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Development and Validation of a Motion and Loading System for A Rat Knee Joint *in vivo*

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Abstract

The influence of biomechanical stimuli on modulating cartilage homeostasis is well recognized. However, many aspects of cellular mechanotransduction in cartilage remain unknown. We developed a computer-controlled joint motion and loading system (JMLS) to study the biological response of cartilage under well-characterized mechanical loading environments. The JMLS was capable of controlling i) angular displacement, ii) motion frequency, iii) magnitude of the axial compressive load applied to the moving joint, and it featured real-time monitoring. The accuracy and repeatability of angular position measurements, the kinematic misalignment error as well as the repositioning error of the JMLS were evaluated. The effectiveness of the JMLS in implementing well-defined loading protocols such as moderate Passive Motion Loading (PML) and increased Compressive Motion Loading (CML) were tested. The JMLS demonstrated remarkable accuracy and reliability for the measurement and kinematics tests. Moreover, the effectiveness test demonstrated the ability of the JMLS to produce an effective stimulus via PML that led to the suppression of the catabolic effects of immobilization. Interestingly, the biological response of the CML group was catabolic and exhibited a pattern similar to that observed in the immobilization group. This novel system may be useful for joint biomechanics studies that require different treatment conditions of load and motion *in vivo*.

Keywords

Articular Cartilage; Rat Knee Joint Loading Device; Continuous Passive Motion; Compressive Motion Loading; Immobilization; Overloading; *in vivo*

INTRODUCTION

Articular cartilage in the knee joint provides low friction, prevents wear and tear at contacting joint surfaces, and enables the knee to absorb shocks. Chondrocytes react to mechanical stimuli due to joint motion and compressive loading by modulating both anabolic and catabolic activities, thus preserving or degrading their extracellular matrix, respectively. The degradation of articular cartilage is the central feature of major joint diseases such as osteoarthritis (OA)

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and involves a complex interaction between mechanical, environmental, anatomical, and biological factors ^{1, 3, 29}.

Biomechanical stresses beyond a physiological range induce cartilage degeneration and alter the ability of cartilage to perform its normal function. On the one hand, prolonged synovial joint immobilization seems to lead to cartilage degradation ¹⁴. On the other hand, if the physiological load-bearing capacity of cartilage is exceeded as a result of overuse or overloading, as seen in intense sports ^{4, 6, 23, 24, 30}, repetitive high-impact activity ^{8, 17} and obesity ^{9, 25}, the tissue is subject to higher risk of breakdown. Once the cartilage is damaged, the tissue may become increasingly vulnerable to further degradation even by physiological-level joint forces, and the progression of tissue breakdown can be accelerated with moderate exercise ².

Physiological joint motion and loading are considered to have a chondroprotective effect preventing cartilage degradation ^{4, 11, 12}. After a period of immobilization, motion of the joint can prevent or overcome joint stiffness by enhancing synovial fluid motion, cartilage nutrition, and stimulating the healing and regeneration of articular cartilage ²⁷. Beneficial results of motion-based physical therapy include the reduction of pain, maintenance of physiological range of joint flexion and extension, and a reduced period of rehabilitation or hospitalization. Meta-analyses have indicated that continuous passive motion or intermittent motion in humans is better for joints and their articular cartilage than is immobilization ⁵. Nevertheless, there is no consensus on either the optimal therapeutic protocol or the long-term clinical effectiveness of continuous passive motion ^{5, 13}. Key to this debate is the diversity of treatment variables (including the frequency, range of motion, etc.) and the complex biomechanical processes associated with those treatment variables.

