Extended Mechanical Stimulation of Cartilage for Growth and Repair

by

Gary C. Lee

Submitted to the Department of Electrical Engineering and Computer Science

in Partial Fulfillment of the Requirements for the Degree of

Master of Engineering in Electrical Engineering and Computer Science

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Abstract

Extended mechanical stimulation of articular cartilage in an *in vitro* model explant system promotes growth and repair. An alternating day mechanical loading protocol consisting of dynamic sinusoidal compression results in long term extracelluar matrix macromolecule biosynthesis and increased biosynthetic rates in the cartilage system. The protocol uses total glycosaminoglycan and DNA content assays to measure matrix macromolecule biosynthesis and uses L-^{3}H-proline and ^{35}S-sulfate radiolabel incorporation rates to measure rates of biosynthesis. The results also demonstrate the feasibility of replacing the fetal bovine serum and L-ascorbic acid components of the typical explant feeding medium with ITS (insulin-transferrin-selenium solution) and acorbyl-2-phosphate in anticipation of future long term studies of the synergistic pathways of dynamic compression and IGF-I stimulation for cartilage growth and repair.

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Title: Professor of Electrical, Mechanical, and Biological Engineering

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Chapter 1 - Introduction

1.1 Background

Articular cartilage is a dense connective tissue that lines the end of synovial or bag-like joints. Cartilage is responsible for the mobility of joints, including the properties of dissipating stresses or shocks and lowering frictional forces within joints. It is comprised of chondrocytes fixed in an extracellular matrix (ECM) consisting of collagen type II, aggrecan, and other highly charged proteoglycans (Figure 1.1). Cartilage proteoglycans are proteins that contain covalently linked glycosaminoglycan chains (chondroitin sulfate and keratin sulfate) attached to a polypeptide backbone known as the core protein [1] and the core proteins are noncovalently bound to a long filament of hyaluronate.

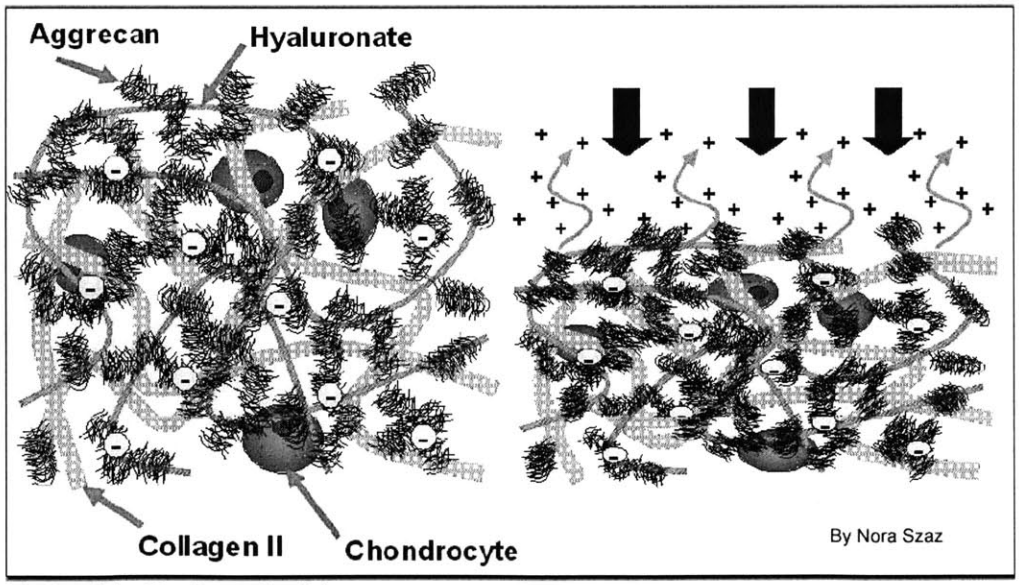

Figure 1.1. Cartilage physiology.

Cartilage is a poroelastic tissue in which proteoglycans contain fixed negative charges that contribute to the overall net negative charge density of the tissue. Compression of cartilage causes deformation of the chondrocytes and the extracellular matrix, which lead to repulsive forces between the negatively charged proteoglycans.

These forces result in fluid flows in the tissue, which induce streaming potentials and currents. The repulsive forces between the proteoglycans also contribute to the stiffness of the cartilage. Thus, cartilage is a complex system in which chondrocytes respond to loading or other physical stimuli by changing rates of biosynthesis of extracellular matrix biomolecules. These changes in the extracellular matrix can, in turn, cause changes in mechanical properties of the tissue, contributing to an overall metabolic feedback system.

Osteoarthritis, a degenerative joint disease, is a common defect of articular cartilage and underlying bone. Symptoms of osteoarthritis include the breakdown of the cartilage in the joint, which leads to pain and a loss of movement as the opposing bones rub against each other. Osteoarthritis results from a combination of risk factors including age, genetics, obesity, and traumatic joint injuries due to sports, accidents, and work-related activities. Articular cartilage defects may require novel regenerative and repair strategies to restore biological and functional activity to the damaged cartilage tissue.

It is generally accepted that physical stimuli in the environment of the tissue can cause significant changes to the synthesis and degradation of matrix macromolecules; however, the cellular mechanisms that influence the response of the chondrocyte to those stimuli are still not well known. Biochemical studies have also shown that growth factors and cytokines also regulate many of the same responses. Thus, recent studies have indicated that there are multiple pathways by which cartilage can sense and respond to physical stimuli [2]. Because these pathways are difficult to study *in vivo*, *in vitro* models such as cartilage explant and chondrocyte/gel systems have been used to investigate these mechanisms.

