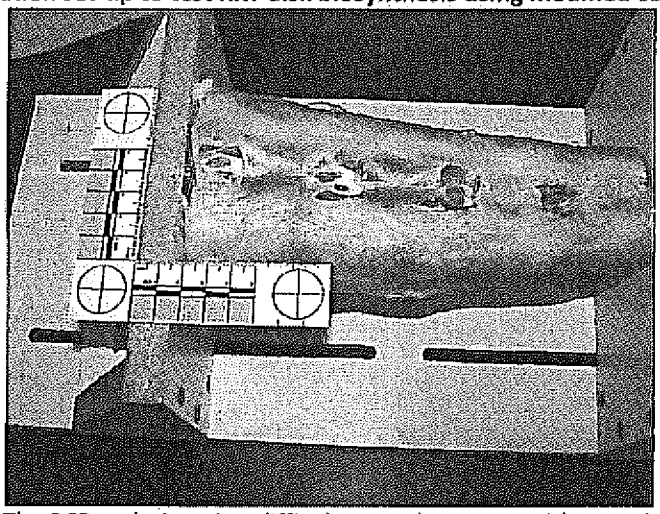
Disk Biosynthesis (Start Date: June 29 – 2009)
Justification: We have described ideal frequency windows causing IVD disk biosynthesis (Desmoulin et al. submitted, Spine) in free axial vibration. Therefore, we need to perform KKT biosynthesis experiments using the modified control program that matches the ideal frequency windows in hopes of demonstrating KKT efficacy for patients with disk degeneration.
Hypothesis: Vibrations delivered by the KKT system at new frequencies windows have a positive effect on biosynthesis in the vertebral column, particularly gene expression by cells in the intervertebral discs.
Aim: Test whether modifications to the KKT control program can increase the positive effects of treatment on IVD gene expression.
Technical Objectives:

1. Using KKT with modified control program load isolated bovine tail segments. Measure the resulting mRNA expression for aggrecan, biglycan, collagen type I, collagen type II, decorin, and versican in the annulus fibrosus and nucleus pulposus of the treated IVD samples vs controls.

Plan of Work: Geoff Desmoulin will carry out the experiments using a KKT unit with a modified control program at the University of Calgary under the supervision of Dr. Christopher Hunter. The KKT stylus will be placed on the vertebrae of a bovine tail segment to emulate human treatment (Fig. 2).
Geoff Desmoulin will load multiple disks, harvest the disks, flash-freeze them in liquid nitrogen, store at -80C and assist Carol Reno in RT-PCR (See below). Carol Reno will extract the total RNA of the stored disks; full details provided in Reno et al, Biotechniques 1997, probe cDNA with custom intron-spanning primers for aggrecan, biglycan, collagen type I, collagen type II, decorin, GAPDH, and versican. Perform real-time RT-PCR using SYBR green chemistry on an iCycler IQ system and normalize all data to GAPDH expression. The gene expression results shall be provided in an EXCEL file by Carol Reno to Optima

Health Research Staff by the project tend date.

Figure 2 Clinical emulation set-up to test KKT disk biosynthesis using modified control program.



Technical Difficulties: The PCR technique is a difficult procedure even with experienced technicians. While Carol Reno has performed RT-PCR many times before and has added to the scientific literature in regards to its methodology, Geoff Desmoulin has not performed this technique previously. Therefore, time will be taken to progress carefully as we move forward since Carol will teach and supervise Geoff's first few times through the protocol.

Description of Work to Date: These experiments were performed previously and we found that the KKT device does not operate in the ideal 'window' for stimulating extracellular matrix gene synthesis in the IVD and no gene expression changes were detected with various intensities. However, tests on free axial vibration of isolated IVDs (Desmoulin et al. submitted, Spine) suggest that two lower frequency windows may be optimal.

Project #2: Optimal Mechanics for Biosynthesis (Design, Build, Validate & Experiment) (Project Date: Nov 30 – 2009 to Oct 15 - 2010)

Justification: We have completed studies investigating the general sensitivity of the IVD to unconstrained axial vibrations (Desmoulin, Reno, and Hunter, Spine, In review). However, the present system only allows vibration in a free state and only in an axial direction. While the KKT applies transverse vibration (which translates into a complex array of axial, transverse, and shear motions) with partially fixed boundary conditions and therefore our current results, while informative, are based on a non-ideal loading pattern. While the ideal system would be to apply vibration to whole cervical spines, this involves considerable complexity and limits some testing options. Therefore, we propose to build a dedicated culture system, which can apply constrained vibration in various axes to multiple samples.