Previous studies have developed useful animal models to evaluate the impact of physical activity on biological changes in cartilage and tendon ^{2, 16, 21, 22, 32}. All of these models have provided important insight into the relationship between mechanical stimuli and subsequent structural, compositional and morphological changes in the tissue. The most commonly used loading devices such as the treadmill and rotary cylinder systems make the animal to run at a controlled speed for a determined period of time; however, the mechanical stimulus applied to the joint may be different across animals and difficult to standardize. In devices that use electrical stimulation to produce muscle contraction of the animal's limb, a rapid muscle twitch is generated to allow precise control of mean peak load and repetition frequency; nevertheless, a smooth motion of joint flexion may not be easy to obtain and the loads are not identical in this approach. In contrast, assisted motion devices such as continuous passive motion (CPM) systems seem to offer a better alternative to control the dose of mechanical loading and range of motion provided to the animal joint ^{16, 18, 19, 20, 26, 28}. Unlike treadmills, rotary cylinders or electrical stimulation-based systems, the CPM devices allow uniform cyclic motion of the joint with more quantifiable loading that can be applied at very low repetition rates. A murine joint loading model has been reported in recent musculoskeletal mechanotransduction studies ^{34, 35}. This novel joint loading device apply the loading stimulus laterally to the knee joint, offering well characterized loads at various waveforms to study the effect of dynamic mechanical loading on intramedullary pressure, bone formation and fracture healing; however, the effect of such loading modality on cartilage has not been reported.

In this study, we designed and constructed a computer-controlled joint motion and loading system (JMLS) capable of performing passive motion loading (PML) and compressive motion loading (CML) on the knee joint of a rat. The system was conceived to provide not only motion-induced mechanical loading but also axial compressive force applied normal to the cartilage surface with real-time monitoring of joint motion and loading ^{21, 22}. Therefore, this JMLS is not directly comparable to continuous passive motion (CPM) devices, and so, to avoid any

confusion with CPM clinical devices, we will use different terminology in describing the system. The novel motion and loading system, in combination with an immobilization-based animal model, allowed the study of key catabolic/anti-catabolic proteins involved in tissue degeneration and cartilage maintenance in response to specific motion and loading protocols.

MATERIALS AND METHODS

Joint Motion and Loading System Design

The JMLS was designed to perform passive motion loading and compressive motion loading on the knee joint of a small animal, providing adjustable control of cyclic motion frequency, range of angular displacement, axial compressive force magnitude between the tibia and femur, and permitting real time monitoring of angular motion and compressive load.

The JMLS consisted of an animal bed and an anesthesia machine integrated into a custom-built knee joint motion/load apparatus, a linear actuator with micro-stepping driver and digital encoder, a magnetic goniometer for real-time joint angle readout, a load cell for compressive load monitoring, a USB analog/digital I/O interface module, and LabView control panel (Fig. 1). The anesthetized animal was placed prone over an acrylic bed, and the hind limbs were introduced through two spaces located at the center of the bed. The right hind limb of the animal was placed in the knee joint apparatus and the animal body was secured to the bed using Velcro straps. Special care was taken during positioning of the limb in the apparatus to align the rotation axes of the knee joint and the structural apparatus. The hip and femur were maintained in a fixed position to minimize lateral displacement or misalignment during the test. Throughout the experiment, the animal was anesthetized using 1–3% isoflurane dissolved in 21% oxygen, while the respiration rate and body temperature were continuously monitored. A gas mask directed the anesthetic to the nose of the animal, and the exhalation gases were circulated toward a carbon-activated filter and exhaust system. The operation of the system did not require any special consideration of the environment (Fig. 2).

The knee joint apparatus transformed the linear motion provided by the actuator into angular displacement at the knee joint (Appendix). Double steel ball bearings were used to minimize the friction during rotation of the structural steel arms of the motion apparatus. A miniature magnetic encoder (US Digital Corp, Vancouver, WA) aligned to the rotation axis of the structural arms provided a pulse width modulated (PWM) signal proportional to the angular position of the system, the PWM signal was digitized and calibrated using a protractor to exhibit a numerical value within 0–360° that represented the angular position. The JMLS had adjustable control of motion frequency and range of angular displacement via a linear actuator with 50 mm travel stroke (bipolar NEMA 13 Hybrid, Haydon Motion, Waterbury, CT) coupled to an incremental rotary encoder (ESS, US Digital Corp, Vancouver, WA). The incremental rotary encoder used an LED and photodetector to identify the angular position, direction and rotation speed of the motor shaft. A micro-stepping motor drive (MD2S-P US Digital Corp, Vancouver, WA) controlled the rate of motion of the actuator, offering a high linear displacement resolution up to 3 micrometers per step. The motion control for the actuator was performed based on the mathematical relationship between angular and linear motion of the apparatus and linear actuator respectively, as shown in the appendix. The linear actuator was able to provide a linear velocity as high as 30 mm/s, which resulted in a maximum cyclic motion frequency of 20 cycles/min for the full range of motion between 65° and 135°.