Insulin-like growth factor-I (IGF-I) is a 7.6 kDa polypeptide synthesized by cartilage via the chondrocytes, as well as by the liver. IGF-I binds to cell surface receptors in the chondrocyte and increases synthesis of extracellular matrix components such as collagens and proteogylcans [3, 4]. Also, IGF-I has been shown to inhibit or counteract catabolic processes or pathways which usually result in cartilage tissue breakdown [5, 6]. Furthermore, Sah et al. have demonstrated that long term treatment of articular cartilage explants with IGF-I have demonstrated increased biosynthetic rates, altered extracellular matrix composition, and improved biomechanical tissue properties [7]. IGF-I levels in joint synovial fluid have also been shown to be elevated in osteoarthritis and rheumatoid arthritis, suggesting a reparative motivation for the presence of IGF-I [8].

Bonnassar et al. have demonstrated the effect of short term dynamic compression and IGF-I on articular cartilage explants [9]. The study showed that IGF-I increased protein synthesis by 90% and proteoglycan synthesis by 120% in bovine patellofemoral cartilage and that short term, low-amplitude, sinusoidal dynamic compression increased protein synthesis by 40% and proteoglycan synthesis by 90%. They also showed that simultaneous dynamic compression and stimulation by IGF-I resulted in increased protein and proteoglycan synthesis by 180% and 290%, respectively. Furthermore, their studies indicated that dynamic compression accelerated the response of the cartilage of IGF-I and increased the amount of IGF-I transported into the extracellular matrix. However, the two different stimuli appeared to act through distinct cellular mechanisms, as suggested by the contrasting kinetics of the two responses.

Using a three-dimensional self-assembling peptide hydrogel system, Kisiday et al. investigated the possibility of using the peptide hydrogel as a scaffold for chondrocyte seeding, resulting in the synthesis of a cartilage-like extracellular matrix in culture [10]. In their study, they investigated the effects of long-term dynamic compression on biosynthetic rates of chondrocyte seeded within the peptide gel, specifically through loading protocols involving a combination of periods of loading and free-swelling culture. They found that an alternate day loading protocol resulted in increased total proteoglycan and protein synthesis in the system.

Previous studies have shown the effect of injurious (high-amplitude) compression on cartilage explants [11, 12]. Injurious mechanical compression typically led to decreased biosynthetic rates, tissue swelling, and chondrocyte apoptosis. This type of injury is representative of the type of traumatic injury believe to be a risk factor for development of osteoarthritis. Cosman et al. showed the effect of IGF-I on the repair of injured cartilage explants [13]. Following injurious compression, they demonstrated that cartilage exposed to IGF-I stimulation showed higher biosynthesis rates than non-treated samples.

Ascorbic acid is an antioxidant and vital to cartilage metabolism. L-ascorbic acid is typically used as a component in the feeding medium for chondrocyte and cartilage explant model systems. However, the aqueous half-life of L-ascorbic acid is known to be approximately two days in medium. A2P, an ascorbyl-2-phosphate magnesium salt derivative, is an alternative source of ascorbate that is a potent antioxidant with a longer half-life than L-ascorbic acid and is internalized, desphophorylated, and concentrated in the aqueous phase of the chondrocyte. Previous studies have shown that A2P can be used

in place of L-ascorbic acid in the feeding medium for guinea pig cartilage in long term metabolism studies [14].

Thus, the previous studies indicate that the synergistic use of long term, dynamic compression and recombinant growth factors has the potential to stimulate long term growth and repair in articular cartilage. The ability to stimulate long term growth and repair in cartilage would be an important step in treating and understanding common articular defects such as traumatic injury or osteoarthritis.

1.2 Objectives and hypotheses

The major goal of this Master of Engineering thesis research project was to develop an experimental protocol to demonstrate long term cartilage growth and repair in articular cartilage explants. The protocol has used a low-amplitude, alternative day sinusoidal dynamic compression loading procedure as the major physical stimulator of growth and repair. The hypothesis was that the protocol would result in elevated levels of extracellular matrix protein biosynthesis rates and total matrix protein content.

In preparation for future work involving synergistic stimulation of cartilage growth and repair by the long term loading protocol and by IGF-I stimulation, another goal was to isolate and observe the effects of IGF-I under both long term free swelling and mechanical loading conditions by replacing fetal bovine serum (FBS), a nutrient rich supplement of the typical explant feeding medium containing numerous growth factors with ITS (an insulin-transferrin-selenium nutrient solution). Furthermore, the possibility of replacing L-ascorbic acid in the feeding medium with a more stable form, A2P was also investigated. The hypothesis was that the replacement of the FBS and L-ascorbic

acid components would have no adverse effects on the long term cartilage growth and would set the stage for future IGF-I studies.

Chapter 2 - Methods

2.1 Materials

Knee joints from freshly slaughtered 1-2 week calves were from Research 87 (Marlborough, MA). High glucose Dulbecco's modified Eagle's medium (DMEM), HEPES buffer solution, phosphate-buffered saline (PBS), and sodium pyruvate were from GIBCO (Grand Island, NY). Fetal bovine serum was from HyClone (Logan, Utah). PenStrep, L-ascorbic acid, proline, non-essential amino acid solution, and ITS were from Sigma-Aldrich (St. Louis, MO). Proteinase K was from Roche Diagnostics (Indianapolis, IN). Sodium sulfate was from Mallinckrodt Specialty Chemicals (Paris, KY). L-[^{3}H]-proline was from Amersham Biosciences (United Kingdom). Sodium [^{35}S]-sulfate was from Perkin-Elmer (Boston, MA). Ecolume scintillation fluid was from Fisher (Boston, MA). Dimethylmethylene blue dye was from Polysciences (Warrington, PA). Hoechst dye was from Hoechst Celanese (Short Hills, NJ).

