

# A Direct Compression Stimulator for Articular Cartilage and Meniscal Explants

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**Abstract**—This paper describes the development and use of a direct compression stimulator for culturing explants from the meniscus of the knee and articular cartilage. Following design and fabrication of the instrument along with its data acquisition system, the function of the machine was verified by both mechanical means and tissue effect. The loading chamber can hold up to 45 5 mm diameter samples. While designed to stimulate samples up to 4 mm thick, axial displacements as little as  $0.127\ \mu\text{m}$  are within the theoretical capacity of the stimulator. In gene expression studies, collagen II and aggrecan expression were examined in explants from articular cartilage as well as medial and lateral menisci subjected to dynamic stimulation and static compression. These results were then compared to free swelling samples. It was found that static compression to cut thickness down-regulated aggrecan and collagen II expression in articular cartilage explants compared to free swelling controls by 94% and 90%, respectively. The application of a dynamic, intermittent, 2% oscillation around the cut thickness returned expression levels to those of free swelling controls at 4 h but not at 76 h. In medial meniscus samples, dynamic compression up-regulated aggrecan expression by 108%, but not collagen II expression, at 4 and 76 h compared to static controls. No difference in gene expression was observed for lateral meniscal explants. Thus, effects of direct compression seen in articular cartilage may not necessarily translate to the knee meniscus. The design of this stimulator will allow a variety of tissues and loading regimens to be examined. It is hoped that regimens can be found that not only return samples to the production levels of free swelling controls, but also surpass them in terms of gene expression, protein synthesis, and functional properties.

**Keywords**—Direct compression, Gene expression, Meniscal explants, Cartilage explants, Dynamic stimulation.

## INTRODUCTION

Articular cartilage and the knee meniscus share many properties, which must be considered when investigating responses to mechanical stimulation. Both of these tissues have shown a susceptibility to a wide range of injuries from automobile accident to sports related trauma. Damage to the menisci of the knee almost invariably leads to degeneration

of the underlying articular cartilage, as greater stresses are subsequently placed upon the cartilage. In the United States alone, it is estimated that osteoarthritis affects 20.7 million Americans; 600,000 surgeries are performed each year due to complications of the meniscus.<sup>38</sup>

The mechanical environment in which a tissue is placed can have a profound effect on that tissue's biology.<sup>42,50,53</sup> It has been shown that articular chondrocytes and meniscal fibrochondrocytes respond to changes in their mechanical environment *in vivo*, but the mechanisms of matrix and mechanical property regulation are as of yet not fully understood.<sup>8,9,19,20,32</sup> Due to the inherent difficulties of study in the *in vivo* environment, several *in vitro* studies have been undertaken to examine the effects of mechanical stimulation on cartilage and the meniscus.<sup>3,5–7,17,18,24,25,36,37,39–41,43–49</sup> More recently, direct compression mechanical stimulation has been used toward the goal of functional tissue engineering.<sup>28–30,39,51</sup>

Several different methods of driving these stimulation devices have been developed, including pneumatics, stepper motors, and cam driven pistons, as well as adaptation of commercially available systems.<sup>11,30,35,40,43,49</sup> The methods of controlling these devices have fallen into two broad categories: load or deformation control. Previous deformation-control studies have used the cut thickness of the explant as the control.<sup>3,18,40</sup> Dynamic compression is then performed by oscillating about that point. Load-control studies have generally used a free swelling control,<sup>21,25,27,33,34,41,43,45,46,48,49</sup> though some studies have used an intermediate value of the applied cyclic force as a static control.<sup>17,25</sup> Some of the load controlled studies have also examined the effect of a statically applied constant load equal to the intermittently applied load.<sup>21,34,48</sup>

There have been a number of previous direct compression studies performed on articular cartilage explants and chondrocytes seeded in gels or on scaffolds. It has been found that static compression is detrimental to gene expression and synthesis in explants.<sup>7,12,25,36,54</sup> In addition, down-regulation of chondrocytes cultured in gels also occurred when statically compressed.<sup>5,15,16,22,37</sup> To date, numerous dynamic loading studies have been performed

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and, as reviewed,<sup>2</sup> the results from these are anything but conclusive. Frequency, strain, stimulation duration, and loading duty cycle all have been shown to contribute to the effects of dynamic stimulation.<sup>4, 25, 33, 40, 41, 44–46, 48</sup> The effects of direct compression also appears to be influenced by the age of the tissue being stimulated, with responsiveness to direct compression diminishing with age.<sup>23, 25, 27, 34</sup>

Far less research has been performed in terms of mechanical stimulation of meniscal cells or explants. Static compression has been shown to decrease radiolabeled proline and sulfate incorporation in bovine menisci.<sup>17</sup> Another experiment found that expression for Col I and II was significantly down-regulated following 24 h of static compression (0.1 MPa) while aggrecan expression was not changed.<sup>49</sup> Dynamic stimulation (0.16 MPa max, 0.08 MPa minimum, 0.5 Hz, 24 h) did not appear to regulate aggrecan expression under this loading protocol.<sup>49</sup> To date, no deformation-controlled dynamic compression studies have been performed on meniscal explants. However, a tensile strain study examining varying frequencies demonstrated meniscal fibrochondrocytes suppress proinflammatory markers when subjected to dynamic tensile forces.<sup>10</sup> Certainly more work is warranted in exploring the role mechanical stimulation may have in tissue maintenance and regeneration of articular cartilage and the knee meniscus.

Both articular cartilage and the knee meniscus have a diminished capacity for complete recovery post injury. As such, they are ideal candidates for tissue engineering attempts. It is hoped that a complete tissue engineering strategy for these tissues can be developed that will use a combination of growth factors, mechanical stimulation, and an appropriate scaffold. The objectives of this study were to develop a direct compression stimulator and examine the effect of direct compression on gene expression in articular cartilage, as well as lateral and medial meniscal explants. We hypothesized that dynamic stimulation of articular cartilage and meniscal explants will up-regulate expression of aggrecan and Col II compared to static and free swelling controls.