In addition to PML, the JMLS facilitated a continuous axial force along the tibia and normal to the plantar sole of the animal throughout the cyclic motion. This compressive motion loading capability was integrated into the JMLS to study the role of overloading on the biological response of the tissue *in vivo*. The anterior aspect of the femur was immobilized at a fixed position, thus, the force applied under the plantar sole was transferred along the major axis of

the tibia to the knee joint cartilage surface at any angular position during the flexion-extension procedure. The adjustable load applied to the plantar sole was created by a pair of extended miniature springs attached to the system at the level of the knee joint and a sliding brace under the animal's foot holder. A subminiature load cell (11BL321 Sensotec-Honeywell) situated between the sliding brace and the foot holder produced a continuous measurement of the reaction force under the plantar sole created by the springs. The compressive load can be adjusted by extending or retracting the springs via a vise below the load cell support. This load sensor had a ± 25 pound linear range of measurement in tension and compression with infinite resolution and 0.1% full-scale non-repeatability performance. The load cell output was amplified using an instrumentation amplifier (INA 122, Texas Instruments), converted into digital signals using a data acquisition interface (USB-6210, National Instruments) and visualized in real time using LabView (V8.5, National Instruments). The load cell output was calibrated using standard weights (ranging from 2.5 to 500 g) to display the measured force in grams-force (data not shown). Motion and loading protocols were executed via a user interface developed in LabView on which the initial and final angle of the knee joint in degrees of flexion, and frequency of the motion in cycles per minute were defined by the user for the test. This visual interface also displayed and recorded in real time the angular displacement and loading magnitude throughout the motion/load experiment.

System Performance Test

The JMLS was designed to produce effective joint kinematic and loading conditions that have been associated with the modulation of joint cartilage biology. The validation test of this new assisted motion/load system included the analysis of the angular positioning error, the repeatability after the animal's hind limb repositioning, and the effectiveness of the JMLS in producing biological responses of cartilage *in vivo*.

Real Time Angular Position Monitoring—Direct *in vivo* assessment of the animal's joint angular position would require an invasive intervention to implant a sensor in the tibia of every animal. However, such invasive procedures may induce a systemic inflammatory response that may obscure or alter the levels of regulatory molecules produced as a consequence of the PML and CML treatments. Instead, the JMLS was designed to provide indirect estimates of the angular displacement of the tibia through the measurement of the kinematics of the mobile apparatus frame, avoiding any open injury to the animal's limb. This approach assumed that the limb of the animal remained aligned with the moving apparatus frame during the cyclic motion, thus requiring an experimental validation test. We validated this indirect approach by characterizing the accuracy and repeatability of the magnetic goniometer attached to the mobile frame, and the angular position error between the frame and the tibia.

First, we determined the accuracy and repeatability of the real-time angular position monitoring offered by the magnetic angular encoder. This magnetic goniometer was aligned with the axis of rotation of the apparatus and provided a pulse width modulated signal that is proportional to the angular position of the apparatus frame. This signal was acquired by LabView via digital ports on the DAQ card and then digitized and transformed into equivalent angular position data in arc degrees. Data of five trials of cyclic motion in full flexion/extension from 65° to 115° at a frequency of 2 cycles/minute for 2.5 minutes each trial were recorded at a sampling rate of 1Hz. The accuracy was assessed by the mean and standard deviation of the error between the recorded measurement data and the set point reference values computed in Matlab using the mathematical modeling equations shown in the appendix. The repeatability was evaluated by comparing the measurement data from five repeated trials; the first trial was defined as the baseline, and the repetition error was calculated as the absolute difference between the baseline and the second to the fifth repeated trials.