2.2 Cartilage explant and culture

Articular cartilage plugs of 1 mm thickness and 3 mm diameter were harvested from freshly slaughtered 1-2 week old calves as described previously [15]. In summary, a drill press and hollow 9 mm drill bit were used to obtain cylindrical cores of cartilage and bone from the bovine femoropatellar groove. A PBS and antibiotic solution was used to prevent dehydration and infection. The cores were inserted into a polysulfone sample holder (Figure 2.1) for a sledge microtome (Figure 2.2, Model 860, American Optical, Buffalo, NY) and the uneven surfaces of the cartilage were removed. The cores were then sliced to obtain the next two 1 mm layers of cartilage. Next, the slices were punched with a 3 mm dermal punch (Miltex Instruments, Lake Success, NY) under

sterile conditions to obtain the final cartilage plugs. The plugs were then placed in 0.5 mL of prepared medium and allowed to reach metabolic steady state in 48-well dishes for 48-60 hours in an incubator at 37° C in 5% CO_2 atmosphere. Figure 2.3 illustrates the explant procedure. The culture medium for dynamic compression initially consisted of high glucose DMEM with phenol red, 10% fetal bovine serum (FBS), 10 mM HEPES buffer, 1 mM sodium pyruvate, 20 ug/mL ascorbate, 0.1 mM non-essential amino acids, 0.4 mM L-proline, and PenStrep (multiple antibiotics).

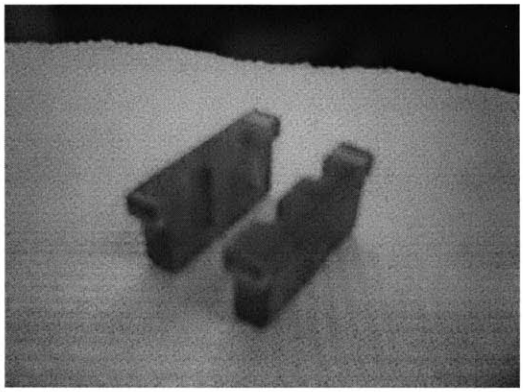

Figure 2.1. Cartilage core polysulfone holder.

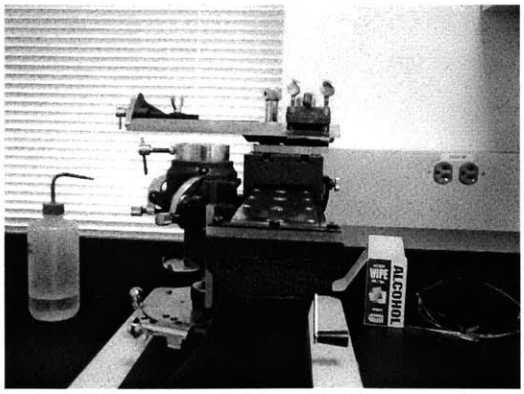

Figure 2.2. Sledge microtome.

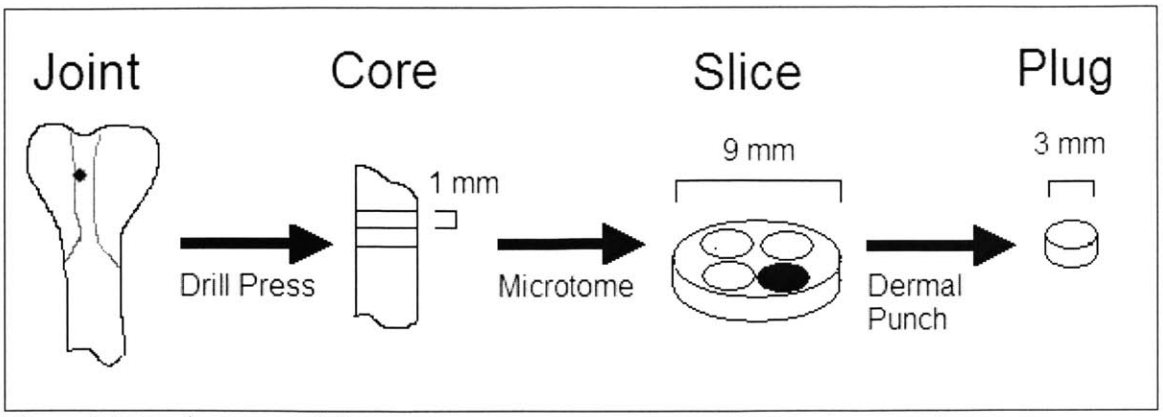

Figure 2.3. Explant protocol diagram.

2.3 Mechanical loading (dynamic compression)

Cartilage plugs were placed into individual wells of a specially machined polysulfone compression chamber (Figure 2.4) with 0.5 mL of media per well. The plugs were held under uniaxial, radially unconfined compression between platens in the base of the chamber and the chamber lid. The compression chamber was then placed inside a custom-built compression apparatus (Figure 2.5) housed in a standard incubator at 37° C in 5% CO_2 atmosphere. For the experiments, cartilage plugs matched from the same slice were used as controls. The general dynamic compression protocol consisted of 4 cycles of 45 minutes of dynamic stimulation per 6 hours every other day. The sinusoidal compression had a magnitude of 3% and a frequency of 0.1 Hz. The number of stimulation days (initially 4), the media composition, and the radiolabel concentration and period were varied during the course of the study. During the course of the compression protocol, the feeding medium for the cartilage plugs was replaced every two days. Figure 2.6 shows a timeline of a typical experiment.