## MATERIALS AND METHODS

### *Mechanical Stimulator Design and Components*

The mechanical stimulator is designed to fit inside a normal incubator and has a large aluminum base for stability (Fig. 1). Two 25.4 mm stainless steel rods serve as both guide rails for the upper platen and as supports for the upper crosspiece. The motion of the upper platen is provided by a linear stepper motor (LA23ECKJ-4, Eastern Air Devices, Dover, NH), which is attached to the upper crosspiece of the system. A threaded rod, which engages a threaded sleeve within the stepper motor, is attached to a cross plate that holds the mounting equipment for the upper platen in the center and is uniaxially restrained at both ends by linear bearings (McMaster-Carr, Atlanta, GA).

The stepper motor is driven by a micro-stepper drive (IM483, Intelligent Motion Systems, Marlborough, CT). The stepper motor driver is powered by a stepper motor amplifier (ISP200, Intelligent Motion Systems). A load cell provides force feedback to ensure contact with explants and for force feedback operation (DSM-100, Transducer Techniques, Temecula, CA). Linear variable displacement transducers (LVDTs) provide positional feedback (500 HCA-200, Schaevitz, Hampton, VA).

The direct compression stimulator is connected to a computer for control. Motion of the upper platen is achieved with the linear stepper motor, which is interfaced with a motion control board (PCI 7344, National Instruments, Austin, TX). Data are collected through a data acquisition board (6048E, National Instruments). This system gives a theoretical positional control of 0.127  $\mu\text{m}$ . The software used to control the components is LabView 7.1 (National Instruments).

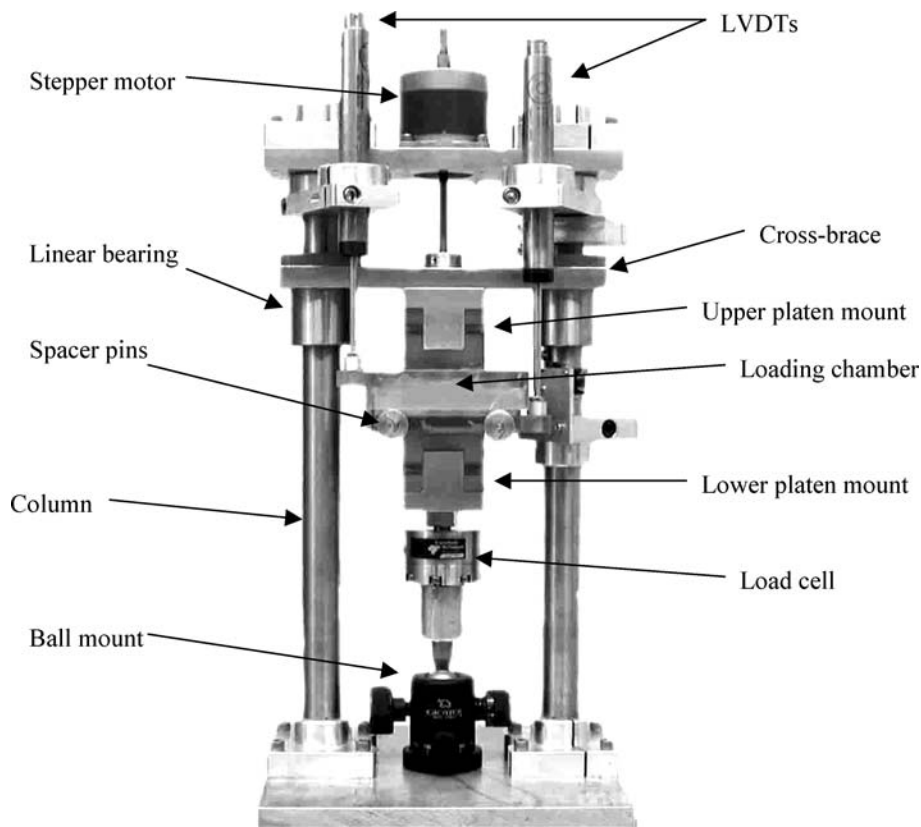
The explant chamber (Fig. 2) is constructed of polysulfone, which was chosen due to its biocompatibility, ease of machining, ease of sterilization, and compressive modulus of 2.6 GPa. The compression chamber can hold up to 45 samples and allows media to circulate amongst the samples. The upper and lower halves of the chamber are separated by safety pins, which allow the chamber to be removed from the stimulator without crushing the samples inside. A quick release system allows the explants to be loaded into the chamber in a sterile environment and then transferred to the direct compression stimulator. After the chamber is mounted in the stimulator, the safety pins are removed, allowing unhindered axial compression of the samples. Due to possible variations in the position of the attachment of the chamber due to play in the quick release mounts, an LVDT for both the upper and lower platens is included, and the difference between the two is calibrated for sample height.

### *Stimulator Validation*

Linear validation of movement of the stepper motor was compared to both LVDT output and to a dial indicator (Last Word Dial Indicator, Starret, Athol, MA). The force transducer was calibrated by known weights. Validation of platen separation was attained by comparing measured separation to known custom made spacers. Validation of depth of wells and upper platen uniformity was performed with a dial indicator.