Second, the difference between the angular position of the apparatus frame and the animal's tibia was characterized using three-axis Microelectromechanical (MEMS) accelerometer sensors (MMA 7260QT, Freescale Semiconductors, Arizona). The sensor simultaneously produced three voltages proportional to the orientation of three orthogonal directions of the sensor relative to earth's gravity. The analog measurement of the three voltages was quantified and digitized for the kinematics analysis. A MEMS sensor chip was attached to the tibia midshaft on a five month old male Sprague Dawley rat right after sacrifice. A small incision was created in the skin and muscle at the level of the mid-diaphysis from the medial aspect of the tibia, a small hole was drilled, and a thin screw was anchored onto the bone. The incision was cleaned free of debris prior to the insertion of the screws and then secured using bone cement. The three-axis MEMS sensor chip was mounted on a small acrylic base attached to the screw head, and the sensor chip was oriented in the same direction as the frame before the cement hardened. A similar sensor chip was installed on the frame of the medial side of the knee joint apparatus frame and aligned with the sensor on the tibia. The voltage output was transformed into equivalent angular position data, and the difference in orientation measured by the two MEMS accelerometers was used to evaluate the alignment between the tibia and the frame during motion. Twenty cycles were recorded from both sensors at a sampling rate of 100Hz, producing voltage signal data points every 10ms. The mean and standard deviation of the difference between the two data series from the frame and the tibia indicate the intrinsic error of our device to indirectly assess the kinematics of the bone using the goniometer that monitored the angular position of the frame.

Repositioning Error—To assess the variability of the measurement results due to the repositioning of the animal's limb, the hind limb of the animal was repositioned five times in the JMLS. Data of the five repeated trials (20 cycles each, 65°~115°, 2 cycles/minute) were recorded at a sampling rate of 100Hz using the same MEMS accelerometer sensors described above for analysis of the repositioning error. In the beginning of each trial, the rat was completely removed from the JMLS before being repositioned by the same operator for the following test. The first trial was defined as baseline, and the repositioning error was defined as the absolute difference between the following four repeated trials (trial 2–5) and baseline.

***In vivo* Test to Produce a Biological Response in Cartilage**—The use of rats for this study was approved by the IACUC committee at Mount Sinai School of Medicine. To assess the effects of reduced, moderate and intense mechanical loading (in the form of immobilization, passive motion loading, and compressive motion loading, respectively) on the acute biological response of cartilage, five month old male Sprague Dawley rats (n=5/group) were randomly assigned to five testing groups and exposed to different motion and loading conditions. The first group of animals maintained normal cage activity as served as a baseline control (CTL). A second group of sham control animals (SHAM) were treated exactly the same way as the following loading groups, except that the device was not activated and the animal's hind limb did not receive any loading. In the immobilization group (IMM), animals were immobilized for six hours using a cast made of cotton and steel mesh which fixed the knee in full flexion (115°) as described previously in the literature¹⁰. A forth group of animals (PML) was immobilized for 2.5 hours, remobilized using passive motion for one hour, and immobilized again for 2.5 more hours. The relative range of angular flexion was 50° with angular displacement from 65° to 115° at a rate of 2 cycles per minute. The fifth group consisted of animals that were treated similarly to the PML group, except their joints were additionally loaded during the one hour motion treatment by applying an axial compressive load (CML) equivalent to approximately twice their body weight (1150±50g). After each rat's respective experiment endpoint, the lateral and femoral condyles were dissected, rinsed in 1X PBS prepared with DEPC-treated water, and frozen in liquid nitrogen. The tissue samples were then homogenized with a Mikro Dismembrator S (S. Braun Biotech International, Germany) for 90

seconds. Lysis buffer was added to the ground tissue and RNA was extracted with the RNeasy Mini kit following manufacturer's instructions. The RNA was reverse transcribed (RT) using Oligo(dT) as a primer, and the RT products were amplified with real-time PCR, using GAPDH for normalization. The primers used for real-time PCR were: MMP-13-F: acatggaggagcatgaaagg, MMP-13-R: gacaggagctaaggcagaca, Collagen2a1F: cctgtctgtcttctgtaaac, Collagen2a1R: agcatctgtaggggtctct, GAPDH-F: aggaccaggtgtctctctg, and GAPDH-R: atgtaggccatgaggtccac.