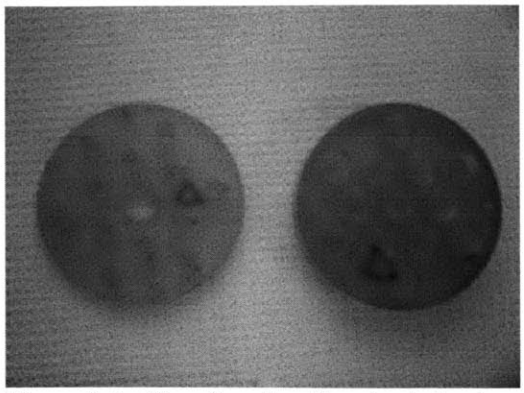

Figure 2.4. 12 well, polysulfone load chamber (Delta series), lid on left and base on right.

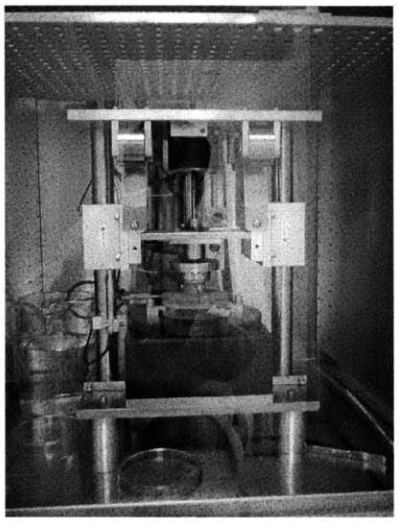

Figure 2.5. Incubator-housed, custom built compression apparatus.

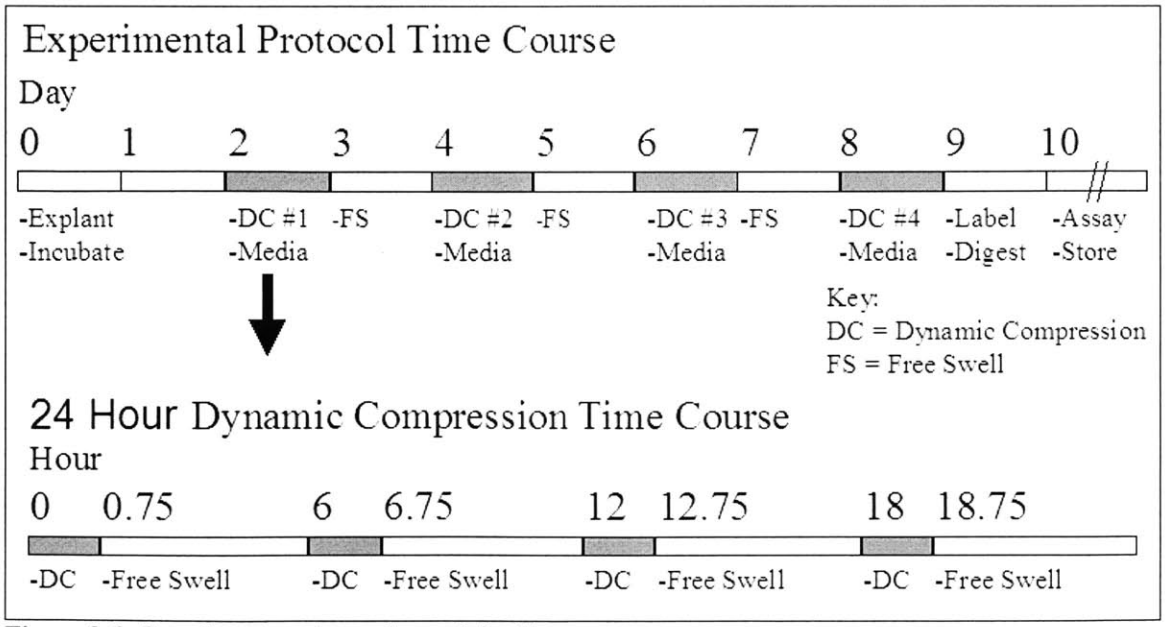

Figure 2.6. Sample protocol experimental timeline.

2.4 Biochemical and biosynthetic analyses

Following the compression protocol, the cartilage plugs were radiolabeled in preparation for the biochemical and biosynthesis assays described below. The total radiolabel incubation time varied across the experiments to observe different time points. The radiolabeled plugs were then digested overnight in a proteinase K solution prior to any of the assays.

2.4.1 Protein and glycosaminoglycan biosynthesis analyses

^{3}H-proline and ^{35}S-sulfate are commonly used as radiolabels in cartilage research to observe protein and glycosaminoglycan (GAG) synthesis rates, respectively. Proline and sulfate isotopes were chosen due to the biochemical composition of the cartilage extracellular matrix. A primary component of the extracellular matrix, Type II collagen, is comprised of a significant amount of proline [ref]. With the presence of ^{3}H-proline in the media, the radioactive marker is incorporated into the new collagen and all other proline-containing proteins in the matrix. Previous studies showed that ~75% of ^{3}H-proline was incorporated into collagen in newborn calf cartilage [15]. Similarly, the composition of the GAG side chains of proteoglycans is dominated by chondroitin sulfate and keratin sulfate. The incorporation of the radiolabeled sulfate enables a method to quantify the amount of GAG synthesized during the radiolabel incubation period. A high rate of radiolabel incorporation, either ^{3}H-proline or ^{35}S-sulfate, signifies a high rate of extracellular matrix biosynthesis in the cartilage plugs. In addition, the incorporation rates of both radiolabels should be correlated.