Aim 1: To design, assemble, and validate a system that can apply controlled vibration in various axes to isolated IVD samples ex vivo for up to 7 days.

Aim 2: Measure the effects of axial, transverse shear and torsional shear vibrations on an expanded

gene expression profile in the IVD using this new system.

OVERALL GOALS

It is known that disk degeneration causes spine related pain. We believe that KKT treatment acts at the cellular level in the intervertebral disks (IVD) to improve the overall health of the disk thus relieving chronic back pain. Our hypothesis is that the IVD is differentially responsive to various envelopes of vibration axis, frequency, amplitude, and duration. In order to investigate this phenomenon so that we may optimize the imparted mechanics of the KKT to maximize disk treatment, we need to assemble a dedicated culture system that can apply controlled vibration to intact IVDs ex vivo. We have a pilot system that can apply controlled axial vibration to a single sample. This request will allow us to build an improved system to culture four samples at a time and apply vibration along various tissue axes for up to two weeks of continuous (Injury Investigation) or intermittent loading (Treatment Investigation).

DESIGN

To date, the vibration experiments have been performed using a single voice coil and a single sample at a time. In order to continue these studies, we now require a more advanced culture system. Basic details are outlined in

Table 2.

The design will be based on the IVD culture systems developed by Drs. Keita Ito, Mauro Alini, and James Iatridis to culture IVDs for extended periods. Our adaptations will include modifying the bioreactor system to provide adequate stiffness for loading at 1-200Hz. The system will utilize a tall culture vessel with a loose-fitting lid (similar to a standard petri dish with an extremely long overlap between the upper and lower pieces) (**Figure 3**). This will allow for considerable motion of the upper and lower assemblies while maintaining an aseptic environment via the labyrinthine path created by the large overlap. Other concepts include a bellows system or a flexible bag to link the upper and lower assemblies. The system will be designed with a self-sealing medium circulation loop to provide fresh nutrients to the disc.

We will design the system so that vibrations can be intermittently delivered to the bioreactor without removing it from the incubator. In this case, the compression actuator will be replaced by a magnetic voice coil to apply static compression and/or vibration.

Vibration will be delivered by a commercially available magnetic voice coil (H2W Technologies NCM Series Moving Magnet) mounted above the culture chamber and rigidly attached to the top of the culture vessel, which will be rigidly attached to the cranial surface of the superior vertebral body (**Figure 3**). The actuator will be driven through sinusoidal motion by a data acquisition system (NI DAQ & Labview). The system will normally operate in closed-loop displacement control via an LVDT sensor and comparator system. Displacement is the ideal measurement to track disk strain values which are important for understanding what mechanics are required at the tissue level to cause maximum benefits. However, while velocity and acceleration can be derived from displacement measurements, we may also wish to incorporate measures of load, velocity and acceleration directly so that the ideal parameters for a KKT control system can eventually be developed. Therefore, whatever disk strain-loading pattern turns out to be the most appropriate for improving disk health can be correlated to the other measurements. The highest correlation with the most practical implementation for KKT control will be chosen for the eventual KKT control system design and its desired values to maintain. This will require additional data input channels for each frame. However, this would allow the system to operate using other parameters for control, increasing the flexibility of possible experiments and increasing the chance that we will find the most appropriate parameter for increasing disk health when used as the control feature in KKT.

In order to allow the system to be reconfigured for various loading axes, a one-chamber, one-frame design will be chosen (**Figure 4**), though other options may be considered as the design process progresses (**Figure 5**). The basic system design will consist of a rigid frame with adjustable space, and guided by two large-diameter steel rods on precision bearings. The upper plate of the frame will support the voice coil and LVDT sensor, which will attach to the lid of the bioreactor chamber. The base of the bioreactor will attach to the lower plate (in the axial loading configuration). For shear loads, the frame will be rotated 90 degrees, and the voice coil/LVDT will attach to the side of the lid. For torsional loads, the bioreactor will be shifted sideways, such that the axis of the voice coil acts eccentrically on the lid.

The bioreactor chamber (**Figure 3**) will contain two porous platens which will rigidly attach to the lid and base of the bioreactor and to the cranial and caudal endplates of the IVD. Culture medium (DMEM containing 10% fetal bovine serum) will be recirculated through the bioreactor with an external peristaltic pump; this will allow for (a) a large volume of culture medium, and (b) periodic changes of culture medium without opening the primary bioreactor chamber. Gas

exchange will be provided via a luer fitting and standard 0.2um syringe filters attached to the lid of the bioreactor.