Statistical Analysis

Two-way analysis of variance (ANOVA) was performed on the data testing the real-time angular position measurement by the goniometer and the data testing the kinematics of the apparatus frame and the animal's limb. The correlations among the five-trial measurements were evaluated. ANOVA and the correlation calculations were performed using the Statistic Toolbox of Matlab 7.2 (The Mathworks). The data were shown as mean $\pm$ standard deviation with the percentage denoting the ratio of the computed value relative to the magnitude of the corresponding angular position. ANOVA with Tukey's post-hoc analysis was used to determine statistical significant differences between SHAM, IMM, PML and CML groups versus CTL. A value of $p < 0.05$ was considered significant.

RESULTS

Accuracy, Precision & Repeatability of Real Time Angular Position Measurement

The magnetic goniometer was found to produce accurate and precise angular positioning measurements. Comparison of measured data by the goniometer and the set point reference values (Fig.3) demonstrated a linear correlation between both quantities ($R^2=0.99$) with an angular position accuracy of 0.56° (0.63%) and a precision of $\pm 2.59^\circ$ (1.06%).

The repeatability test showed that the mean of the repetition errors between trial 2–5 and baseline was $1.21^\circ \pm 1.55^\circ$ ($1.40\% \pm 1.78\%$) (Fig.4). Two-way ANOVA results showed there was no repetition effect or cycle effect ($p \gg 0.05$) and there was no interaction between the two effects ($p \gg 0.05$), indicating that the five trials of data were essentially the same. This conclusion was further supported by the correlation coefficients between any two of the five trials that were exclusively greater than 0.99 (data not shown), indicating that the five trials of data were essentially the same.

Misalignment between Apparatus Frame and Animal's Tibia during Dynamic Test

The angular position error between the apparatus frame (or the goniometer) and the animal's tibia measurements indicated a linear correlation between the animal's tibia and the apparatus frame ($R=0.99$) within the 70° - 110° range of motion (Fig.5) and an averaged misalignment error of $1.56^\circ \pm 3.37^\circ$ ($1.69\% \pm 3.78\%$). A slight deviation at the end of the angular motion range was noticed, which may be caused by the natural rotation of the joint during the flexion of the tibia.

Repositioning Error

Repositioning of the animal by the same operator in the JMLS produced similar results ($<1\%$ difference). Mean and standard deviation of the averaged errors between the measurement trial 2–5 and baseline were plotted out against the corresponding angular position reference values in an increment of 5° (Fig.6). Repeated-trial data showed that the average mean and standard deviation of the absolute repositioning error from baseline was $0.17^\circ \pm 0.37^\circ$ ($0.18\% \pm 0.42\%$) and $0.24^\circ \pm 0.73^\circ$ ($0.27\% \pm 0.83\%$) for the frame motion and tibia motion, respectively. Two-way ANOVA results performed for repetition effect of the five individual trials showed the p-

value was 0.9572 and 0.8169 for the frame and tibia motion measurements, respectively, demonstrating that repetitions of the tested procedure do not significantly vary from one trial to another. The p-value for the repetition effect of 20 cycles within a trial was less than 0.001 for both the frame motion and the tibia motion, indicating that the angular cyclic movement between successive cycles was truly equal to each other over the full flexion range. The correlation coefficients between any two of the five trials were found greater than 0.9970 for the frame measurement and greater than 0.9771 for the tibia measurement, with the p-values < 0.005, demonstrating the high resemblance of the repeated trials.