Radiolabel incorporation, wash, and plug digestion

Immediately, following the completion of the compression protocol, the cartilage plugs of both the control and experimental groups were radiolabeled and assayed for marker incorporation. The radiolabels, L-^{3}H-proline and sodium ^{35}S-sulfate were added to the respective feeding media to create radioactive media at concentrations of 10 μCi/mL ^{35}S-sulfate and 20 μCi/mL ^{3}H-proline or twice that in specific experiments (experiments with short radiolabel incubation periods). 0.5 mL of the radiolabeled media was added to each plug in a 48-well plate and incubated for T_{label} hours (1, 2, 4, or 24 hours, depending on experiment).

After the incubation period, the plugs were washed in cold PBS solution containing 0.4 mM L-proline and 0.8 nM sodium sulfate. Following the removal of the radioactive media, the plugs were each washed 4 times for a period of 15 minutes per wash, replacing the 1.0 mL of fresh cold PBS solution per plug per wash. Next the plugs were placed in individually labeled screw topped vials. Finally, 1.0 mL of a proteinase K digest solution (0.2 mg/mL proteinase K in Tris-HCl buffer) was added to each plug and allowed to digest overnight at 55C overnight. A more detailed version of this protocol can be found in Appendix B.

Liquid scintillation counting

A liquid scintillation counter (RackBeta 1211, LKB) was used to measure the amount of radiolabeled markers present in the digested cartilage samples. 20 μL aliquots of radiolabel standards and 100 μL aliquots of cartilage samples were mixed with 2 mL of scintillation fluid per sample in scintillation vials. Each sample was analyzed by the counter for 3 minutes.

Natural radioactive decay in the vials causes an emission of light ("scintillation") due to components of the scintillation fluid. Each scintillation event is counted by a photomultiplier in the scintillation counter. Different radioisotopes scintillate at different wavelengths of light; thus, the detection of multiple isotopes requires the use of different ranges of minimum and maximum wavelengths. In general, following the detection of an emission event within a specific range, or "window", the count for that range is incremented.

Dual radiolabel incorporation rate determination

During the liquid scintillation counting procedure, the two radiolabels, ^{3}H-proline and ^{35}S-sulfate, have emission spectra that overlap (crossover) in their respective counting spectral windows. Empirically, the overlap of ^{35}S-sulfate label dominates that of the ^{3}H-proline label; thus, it is sufficient to consider only the ^{35}S-sulfate crossover. Figure 2.7 illustrates the dual radiolabel emission spectra and the two counting windows, W1 and W2.

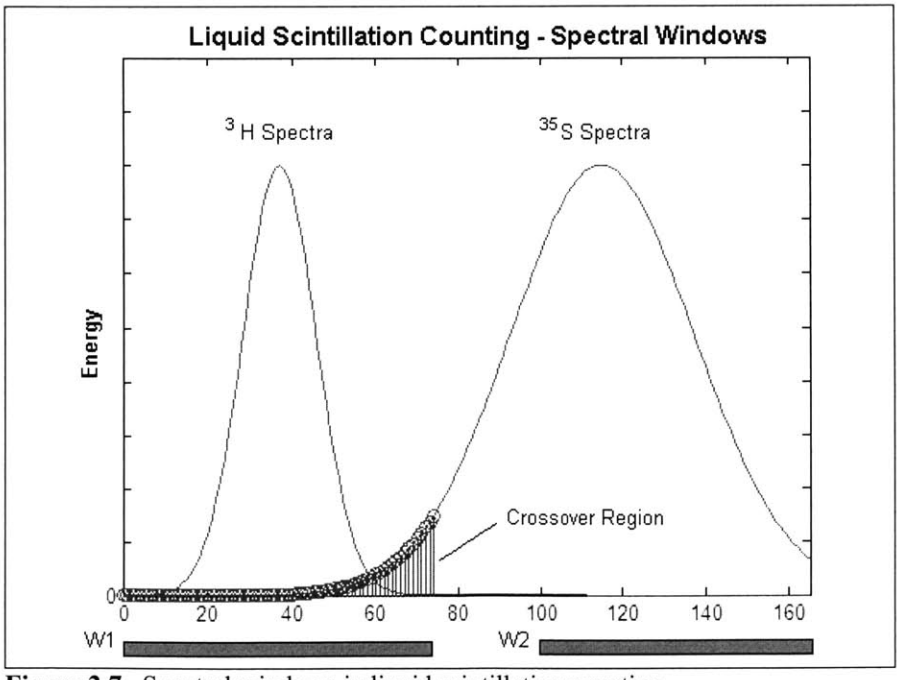

Figure 2.7. Spectral windows in liquid scintillation counting.

In order to determine the crossover ratio for the ^{35}S-sulfate label, two sets of 20 μL standards were counted: 1) ^{35}S only and 2) $^{35}S + {}^{3}H$. To calculate the radiolabel incorporation rates, 100 μL of each sample were also counted. Table 2.1 shows the measured values of the two standards and a sample in each window, in terms of the variables S1, S2, SH1, SH2, A1, and A2.