The control system will consist of an LVDT sensor for position, fed to a custom-designed LabView package. The current position will be compared to the command signal (a standard sine wave at a user-set frequency and amplitude), and the resulting error term will be used to tune the command signal for voice coil motion. The LabView package will include a routine for setting the load duration and the offset; in other words, the user will have the option of setting the system to load for a certain amount of time, and then apply a rest phase at a tare load.

The goal of the medium recirculation and control system will be to allow the system to operate without user intervention for at least 48 hours, thereby reducing the human resources required for operations.

Table 2. Must-Should-Could design criteria for the new culture system.

MUST	Fit within a standard culture incubator
	Maintain an aseptic environment
	Maintain 4 samples independently for up to 3 days
	Apply a known and adjustable tare load to each sample
	Apply controlled motion to each sample independently
	Apply motion in one of: shear, axial compression, or axial rotation
	Apply controlled motion at 0-200Hz and 0-1mm stroke
	Not cause overheating of the incubator
SHOULD	Maintain 4 samples independently for up to 7 days
	Allow for simple conversion between loading axes
	Operate under closed-loop control
	Operate under displacement control
COULD	Be modular and scalable
	Permit independent removal of a single sample without disrupting the others
	Allow for medium sampling and inline measurement of soluble factors
	Operate under load control

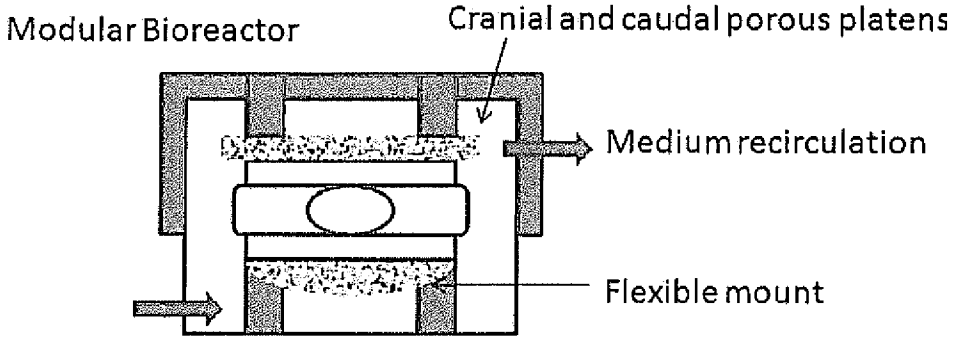


Figure 3. Concept design for the proposed bioreactor system. Each IVD will be maintained in a self-contained chamber with porous platens to ensure mass transfer through the cranial and caudal endplates. A flexible mount will allow for self-alignment in the event of non-parallel surfaces. Medium will be circulated through the bioreactors with an external pump. The bioreactors (n=4 per frame) will be mounted on custom-designed frame(s) which can deliver vibration loads in a controlled manner throughout the culture period.

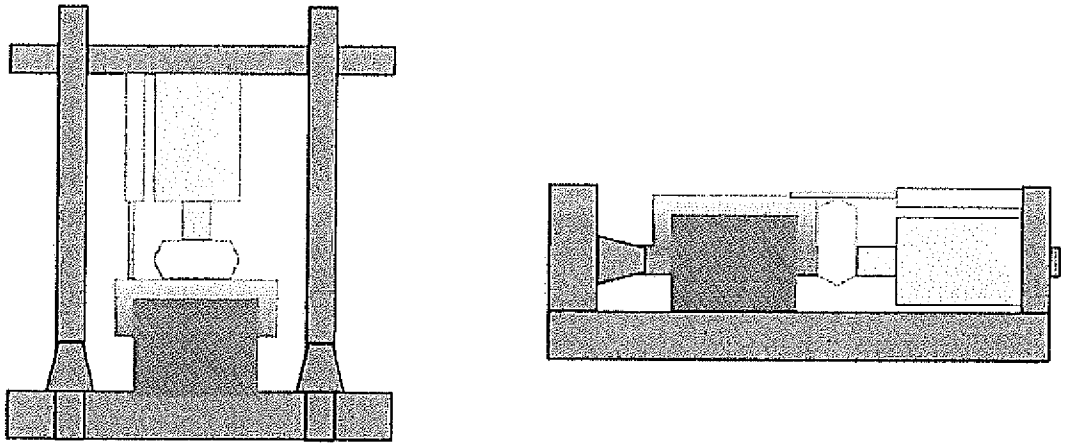


Figure 4. Conceptual design of a single-chamber system, which can be configured for axial load (left) or shear/torque load (right) by rotating the frame around the bioreactor chamber. Shear load will be applied by directly manipulating the chamber lid, while torque load will be applied by attaching to an eccentric point to the side of the IVD's rotational center. Only one of the two axial guide rails is shown at right for clarity.