Tissue Responses to the *in vivo* Motion and Loading System

Motion and loading provided by the JMLS can effectively modulate MMP-13 and Col II expressions in chondrocytes (Fig. 7). The immobilization of the knee joint for 6 hours resulted in i) increased expression of MMP-13, a protease known to cleave type II collagen, and ii) reduced expression of Col II, the predominant structural protein in cartilage. Compared with immobilized knee joints, cartilage exposed to 1 hr of PML inhibited the synthesis of MMP-13 by 2.7 fold and up-regulated the expression of Collagen II by 2.2 fold. This result demonstrated the ability of the JMLS to produce an effective chondroprotective stimulus via PML that led to suppression of the catabolic effects of immobilization. In contrast, the biological response of the group of animals treated with compressive motion loading was catabolic and exhibited a pattern similar to that observed in the immobilization group.

DISCUSSION

The JMLS described here provides a reliable, non-invasive way to produce desired angular displacements of a rat knee joint *in vivo*. The angular position generated by the magnetic goniometer was shown to produce accurate, precise and repeatable measurements to monitor in real time the angular displacement of the mobile apparatus. In the accuracy test, the majority of errors between the recorded data and the reference values were < 1% with corresponding standard deviations < 3%, demonstrating profound accuracy and precision of the measurement offered by the goniometer. In the repetition test, the mean (1.40%) and standard deviation (1.78%) of the overall repetition error between the baseline trial and the four subsequent trials indicated the reliability of the goniometer measurement over repeated trials. The repetition error was found to be largely related to the action taken by the operator to position the JMLS to the exact initial angle before starting the motion. Nevertheless, the discrepancy may be considered reasonable for the proposed experiments. In the test for the mobile alignment of the apparatus frame and the animal's tibia during motion, the maximum angular position difference between the frame and the tibia was shown to be smaller than 3°, exhibiting a high correlation between the displacement of the animal's tibia and the apparatus frame. These results demonstrate that the goniometer measurement of the apparatus angular position can adequately monitor the angular position of the animal's tibia indirectly. This approach avoids any invasive intervention (i.e. intra-skeletal fixators) that may generate an inflammatory response and obscure the assessment of the biological markers of interest.

Repositioning of the animal in the JMLS resulted in a measurement error < 1%. The absolute difference between the repeated trials 2–5 and the baseline trial was observed to be very small, with the maximum error less than 1° (0.68% for the tibia measurement and 0.50% for the frame measurement, respectively) and an average close to zero (0.24% for the tibia measurement and 0.18% for the frame measurement, respectively), demonstrating excellent reproducibility in repositioning the animal in the loading apparatus. The standard deviation of measurements in the frame was much smaller than that in the tibia, which was expected since the frame rotates stably on ball bearings, while the tibia has some freedom to move laterally during the rotation of the frame. Nonetheless, the standard deviations can be considered small when compared

with the magnitude of the corresponding angular positions. Overall, the motion kinematics of the apparatus and the animal's limb demonstrate remarkable alignment consistency. The performed tests give confidence that the JMLS can facilitate reliable control of the range of flexion/extension and frequency of the cyclic motion applied to the animal's knee joint. Real time monitoring of the angular position, axial loading magnitude, as well as frequency and direction of angular displacement ensured the designed protocols to be accurately implemented.

Associations between motion/loading and the biological response of chondrocytes have been extensively investigated; however, the acute biological response in whole cartilage tissue has only been explored *in vitro* partially due to the lack of suitable *in vivo* loading devices. In the current study, we developed the JMLS, an assisted joint motion and loading system capable of down-regulating MMP-13 as well as up-regulating the expression of Col II during passive motion loading. The synthesis of MMP-13 exhibited a U-shaped response as a function of load intensity. MMP-13 was increased in response to reduced load (immobilization), and this increase was suppressed by a moderate level of load (passive motion loading) on the background of immobilization. When this moderate load was replaced with a high load (compressive motion loading), a decrease in MMP-13 was not observed; levels of MMP-13 due to compressive loading were comparable to levels after immobilization. The opposite behavior was seen for the production of Col II, demonstrating acute mechanosensitivity of both synthetic and degradative processes *in vivo*. These findings were consistent with our previous *in vitro* studies and reported literature where moderate load had a chondroprotective effect, while immobilization or excessive impact load may have a deleterious effect in cartilage^{15, 31-33}. These findings were also consistent with recent studies by Ferreti et al.^{15, 16} that showed anti-catabolic effects of continuous passive motion for 24h and 48h. We speculate that passive motion loading (PML) as provided by the JMLS produces a moderate shear loading at the contact cartilage surfaces of the knee joint, while compressive motion loading (CML) generates both increased shear and compression loading, and that shear and compressive loading seem to have opposite effects on acute MMP-13 and Col II synthesis *in vivo*.