Table 2.1. Key to measured variable values for radiolabel incorporation rate calculations.

Isotope	W1 [CPM]	W2 [CPM]
^{35}S	S1	S2
$^{35}S + {}^{3}H$	SH1	SH2
Sample A	A1	A2

The crossover ratio, X, is defined in Equation 1 as the CPM of S in Window 1 divided by the CPM of S in Window 2:

$$X = S1/S2 \quad \text{[Eq. 1]}$$

Next, the total S, S_{tot}, and total H, H_{tot}, can be calculated via Equations 2 and 3 as follows:

$$S_{tot} = S2 + S2 \cdot X = S2 \cdot (1 + X) \quad [CPM] \quad \text{[Eq. 2]}$$
$$H_{tot} = SH1 - S2 \cdot X \quad [CPM] \quad \text{[Eq. 3]}$$

Equations 4-9 define parameters previously measured, empirically determined, or intrinsic to a given experiment:

$$PAF \equiv proline\ activity\ factor = 5 \cdot 10^5 [pmol] \quad \text{[Eq. 4]}$$
$$SAF \equiv sulfate\ activity\ factor = 8.2 \cdot 10^5 [pmol] \quad \text{[Eq. 5]}$$
$$A_{DNA} \equiv DNA\ in\ Sample\ A \quad [\mu g\ DNA] \quad \text{[Eq. 6]}$$
$$T_{label} \equiv total\ radiolabel\ time \quad [hr] \quad \text{[Eq. 7]}$$
$$V_{std} \equiv std.volume = 20 \quad [\mu L] \quad \text{[Eq. 8]}$$
$$V_{samp} \equiv sample\ volume = 100 \quad [\mu L] \quad \text{[Eq. 9]}$$

Then the total ^{3}H and ^{35}S counts for Sample A, $A_{H,tot}$ and $A_{S,tot}$, respectively, are

calculated from Equations 10 and 11:

$$A_{H,tot} = A1 - A2 \cdot X \quad [CPM] \quad \text{[Eq. 10]}$$

$$A_{S,tot} = A2 + A2 \cdot X = A2 \cdot (1 + X) \quad [CPM] \quad \text{[Eq. 11]}$$

Finally, the ^{3}H and ^{35}S radiolabel incorporation rates for Sample A, R_H and R_S, respectively, are given by Equations 12 and 13.

$$R_H = \frac{A_{H,tot} \cdot PAF \cdot V_{std}}{H_{tot} \cdot A_{DNA} \cdot T_{label} \cdot V_{samp}} \quad \left[\frac{pmol\ proline}{\mu g\ DNA \cdot hr}\right] \quad \text{[Eq. 12]}$$

$$R_S = \frac{A_{S,tot} \cdot SAF \cdot V_{std}}{S_{tot} \cdot A_{DNA} \cdot T_{label} \cdot V_{samp}} \quad \left[\frac{pmol\ sulfate}{\mu g\ DNA \cdot hr}\right] \quad \text{[Eq. 13]}$$

2.4.2 Glycosaminoglycan content assay

Proteoglycans are macromolecules containing glycosaminoglycan (GAG) side chains of fixed negative charges; aggrecan is the predominant GAG-bearing proteoglycan of cartilage. The sulfated glycosaminoglycan content of each plug was measured using the DMMB dye assay described previously [15]. GAG standards of known concentrations were prepared by serial dilutions in Tris-HCl. 20 μL of each sample or standard was aliquoted into a 96 well plate. 200 μL of DMMB dye was then added to each sample, taking care to eliminate pockets of air. The light absorbance for each sample was measured by the use of a plate-reading spectrophotometer (Vmax kinetic microplate reader, Molecular Devices). Using the readings from the known GAG standards, the GAG concentrations of the samples were calculated. A side study was also conducted to determine the precision and efficacy of the GAG content assay in the context of this long-term dynamic compression treatment protocol. Appendix A describes the protocol and the results of the side study. A more detailed version of the GAG assay protocol can be also found in Appendix B.

The measured GAG concentrations were used as a measure of proteoglycan synthesis and extracellular matrix activity. For example, low levels of GAG in a sample plug may have indicated a loss of proteoglycans from the extracellular matrix to the media. GAG levels in control and experimental groups may have given an indication of long-term changes or remodeling in the extracellular matrix due to the dynamic stimulation protocol or changes in media composition. Furthermore, increased GAG levels indicated higher levels of proteoglycan synthesis, which may be corroborated by the results of the radiolabel biosynthetic rate assays described below.

2.4.3 DNA content assay

The DNA content of each plug was measured using the Hoechst dye assay described previously [15]. DNA standards of known concentrations were prepared by serial dilutions in Tris-HCl. 20 μL of each sample or standard was aliquoted into a 96 well plate. 200 μL of Hoechst dye was then added to each sample, taking care to eliminate pockets of air. The fluorescence due to ultraviolet light stimulation for each sample was measured by the use of a plate-reading spectrofluorometer (Victor). Using the readings from the known DNA standards, the DNA concentrations of the samples were calculated. A side study was also conducted to determine the precision and efficacy of the DNA content assay in the context of this long-term dynamic compression treatment protocol. Appendix A describes the protocol and the results of the study. A more detailed version of the DNA assay protocol can be found in Appendix B.