### *Explant Harvest and Culture*

Five knees from young calves were acquired within 24 h of slaughter (Research 87, Inc.). The joint capsule was opened using sterile technique. Medial and lateral menisci were isolated and transferred to sterile PBS. Full thickness explants of 5 mm diameter were taken using a dermatological punch in a manner similar to that which has been described previously.<sup>17</sup> Plugs were taken



**FIGURE 1.** Direct compression stimulator. The loading chamber is held in place by 8 pins. This allows for loading in a sterile environment and then transfer to the machine in the incubator.

in the axial direction, from the femoral surface to the tibial surface, and thus all had the same orientation when placed in the stimulation chamber. The surfaces of the menisci were removed, leaving only a 2 mm thick disc from the deep zone of the meniscus. Cartilage explants from the femoral condyles were harvested in the same manner, leaving only cartilage from the middle deep zone. These explants were then washed with PBS and placed in medium, which contained Dulbecco's Modified Eagle Medium (DMEM) with Glutamax™ (Invitrogen, Grand Island, NY) supplemented with 100 units/ml Penicillin (Invitrogen), 1% Fetal Bovine Serum (FBS) (Biowhittaker, Frederick, MD), 2.5 mg/ml Fungizone (Gemini Bioproducts, Woodland, CA), 0.1 mM non-essential amino acids (NEAA) (Invitrogen), and ascorbic acid (25 mg/L). One percent FBS was chosen so as to provide a minimum of progression and competence growth factors without saturating the sample and overshadowing the effects of mechanical stimulation. Samples were allowed to equilibrate for 4 days post harvest prior to stimulation.

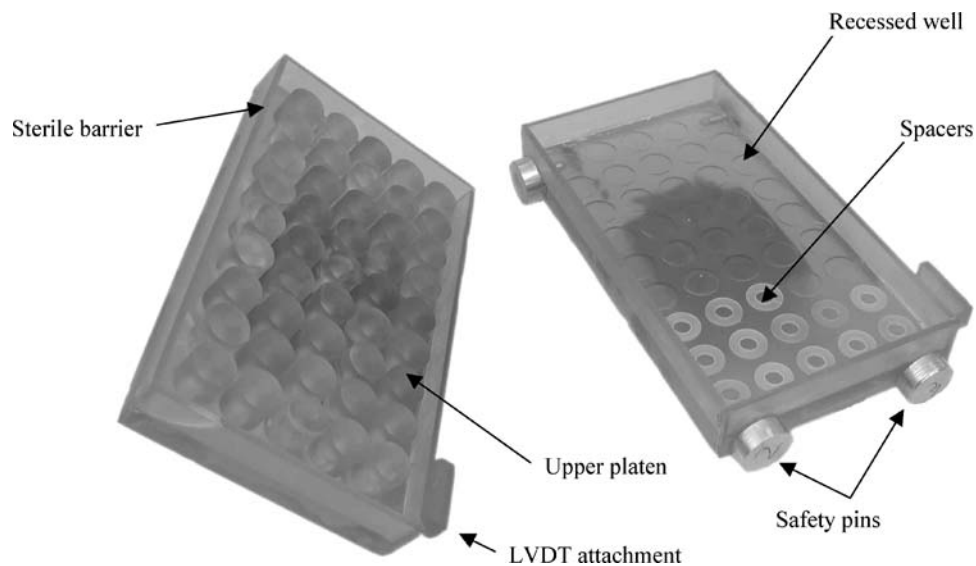
#### *Mechanical Stimulation*

All loading experiments were conducted in a tissue culture incubator at 37°C, 10% CO<sub>2</sub>. The loading chamber

was sterilized with ethylene-oxide prior to the insertion of explants. Dynamic stimulation (compressed to the cut thickness of 2 mm with a 2% oscillatory strain, 1 Hz, 60 s on 60 s off duty cycle) was applied for 4 h per day for 4 consecutive days to articular cartilage and medial and lateral meniscal explants. Compression back to cut thickness was necessary, as the samples from all groups had swollen since harvest. Force readings were obtained to ensure that liftoff, or platen separation between the samples and the platens, did not occur. At all measured times, the compressive force was greater than 0.5 N. Static controls were compressed to the cut thickness, but no oscillation was applied. Free swelling controls were placed in the same chamber for 4 h as well but were not compressed. Samples were harvested immediately after the first 4 h stimulation and on the 4th day immediately after the stimulation had been completed. These two groups are termed 4 and 76 h respectively.

#### *RNA Isolation*

The explants were placed in RNAlater (Ambion, Austin, TX) and frozen at -20°C for no longer than 2 weeks. Articular cartilage RNA was then isolated using TriZol Reagent (Invitrogen, Grand Island, NY). Meniscal RNA was isolated using the FastRNA Pro Green Kit (Qbiogene Inc.,



**FIGURE 2. Chamber components.** The entire chamber is composed of polysulphone that is autoclaveable. Samples are placed in wells of uniform depth so position is maintained under the loading heads.

CA) and FastPrep Instrument (Qbiogene Inc.). Meniscal explants were placed into a tube containing beads and 1 ml of FastRNA Pro solution (Qbiogene Inc). The total amount of RNA was normalized across all samples by using a spectrophotometer (ND-1000, NanoDrop, Wilmington, DE). The RNA was reverse transcribed to DNA using Stratascript™ First Strand Synthesis System (Stratagene, La Jolla, CA).

Quantitative reverse transcriptase-polymerase reaction was performed for Col II, aggrecan and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to determine gene expression levels. For each gene a forward primer, reverse primer, and a gene-specific probe were used. The primers and probes are listed in Table 1, and the primers were designed from bovine and human mRNA sequences from The National Center for Biotechnology Information (NCBI). qRT-PCR was performed using HotStarTaq Master Mix Kit (Qiagen, Malencia, California). The concentration of the MgCl was 3.5 mM for all reactions.

#### *Gene Expression*

GAPDH was used to verify the presence of cDNA in the samples as a positive control. The efficiencies for each gene were found by running standard curves for the qRT-PCR tests. The abundance of each gene of interest ( $A_{GOI}$ ) was found using the equation:

$$A_{GOI} = \frac{1}{(1 + E_{GOI})^{C_{t,GOI}}}$$

where  $E_{GOI}$  and  $C_{t,GOI}$  are the efficiencies and take off cycle numbers of the gene of interest, respectively.