Altogether, the JMLS differs from the previous *in vivo* loading devices in several respects: 1) It creates a motion and loading environment that is both highly controlled and well quantified. Our intention was not only to provide joint loading levels varying from slow motion to moderate and more intense ranges, but also to quantify the specific loading variables. 2) The adjustable axial compressive force applied on the joint in motion facilitates the characterization of weight bearing on a moving joint. It has been reported that the weight bearing load on a human joint at certain positions can be as high as 10 times the body weight, which may cause cell death, rupture of the collagen fiber matrix, and increase in tissue water content⁷. Our system can supply a sustained compressive loading condition to examine the detrimental effect of clinically relevant overloading conditions at different magnitudes. 3) It features real-time monitoring for proper implementation of a wide variety of motion and loading protocols.

In conclusion, this study provided evidence on the effectiveness of a new joint motion and loading system (JMLS) to create the biomechanical environment necessary to induce a clear biological response of cartilage *in vivo*¹⁹. The flexibility of the JMLS provides a versatile tool for a broad range of biomechanics and mechanotransduction-related experiments on small animals. To our knowledge, this is the first device designed to study the acute response of chondrocytes *in vivo* to mechanical stimuli generated by both passive motion loading and compressive motion loading.

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Appendix

According to the designed motion protocol, the user will input the initial and final angles (in deg) as well as the cyclic frequency (in cycles/minute). Given the initial and final angles, the

calculation of the mathematic relationship between the sides of the triangle formed by the mobile tibia and fixed femur will provide the linear displacement that the actuator needs to travel for the cyclic motion (Fig.8). The speed of the linear actuator can thus be obtained by associating this travel length with the cyclic frequency also inputted by the user for the motion control. The linear cyclic motion generated by the computer-controlled linear actuator is transformed by an adaptor attached on the shaft of the actuator into the repeated angular displacement of the animal's joint between the initial and final angle.

The derivation of the travel length as well as the speed of the actuator for motion control of the system are shown by the equations below:

HK: Length of Femur;

KF: Length of Tibia in Initial Position;

KF': Length of Tibia in Final Position;

VF': Travel Length of Linear Actuator;

A: Femur Fixation Angle;

B: Initial Angle (*inputted by a user*);

C: Angular Displacement;

D: Final Angle (*inputted by a user*);

N: Cyclic Frequency in cycles/min (*inputted by a user*).

Given the angles α , β , and C that can be obtained by A , B and D to be defined or inputted by a user,

$$\begin{aligned}\therefore \alpha &= 2\pi - \frac{\pi}{2} - (\pi - A) - B \\ &= \frac{\pi}{2} + A - B;\end{aligned}\quad (1)$$

$$\beta = \frac{1}{2}(\pi - C); \quad (2)$$

$$C = D - B; \quad (3)$$

the range of angular displacement is given by:

$$\begin{aligned}\therefore \theta &= \pi - \alpha - \beta \\ &= \frac{1}{2}(B + D) - A;\end{aligned}\quad (4)$$

where the distance FF' is described as a function of the initial and final angles B and D defined by the user,

$$\therefore FF' = 2 \times FK \times \sin \left[\frac{1}{2}(D - B) \right]; \quad (5)$$

The linear displacement of the actuator is given by the segment VF' as a function of the angular displacement FF',

$$\begin{aligned} \therefore VF' &= FF' \times \sin \theta \\ &= 2 \times \sin \left[\frac{1}{2}(D - B) \right] \times \sin \left[\frac{1}{2}(B + D) - A \right]; \end{aligned} \quad (6)$$