The measured DNA contents were an indication of the number of viable cells in the sample tissue because chondrocytes maintain their DNA until their nuclei are cleaved, as in apoptosis. Thus, a significantly reduced level of DNA content in a sample indicated

a loss of chondrocytes due to some experimental condition. The DNA contents allowed the biosynthesis rates of each sample to be normalized to cell number via the DNA content and enabled the samples to be compared statistically.

2.5 Medium comparison

In order to isolate the effects of the growth factor IGF-I, the feeding medium for the cartilage plugs must be free of other growth factors. Because the FBS in the regular feeding medium contains natural growth factors, it was necessary to investigate the use of a "serum-free" medium for feeding. In this protocol, FBS was replaced by ITS, as was done in previous IGF-I experiments and the effects of the media change was observed in conjunction with the dynamic compression.

In addition, the ascorbate in the regular feeding medium is known to break down after 48 hours, one factor leading to the medium change every 2 days in the protocol. However, Clark et al. have demonstrated that ascorbyl-2-phosphate (A2P), a different ascorbic acid compound that is stable over significantly longer periods of time, can replace the typical ascorbate (L-ascorbic acid) in the feeding medium of guinea pig articular cartilage explants [14]. In this protocol, the effects of replacing the normal ascorbate with A2P were observed in several experiments, with the goal of demonstrating the efficacy and practicality of using more stable feeding medium components due to the long-term nature of the protocol in general, as well as saving time and cost in medium preparation.

2.6 Statistical analyses

Statistical Analyses were performed using Microsoft EXCEL (with the Analysis ToolPak) and Mathworks MATLAB. Standard curves for the photometric GAG and

fluorometric DNA data were constructed by statistical curve-fitting functions. The standard curves were used to convert the measured assay data into ug/mL GAG or DNA present in the digested cartilage plug solutions. Liquid scintillation count data were converted into biosynthetic incorporation rates as described above. Then calculated total DNA data were used to normalize the GAG and biosynthetic incorporation rate data for each plug.

Within each experiment, data for each experimental condition were reported as the mean (size N plugs) ± standard error. Finally, Student T-tests or multifactorial analyses of variance (ANOVA) [16] were then performed to examine the effects of independent experimental parameters (dynamic compression or variable media).

Chapter 3 – Results

3.1 Overall Experiment Parameters Summary

Table 3.1 shows a summary of the parameters of each experiment.

Table 3.1. Experimental Parameters Summary.

		Experiments							
		1	2	3	4	5	6	7	8
Conditions	Free Swell vs. DC	x	x	x	x	x	x	x	x
	Regular vs. ITS Media						x	x	
	Regular vs. A2P Media						x		
	Regular vs. ITS-A2P Media								x
Radiolabel Period	1 Hour Timepoint			x	x	x			
	2 Hour Timepoint			x	x	x	x	x	x
	4 Hour Timepoint			x					
	24 Hour Timepoint	x	x			x			
Compression Days	4 Day	x	x	x	x	x		x	x
	6 Day						x		
Slice Preference	Nearest to surface	x	x	x	x				
	Nearest to 1 mm					x	x	x	x

3.2 Experiment 1

Design

During the explant procedure, 6 cartilage slices were obtained from one joint. The slices taken closer to the superficial core surface were chosen over those that were deeper and more vascularized. The cartilage plugs were all fed with the regular medium. One group of plugs (Control) was allowed to remain in free swell conditions. Another group of plugs (DC) was mechanically loaded using the dynamic compression protocol over 4 compression days. From each slice, one plug went into the Control group and one plug went into the DC group. The remaining two plugs per slice were not used. Following the loading protocol, the plugs were radiolabeled for 24 hours. There were a total of 2 experimental groups (Control, DC) with N = 6. Details of the experimental design are found in Appendix C.

Assays

Figure 3.1a shows the results of the sulfate incorporation (mean ± SEM) assay. Figure 3.1b shows the results of the proline incorporation (mean ± SEM) assay. Figure 3.1c shows the results of the total GAG (mean ± SEM) assay. The experiment did not demonstrate significant statistical differences ($p > 0.05$) in any of the three assays. Details of the data and analyses of the assays are found in Appendix C.

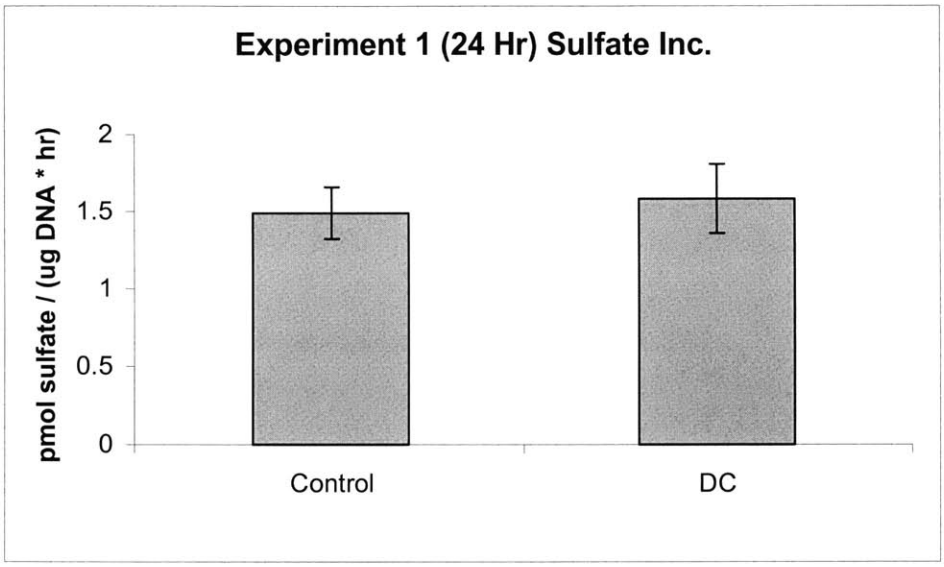

Figure 3.1a. Experiment 1 (24 hour): rate of ^{35}S-sulfate incorporation (mean ± SEM).