#### *Statistical Analysis*

A sample size of  $n=4$  or 5 was used for all gene expression experiments. Samples from each of the five bovine knees were randomized throughout all experimental groups. Each sample was run in triplicate. A multifactor ANOVA with repeated measures was performed on the qRT-PCR results using statistics software (JMP IN 5.1, SAS Institute Inc, Cary, NC). If significance existed, a Tukey's HSD post-hoc was performed with  $p < 0.05$  being considered significant. All data are expressed as mean  $\pm$  standard deviation.

## **RESULTS**

#### *Stimulator Design*

The direct compression stimulator proved easy to use inside the incubator. Samples were loaded into the sterile direct compression chamber in a laminar flow hood. The entire chamber was then transferred into the frame of the direct compression stimulator. The machine had a 3 mm maximum axial displacement and linear resolution of  $\pm 1 \mu\text{m}$ . The machine is also capable of applying 445 N with a resolution of 0.02 N. The maximum frequency of stimulation as configured is 5 Hz.

#### *Gross Inspection*

Upon gross inspection, articular cartilage explants maintained a smooth, shiny surface for the 4 days in culture (Fig. 3). The free swelling group at 4 h ( $2.4 \pm 0.2$  mm, Fig. 3A) was noticeably thicker than the statically compressed samples ( $2 \pm 0.1$  mm, Fig. 3B). The dynamically

**TABLE 1. Target gene sequences, dyes, and quenchers.**

Gene* (accession number, product size)	Forward primer, reverse primer, probe	Dye, quencher
Glyceraldehyde-3-phosphate dehydrogenase (U85042, 86bp)	ACCCTCAAGATTGTCTAGCAA ACGATGCCAAAGTGGTCA CCTCCTGCACCACCAACTGCTT	FAM BHQ-1
Collagen type II (NM_174520, 69bp)	AACGGTGGCTTCCACTTC GCAGGAAGGTCATCTGGA ATGACAACCTGGCTCCCAACACC	ROX BHQ-2
Aggrecan (U76615, 76bp)	GCTACCCTCACCTTCATC AAGCTTTCTGGGATGTCCAC TGACGCCATCTGCTACACAGGTGA	Cy5 BHQ-2

Note. GAPDH was used as a positive control for qRT-PCR.

\*All sequences are 5'-3'.

compressed samples ( $2.3 \pm 0.1$  mm, Fig. 3C) appeared similar to the free swelling group (Fig. 3A). The stimulated samples recovered overnight to greater than their cut thickness of 2 mm. At 76 h, the free swelling group was thicker ( $2.5 \pm 0.2$  mm, Fig. 3D) than the free swelling group at 4 h. The dynamic group at 76 h was approximately the same thickness ( $2.4 \pm 0.1$  mm, Fig. 3F) as the free swelling group. The statically compressed group had also increased slightly in thickness ( $2.1 \pm 0.1$  mm, Fig. 3E).

Similarly, immediately after the first 4 h of compression, both the cut thickness ( $2.1 \pm 0.0$  mm) and dynamically compressed ( $2.1 \pm 0.1$  mm) medial meniscal samples were visibly thinner than free swelling samples ( $2.2 \pm 0.1$  mm, Fig. 4 A–C). Only the free swelling meniscal group increased in thickness over the 4-day experiment ( $2.3 \pm 0.2$  mm, Fig. 4D), while both the dynamic and statically compressed groups remained at approximately 2.1 mm thick (Fig. 4E and F). The direct compression stimulator left no visible marks or damage to the surfaces of compressed samples. Lateral menisci were similar upon inspection to the medial meniscus (not shown).

### Gene Expression

Initially, a three factor ANOVA was run to examine the effects of tissue type, time, and culture condition. This demonstrated that the tissue types were significantly different ( $p < 0.0001$ ) in terms of aggrecan and Col II abundance. Thus, the groups were divided by tissue and a two factor ANOVA was performed for each tissue separately to further elucidate the effects of culture condition and time. When an individual term was significant, an interaction test of time and culture condition was analyzed for significance. The result of the interaction test shows differences in aggrecan and Col II expression between the six treatment groups (two times and three culture conditions).

A two factor ANOVA demonstrated that time was not a significant factor between 4 and 76 h for Col II expression ( $p = 0.38$ ) or aggrecan expression ( $p = 0.53$ ). However, culture condition for both genes was significant ( $p <$

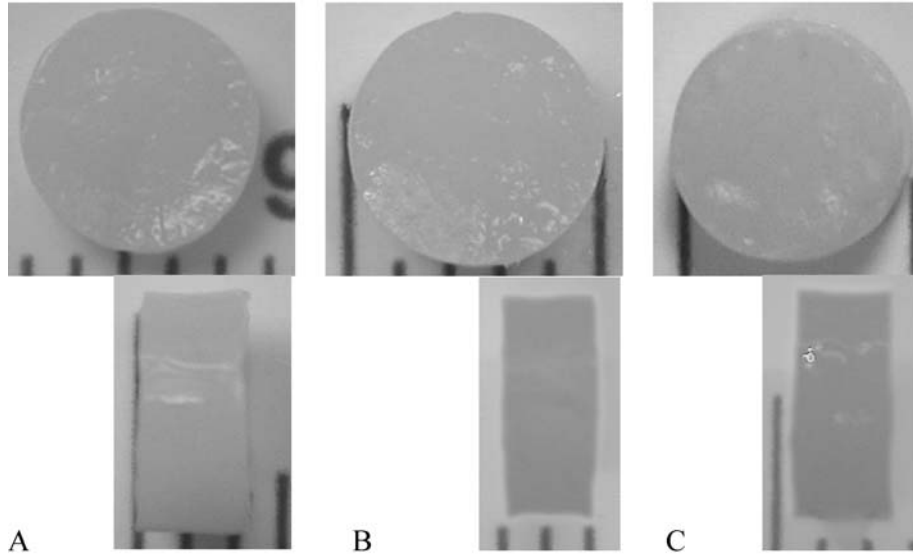
0.01). An interaction test between the two for Col II was performed and is shown in Fig. 5. A similar graph is shown for aggrecan in Fig. 6. Following 4 h of direct dynamic compression (1 Hz, 60 s on, 60 s off), expression of Col II in articular cartilage explants did not differ significantly from free swelling controls (Fig. 5,  $0.87 \pm 0.38 \times 10^{-4}$  and  $1.18 \pm 0.19 \times 10^{-4}$ , respectively,  $p > 0.05$ ), while statically compressed samples were much lower ( $1.20 \pm 2.18 \times 10^{-5}$ ,  $p < 0.05$ ). However, at 76 h (Fig. 5B) after the first compression began, the abundance of Col II in dynamically stimulated samples ( $0.39 \pm 0.19 \times 10^{-4}$ ) had decreased to that of cut thickness controls ( $0.38 \pm 0.18 \times 10^{-4}$ ) while the free swelling group ( $1.15 \pm 0.25 \times 10^{-4}$ ) had not changed significantly compared to 4 h free swelling group ( $p > 0.05$ ).