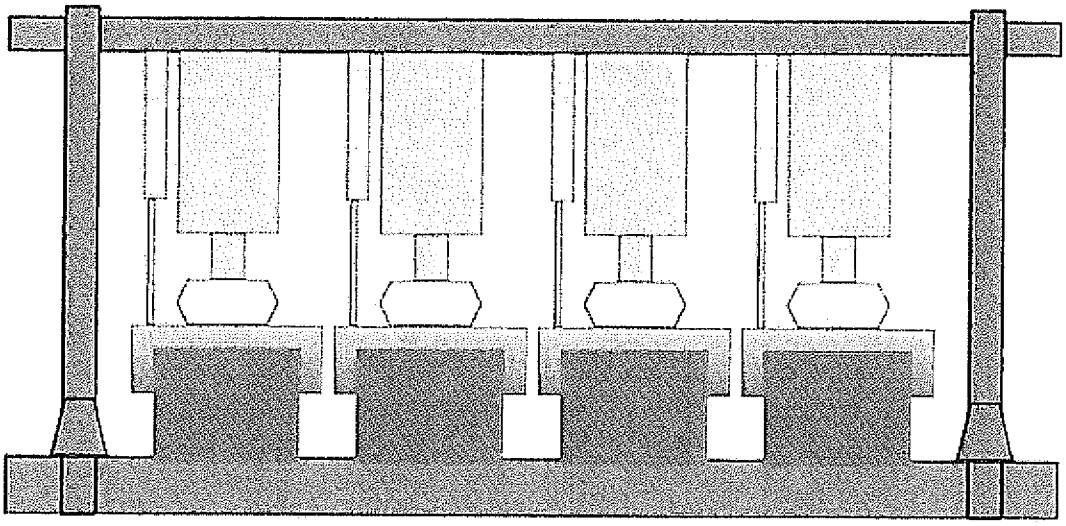


Figure 5. Conceptual design of a parallel four-chamber system. Light gray: voice coils; Pink: LVDT; Green: coupling; Blue: bioreactor chamber, top; Red: bioreactor chamber, bottom; Dark gray: rigid frame.

ASSEMBLY

Assembly will be performed at the University of Calgary (UofC) and be executed in three stages (Hardware, Electronics, and Software). First, the desired hardware specifications from the design phase will be sought out so that Matlab (Simulink) modeling of the response of the system can be

known prior to assembly. Then the hardware will be purchased and tested individually to ensure quality and response verification. Then the hardware for the apparatus itself will be purchased and assembled in accordance to the design specifications. The UofC machine shop will assist in the total assembly and tooling of any special parts. Second, the electronics involved in the control system will be purchased and assembled with the help of UofC electronics shop to ensure robust and accurate connections. Lastly, the software will be programmed to run the voice coils at the specified protocols with displacement control and tested.

VALIDATION

System validation will be provided by (a) the internal LVDTs for closed-loop monitoring of actuator motion, and (b) independent validation and calibration of the system using accelerometers (as previously used for the KKT motion analysis). The signal from the internal LVDTs can be monitored separately from the Labview software program in a completely different computer and translated to displacement remotely. Validation will occur when the remote measure of displacement matches to within 5% of the programmed control displacement in Labview. Further, the displacement can be tracked using a double integrated signal from an accelerometer. We expect less accurate results (~7%) using this method but if we can show that two different and separate methods are achieving similar displacement values we will consider the validation complete.

EXPERIMENTATION

Our initial results have suggested the ideal windows for axial load (>0.5g), frequency (~16Hz and 50-80Hz), and duration (10 min.). The KKT stylus control will be modified to match these numbers and we will again measure the Imparted Mechanics of KKT in situ (See Imparted Mechanics section). Knowing these ideal axial loads in situ, we will then determine the most effective loading axis. Therefore, these experiments will look at various axis angles at the specified loads, frequencies, and durations found during the previous axial experiments. The KKT treatment will then be updated to apply what we think is a 'better' protocol. We will iterate this process until we find the window of KKT parameters which matches our 'best' conditions demonstrated with the ex vivo test system. Further, we will expand the number of genes we track expression in to include TIMP-1, TIMP-3, MMP-2, MMP-3, MMP-13, and ADAMTS-4 in addition to the other 6 genes we monitored in previous experiments. This will allow us to monitor both beneficial and detrimental changes more accurately and in greater detail.