Therefore, the speed of the linear actuator (mm/s) is determined upon the required linear displacement to achieve the range of angular motion (characterized by A , B , and D) and cyclic frequency (N) selected by the user:

$$\begin{aligned} \text{Speed of Actuator} &= \frac{1}{60} (2N \times VF') \\ &= \frac{1}{15} N \times \sin \left[\frac{1}{2}(D - B) \right] \times \sin \left[\frac{1}{2}(B + D) - A \right] \end{aligned} \quad (7)$$

FIGURE 1. Block diagram of the components of the *in vivo* computer-controlled joint motion and loading System (JMLS)

The JMLS consisted of an animal bed and the anesthesia device, a knee joint motion apparatus with a monitoring goniometer, a linear actuator with micro-stepping drive and a monitoring encoder, a compressive load apparatus with a monitoring load cell, and the hardware and software interfaces.

FIGURE 2. (Left) The schematic diagram of the JMLS design and (Right) Photograph of the experimental setup with an animal undertaking the motion and loading protocol

FIGURE 3. Comparison between the angular position measurement from the goniometer and the reference values designated by the control algorithm

There was a linear correlation between the two quantities ($R^2=0.99$) with an angular position accuracy of 0.56° (0.63%) and a precision of $\pm 2.59^\circ$ (1.06%).

FIGURE 4. Five-trial repetition test result of the angular position measurement offered by the goniometer

Repetition error was defined as the absolute difference between the first trial (baseline trial) and the repeated four trials (trial 2–5). The averaged mean and standard deviation of the errors calculated from trial 2–5 against baseline trial was $1.21^{\circ} \pm 1.55^{\circ}$ ($1.40\% \pm 1.78\%$).

Angular Position Difference between Frame & Tibia

FIGURE 5. Comparison between the angular displacements of apparatus frame and the animal's tibia

The data were measured by the MEMS sensors attached on the frame and the tibia. There was a linear correlation between the two quantities ($R^2=0.99$) and the averaged misalignment error is $1.56^\circ \pm 3.37^\circ$ ($1.69\% \pm 3.78\%$).

FIGURE 6. Five-trial repositioning test result of the angular displacement of the apparatus frame and the animal's tibia

The data were measured by the MEMS sensors attached on the frame and the tibia.

Repositioning error was defined as the absolute difference between the first trial (baseline trial) and the repeated four trials (trial 2–5). The averaged mean and standard deviation of the errors calculated from trial 2–5 against baseline was $0.17^{\circ} \pm 0.37^{\circ}$ ($0.18\% \pm 0.42\%$) for the frame, and $0.24^{\circ} \pm 0.73^{\circ}$ ($0.27\% \pm 0.83\%$) for the tibia.

FIGURE 7. Gene expressions of pro-inflammatory effector MMP-13 and major structural protein in cartilage Collagen II in response to immobilization (IMM), moderate passive motion loading (PML), and compressive motion loading conditions (CML)

The results showed that IMM and CML groups exhibited an up-regulation of MMP-13 and a down-regulation of Collagen II, while PML reversed the catabolic responses caused by immobilization by showing reduced gene expression of MMP-13 and increased gene expression of Collagen II.

FIGURE 8. Control algorithm of the joint motion and loading system

The animal's femur (HK) is maintained at a fixed position, and the tibia is displaced by the actuator back and forth from KF to KF' that corresponds to the initial angle B and final angle D . The vertical displacement for the actuator to travel in a half cycle is VF' that corresponds to the angular displacement C . Given the parameters (initial angle B , final angle D , and cyclic frequency N) that the user inputs on the LabView control panel, the software computes the travel length VF' using FF' and θ that can be described as a function of known variables to obtain the speed for the linear actuator. HK: Length of Femur; KF: Length of Tibia in Initial Position; KF': Length of Tibia in Final Position; VF': Travel Length of Actuator; A: Femur Immobilization Angle; B: Initial Angle (*inputted by a user*); C: Angular Displacement; D: Final Angle (*inputted by a user*).

Table