Aggrecan expression was also measured at 4 and 76 h. Free swelling and dynamically stimulated cartilage explants (Fig. 6,  $1.05 \pm 0.48 \times 10^{-8}$  and  $0.94 \pm 0.53 \times 10^{-8}$ , respectively) were not significantly different from each other at 4 h ( $p > 0.05$ ), but were both significantly higher than statically compressed samples ( $0.06 \pm 0.10 \times 10^{-8}$ ,  $p < 0.05$ ). At 76 h, statically compressed samples were not significantly different from dynamically compressed samples ( $0.44 \pm 0.33 \times 10^{-8}$  and  $0.52 \pm 0.50 \times 10^{-8}$ , respectively,  $p > 0.05$ ) but both were significantly less than free swelling samples ( $1.60 \pm 1.05 \times 10^{-8}$ ,  $p < 0.05$ ) which had not changed significantly from 4 h ( $p > 0.05$ ).

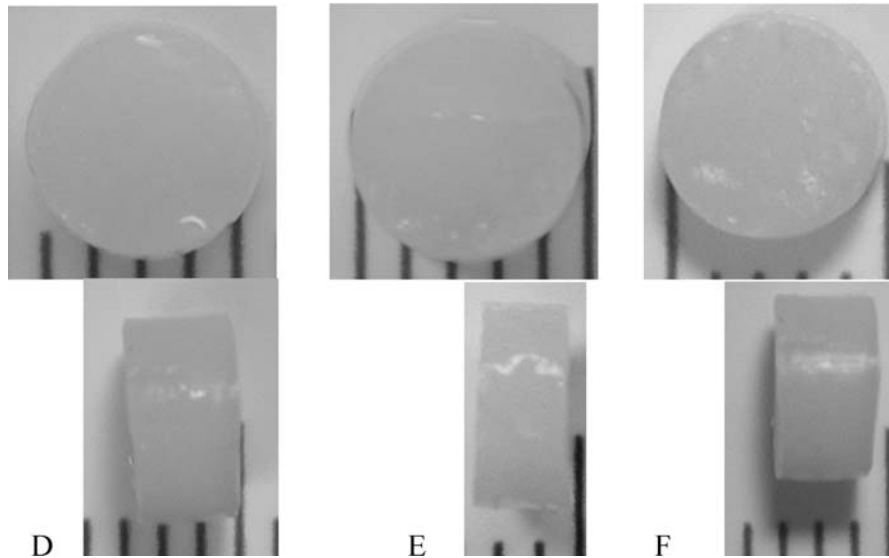
The same loading regimen was used for both medial and lateral menisci. Time was significant for Col II expression ( $P = 0.043$ ) but culture condition was not significant ( $p = 0.46$ , Table 2) and the cross of these effects was not significant ( $p > 0.05$ ) in the medial meniscus. Time and culture condition were both significant for aggrecan ( $p < 0.001$ , Fig. 7) for the medial meniscus. Dynamically loaded medial meniscal tissue (4 h =  $1.65 \pm 1.08 \times 10^{-8}$ , 76 h =  $2.4 \pm 2.26 \times 10^{-10}$ ) did not demonstrate any significant difference for Col II expression from cut thickness (4 h =  $4.14 \pm 3.44 \times 10^{-8}$ , 76 h =  $2.65 \pm 4.56 \times 10^{-10}$ ,  $p > 0.05$ ) or free swelling controls (4 h =  $0.86 \pm 1.33 \times 10^{-7}$ , 76 h =  $2.74 \pm 1.55 \times 10^{-10}$ ) at 4 or 76 h.



4 hrs: Free swelling (A), cut thickness (B), dynamic (C)



100 hrs: Free swelling (D), cut thickness (E), dynamic (F)



**FIGURE 3.** Articular cartilage explants. A) 4 h, free swelling articular cartilage explants. B) 4 h, statically compressed articular cartilage explants. C) 4 h, dynamically stimulated articular cartilage explants. D) 76 h, free swelling articular cartilage explants. E) 76 h, statically compressed articular cartilage explants. F) 76 h, dynamically stimulated articular cartilage explants.

The abundance values over this period did not decrease significantly ( $p > 0.05$ ).