The system as proposed here will be capable of exploring these questions in turn by isolating the various parameters. Further, future experiments with the device could include a routine such as: load 0% for 6 hours, load 5% plus a 1Hz sine wave at 0.5% for 2 hours, load 5% plus an 80Hz sine wave at 0.5% for 20 minutes, load 5% plus a 1Hz sine wave at 0.25% for 13 hours, 40 minutes, load 0% for 2 hours (simulating 8 hours of rest, 16 hours of activity, and 20 minutes of KKT therapy).

Sample Sizes: For Aim 2 testing of this section of the proposal (multi-axis vibration), general conditions are: control (unloaded) and treated (using the new apparatus from Aim 1), which yields a total of 50 samples for each set of conditions since multiple conditions can utilize the same control disks. Specifically, treatment conditions (independent variables) are: horizontal plane constrained vibration at various angles (0, 22.5, 45, 67.5, 90deg) and vertical plane constrained vibration at various angles (0, 22.5, 45, 67.5, 90) with constant amplitude (>0.5 m/s^2 r.m.s.), a constant frequency window (0, ~16/50-80Hz), and a constant duration (10 min.). Each angle from

each plane will be tested -> (5x5 = 25 conditions with n=9 (treatment) and n=5 per set of conditions (control), yielding 250 total samples.

Geoff Desmoulin will load the 250 disks, harvest the disks, flash-freeze them in liquid nitrogen, and store at -80C and assist Carol Reno in RT-PCR (See below). Carol Reno will extract the total RNA of the stored disks; full details provided in Reno et al, Biotechniques 1997, probe cDNA with custom intron-spanning primers for aggrecan, biglycan, collagen type I, collagen type II, decorin, GAPDH, and versican. Perform real-time RT-PCR using SYBR green chemistry on an iCycler IQ system and normalize all data to GAPDH expression. The gene expression results shall be provided in an EXCEL file by Carol Reno to Optima Health Research Staff by the project end date.

Table 3 Experimental Plan

Load (g)	Duration (min)	Frequency (Hz)	Number of disks	Axes to test (transverse plane (deg))	Axes to test (vertical plane (deg))
0.6	10	16/50-80	9	0	0
0.6	10	16/50-80	9	22.5	0
0.6	10	16/50-80	9	45	0
0.6	10	16/50-80	9	67.5	0
0.6	10	16/50-80	9	90	0
0.6	10	16/50-80	9	0	22.5
0.6	10	16/50-80	9	22.5	22.5
0.6	10	16/50-80	9	45	22.5
0.6	10	16/50-80	9	67.5	22.5
0.6	10	16/50-80	9	90	22.5
0.6	10	16/50-80	9	0	45
0.6	10	16/50-80	9	22.5	45
0.6	10	16/50-80	9	45	45
0.6	10	16/50-80	9	67.5	45
0.6	10	16/50-80	9	90	45
0.6	10	16/50-80	9	0	67.5
0.6	10	16/50-80	9	22.5	67.5
0.6	10	16/50-80	9	45	67.5
0.6	10	16/50-80	9	67.5	67.5
0.6	10	16/50-80	9	90	67.5
0.6	10	16/50-80	9	0	90
0.6	10	16/50-80	9	22.5	90
0.6	10	16/50-80	9	45	90
0.6	10	16/50-80	9	67.5	90
0.6	10	16/50-80	9	90	90
Control (0)	10	0	25	0	0

TOTAL: 250 samples (PCR)

****Note: Optima reserves the right to edit this experimental plan should clinical data or early basic research results dictate a change.****

Technical Difficulties: The main difficulty of this project is its multiple components. Multiple signals, data acquisition, computer control, fluid pumps, cooling fans, the necessary validation equipment, machine shop tools requirements, design software, etc. Optima research staff will have no choice but to include many resources and team members available to contractors doing work at a University under a contract agreement. For example, engineering machine shop tools, machinist assistance, electrical and mechanical technician's assistance, and weekly progress reports to Dr. Christopher Hunter will work to include the necessary expertise on the project to avoid these complications.

Project	Personell		Unit Cost	Qty.	Total
#1 (See Research Agreement)	Carol Reno	PCR Technician	$41.80 + 22% = $51/hr ~1hr/sample	25	$1,275.00
#2 (See Research Agreement)	Carol Reno	PCR Technician	$41.80 + 22% = $51/hr ~1hr/sample	226	$11,526.00
			Sub-Total		$12,801.00
#1 and #2	40% Overhead		Sub-Total		$5,120.40
			Total		**$17,921.40**