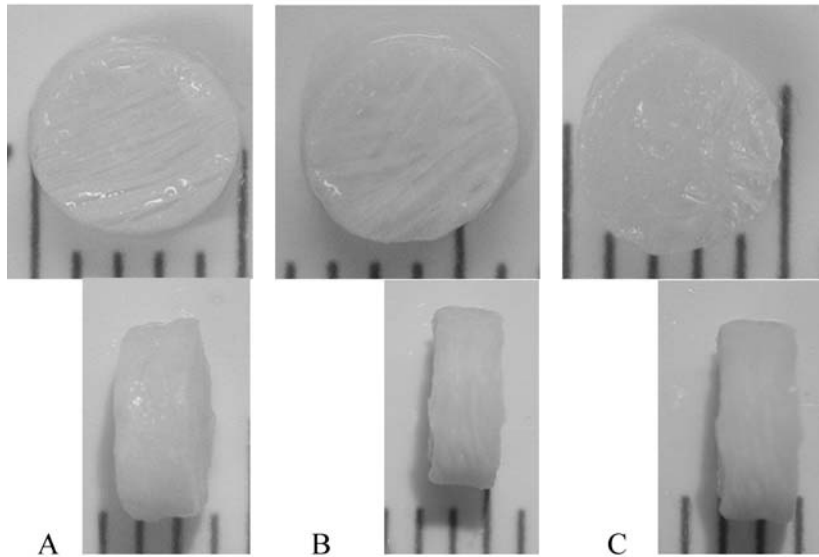
Static compression of the medial meniscus (Fig. 7) significantly decreased aggrecan expression ( $6.66 \pm 1.53 \times 10^{-9}$ ) compared to dynamic ( $1.39 \pm 0.41 \times 10^{-8}$ ) and free swelling controls ( $1.28 \pm 0.41 \times 10^{-8}$ ) after 4 h of stimulation ( $p < 0.05$ ). After 76 h of total culture time, dynamically compressed medial meniscus samples ( $6.88 \pm 5.31 \times 10^{-9}$ ) demonstrated significantly more aggrecan expression than statically compressed samples ( $0.938 \pm 0.791 \times 10^{-9}$ ,  $p < 0.05$ ), however the free

swelling group at 76 h expressed significantly less aggrecan than at 4 h. Neither time nor culture condition was significant for aggrecan or Col II in the lateral meniscus ( $p > 0.05$ , Table 3).

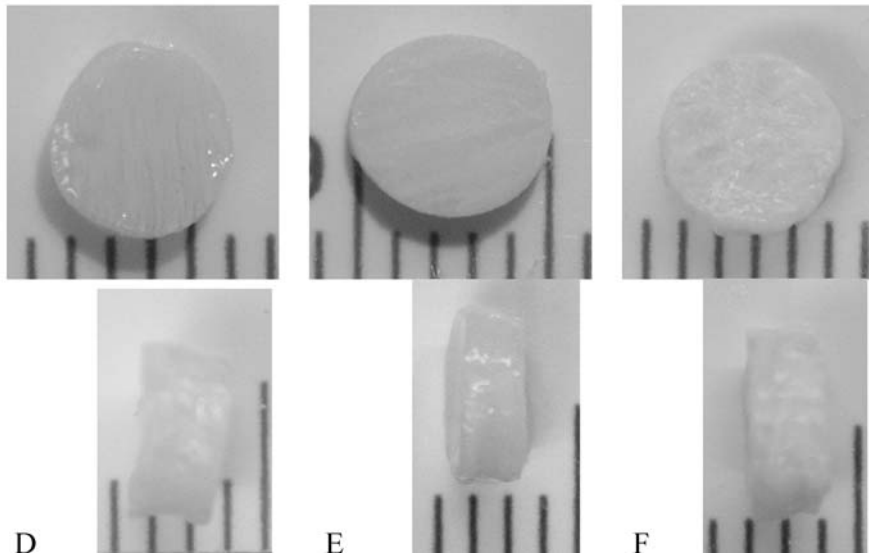
## DISCUSSION

In this study, we have successfully designed, fabricated, and validated a direct compression stimulator capable of culturing cartilaginous tissues. This is critical for the pursuit of the goal of a tissue engineered articular cartilage

4 hrs: Free swelling (A), cut thickness (B), dynamic (C)



76 hrs: Free swelling (D), cut thickness (E), dynamic (F)

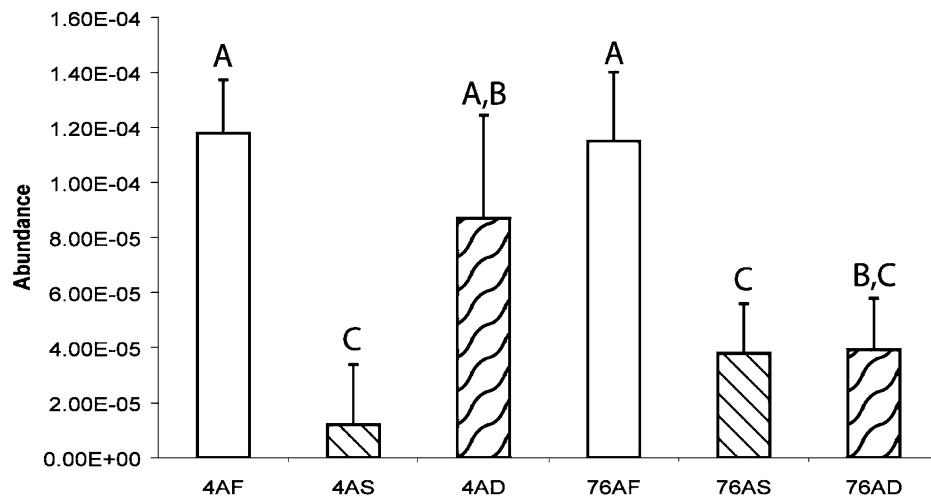


**FIGURE 4. Medial meniscus explants. A) 4 h, free swelling medial meniscus explants. B) 4 h, statically compressed medial meniscus explants. C) 4 h, dynamically stimulated medial meniscus explants. D) 76 h, free swelling medial meniscus explants. E) 76 h, statically compressed medial meniscus explants. F) 76 h, dynamically stimulated medial meniscus explants.**

or knee meniscal construct, where mechanical stimulation is believed to be essential.<sup>2</sup> With this stimulator, we can examine a wide range of loading modalities, probing such factors as frequency, duration, strain, and duty cycle, with the overall goal to find a treatment that exceeds the results shown in free swelling culture. We chose a modular design for the construction of our stimulator to easily allow for changing requirements. For instance, changing the load cell allows for more precise work or for greater loads and changing the loading platens allows for different, novel shaped constructs to be stimulated. We validated our ma-

chine by both verification of mechanical operation and by gene expression measurements in articular cartilage and meniscal explants.

One question that arises during any mechanical loading experiment is the appropriate use of a control for the dynamic loading samples. The obvious first choice is a free swelling, unloaded control. This, however, has problems. A free swelling control is an artifact of the *in vitro* environment. In a previous study, free swelling articular cartilage control discs were found to swell 25–45% in the axial direction.<sup>40</sup> In a worst case scenario (45% swelling

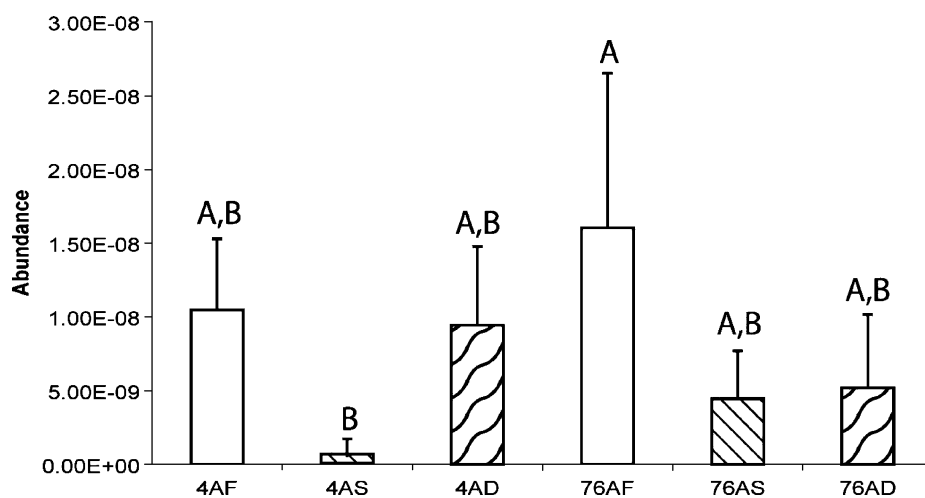


**FIGURE 5.** Articular cartilage collagen II expression shown at 4 and 76 h. Data shown as *mean ± standard deviation*. Groups not connected by same letter are significantly different ( $p < 0.05$ ). AF: Articular cartilage free swelling, AS: Articular cartilage compressed to cut thickness, AD: Articular cartilage dynamically stimulated.

from cut thickness, no upper platen) for a 5 mm diameter, 2 mm cut thickness sample, the free swelling control has 108% greater surface area available for nutrient transport than the cut thickness sample placed between two platens. Even if no load is applied, the use of a non-porous upper platen reduces surface area by 30%. Some studies using force-controlled methods of applying load have used some constant force, usually the mean value of the applied cyclic force, as their static controls in addition to free swelling controls.<sup>25,26</sup> Previous studies using the deformation-control method have often used the cut thickness of the explants as the control. Dynamic compression is then performed by oscillating about that point.<sup>3,40</sup> Thus, for the validation

of our machine, we included both a free swelling positive control and cut thickness (static) control.

We chose 1% FBS, compared with other studies that have used 10% FBS, so as to provide a minimum of necessary growth factors, but not so much as to saturate the effects of dynamic stimulation. Research with growth factors has shown that normal human serum can contain as much as 153 ng/ml of insulin-like growth factor-I and 50 ng/ml of platelet derived growth factor.<sup>1,55</sup> More recently, it has been shown that TGF- $\beta$ 1 levels in fetal bovine serum can range as high as 13 ng/ml.<sup>52</sup> These same growth factors modulate collagen production and cell proliferation.<sup>13,31</sup> Thus, it was thought prudent to have a minimum of



**FIGURE 6.** Articular cartilage aggrecan expression shown at 4 and 76 h. Data shown as *mean ± standard deviation*. Groups not connected by same letter are significantly different (ANOVA:  $p < 0.05$ ). AF: Articular cartilage free swelling, AS: Articular cartilage compressed to cut thickness, AD: Articular cartilage dynamically stimulated.



**TABLE 2. Lateral meniscus gene expression.**

Group, time	Col II abundance	AGC abundance
Free swelling, 4 h	3.93E-08 ± 1.78E-08	5.70E-08 ± 4.20E-08
Statically compressed, 4 h	5.81E-07 ± 8.07E-07	2.78E-08 ± 2.42E-08
Dynamically stimulated, 4 h	1.07E-07 ± 1.15E-07	2.95E-08 ± 1.48E-08
Free swelling, 76 h	5.72E-08 ± 5.65E-08	2.33E-08 ± 1.63E-08
Free swelling, 76 h	4.27E-08 ± 4.10E-08	1.69E-08 ± 6.95E-09
Dynamically stimulated, 76 h	1.98E-07 ± 3.09E-07	4.07E-08 ± 2.39E-08

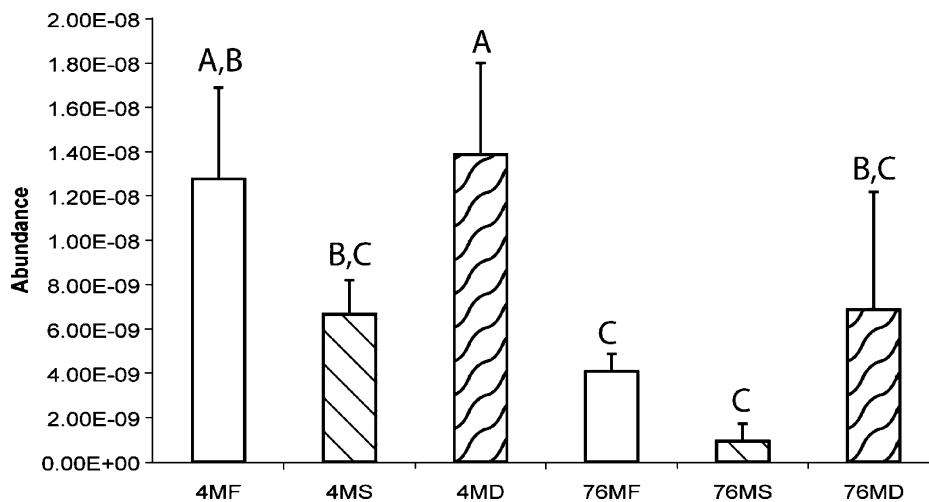
Note. All data are mean ± S.D. Neither culture time nor culture condition was significant. (ANOVA:  $p > 0.05$ ).

exogenous growth factors in the medium so as not to overshadow the effects of the compression loading.

Another difference between our study and previous direct compression research is the CO<sub>2</sub> level in the incubator. Previous research has shown that decreased oxygen tension is chondrogenic and has an additive effect when combined with intermittent hydrostatic pressure.<sup>14</sup> This decreased oxygen tension may have provided an added benefit to the articular cartilage explants. It is also reasonable to culture the meniscal explants in the same condition as it is generally accepted that the inner one third of the meniscus, which is most like articular cartilage, is the most likely to benefit from tissue engineering approaches. It would be interesting in the future to examine the effect of direct compression and varying levels of CO<sub>2</sub> with meniscal explants.

Our strain and frequency were chosen from published literature where beneficial effects were noted.<sup>3,30,40</sup> We chose a 60 s on, 60 s off duty cycle as there is some evidence for an up-regulation of synthesis with a resting period following stimulation and that the optimum timeframe for unloading between loading periods is less than 100 s.<sup>41</sup> Static compression has been shown to down-regulate Col II and ag-

grecan expression in chondrocytes.<sup>36</sup> Our articular cartilage results correlate well with previous research.<sup>3,36,40</sup> In response to static compression to cut thickness, a clear down-regulation of aggrecan and Col II gene expression was observed in articular cartilage compared to free swelling controls, more so than previously published.<sup>36</sup> Previous research compressing explants from free swelling back to cut thickness has not shown a significant decrease in gene expression.<sup>36</sup> However, this phenomenon has been observed in alginate cultures.<sup>37</sup> Ragan *et al.*<sup>36</sup> demonstrated that samples compressed to cut thickness were not significantly different from free swelling controls. A possible explanation for this is our use of 1% rather than 10% FBS. This may imply that the growth factors in FBS mask or in other ways cover the effects of static direct compression. In our study for articular cartilage, a 2% dynamic oscillation returned expression levels to those of free swelling controls at 4 h. After 4 h, dynamically stimulated articular cartilage explants had a 7.3 times higher Col II and a 15.7 times higher aggrecan expression than static groups. The free swelling articular cartilage groups were not significantly different from each other at 4 or 76 h. On the 4th consecutive day of



**FIGURE 7. Medial meniscal aggrecan expression shown at 4 and 76 h. Data shown as mean ± standard deviation. Groups not connected by same letter are significantly different (ANOVA:  $p < 0.05$ ). MF: Medial meniscus free swelling, MS: Medial meniscus compressed to cut thickness, MD: Medial meniscus dynamically stimulated.**

**TABLE 3. Medial meniscus gene expression.**

Group, time	Col II abundance
Free swelling, 4 h	8.63E-08 ± 1.33E-07
Statically compressed, 4 h	4.14E-08 ± 3.44E-08
Dynamically stimulated, 4 h	1.65E-08 ± 1.08E-08
Free swelling, 76 h	2.74E-10 ± 1.55E-10
Free swelling, 76 h	2.65E-10 ± 4.56E-10
Dynamically stimulated, 76 h	2.40E-10 ± 2.26E-10

*Note.* All data are mean ± S.D. Neither culture time nor culture condition was significant. (ANOVA:  $p > 0.05$ ).

compression, both dynamic and static articular cartilage compression groups were not significantly different from each other for either gene measured. This may be due to a greater strain in the dynamic samples as they continued to swell, thus the down-regulation of gene expression. Our dynamic loading regimen dictated that explants were compressed to their cut thickness of 2 mm and then a 2% intermittent, oscillatory strain was superimposed. On the 4th day, the free swelling group had swollen to an average of 2.5 mm with a maximum of 2.7 mm. The dynamic group had swollen to a similar thickness and this corresponds to approximately 26% maximum static strain. This is within the detrimental range observed previously for gene expression.<sup>17,36</sup> During the same loading conditions, the lateral meniscus exhibited no change in gene expression. Interestingly, the medial meniscus did exhibit a change in aggrecan expression at both 4 and 76 h (2 and 7 times static controls, respectively). The free swelling group did significantly decrease in aggrecan gene expression between 4 and 76 h. A previous meniscal gene expression study demonstrated a down-regulation of ColII, Col II and decorin during static compression.<sup>49</sup> In the same study, dynamic stimulation appeared to down-regulate Col II while it did not change aggrecan expression. This is in contrast to our work, which showed a down-regulation of aggrecan due to static compression, but no difference in aggrecan expression in dynamically stimulated samples when compared to the free swelling controls. There are many possible explanations for these differences including different frequency (1 Hz vs. 0.5 Hz), duration (4 h vs. 24 h), duty cycle, percent FBS (1% vs. 10%), as well as deformation versus load control of the stimulation.

It has been thought that stimulation techniques used for articular cartilage may also prove beneficial for tissue engineering the knee meniscus. Our results suggest that while direct compression does have an effect on both articular cartilage and the knee meniscus, the results are not directly comparable. Further work is required to determine whether the initial thickness or swollen thickness is most appropriate for calculating beneficial strain on explants. Longer-term studies must be done to examine the effects of dynamic compression not just on gene expression, but also on protein synthesis and mechanical properties. Following

design, fabrication, and validation of this direct compression stimulator, future goals will be to establish a loading regimen that produces more matrix and greater mechanical properties than controls in both articular cartilage and the knee meniscus.

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