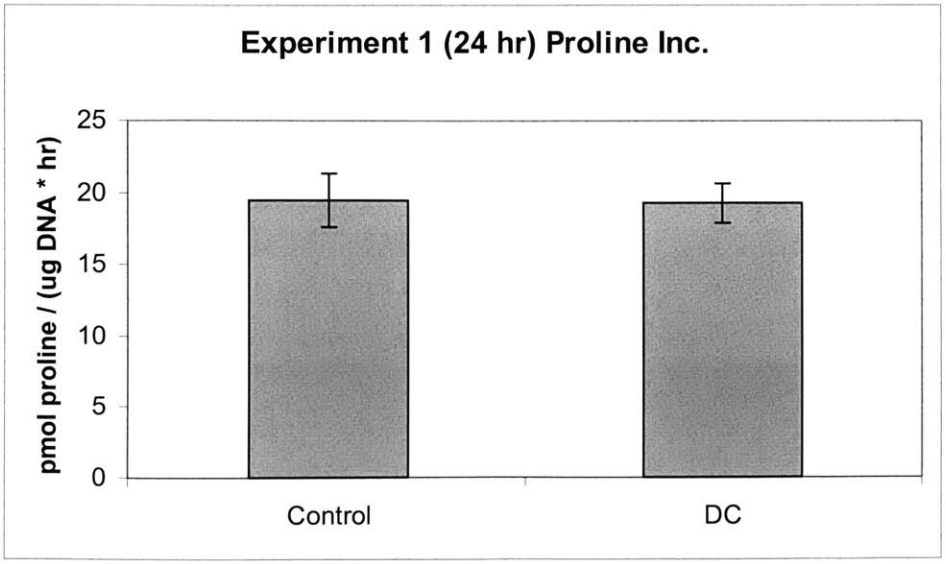

Figure 3.1b. Experiment 1 (24 hour): rate of ^{3}H-proline incorporation (mean ± SEM).

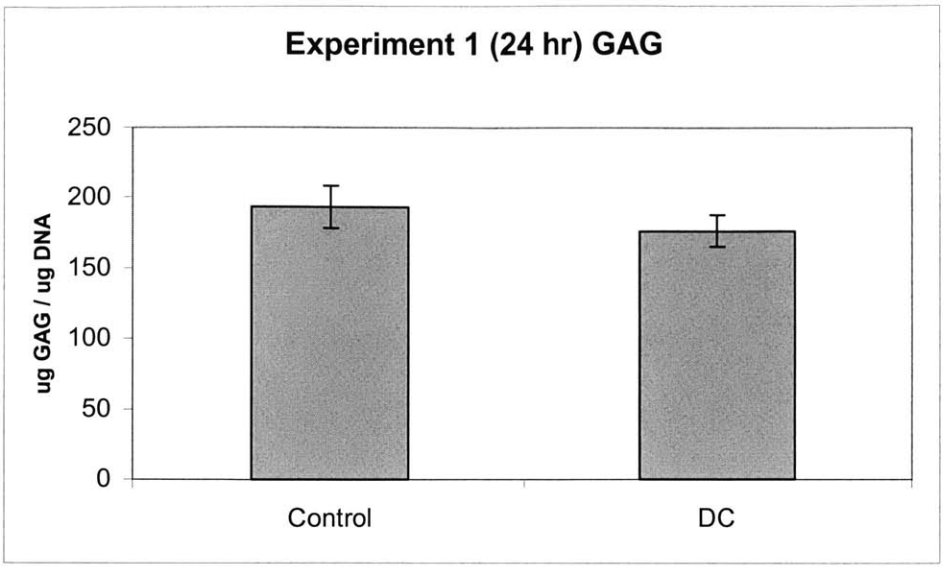

Figure 3.1c. Experiment 1 (24 hour): total GAG content (mean ± SEM).

3.3 Experiment 2

Design

During the explant procedure, 9 cartilage slices were obtained from one joint. The slices taken closer to the superficial core surface were chosen over those that were deeper and more vascularized. The cartilage plugs were all fed with the regular medium. Three groups of plugs (Control) were allowed to remain in free swell conditions. Another three groups of plugs (DC) were mechanically loaded using the dynamic compression protocol with 4 compression days. Two groups each (four total) of the Control and DC groups were obtained from the first 5 slices, and the other Control and DC groups (remaining two) were obtained from the remaining 4 slices. Following the loading protocol, the plugs were radiolabeled for 24 hours. The plugs were then pooled together for a total of 2 experimental groups (Control, DC) with N = 14. The goal of this experiment was to repeat Experiment 1 with a larger data set. Details of the experimental design are found in Appendix C.

Assays

Figure 3.2a shows the results of the sulfate incorporation (mean +/- SEM) assay. The sulfate incorporation in the DC group was 1.61 times that of the Control group with $p < 0.05$ (Student T test). Figure 3.2b shows the results of the total GAG (mean +/- SEM) assay. The experiment did not demonstrate significant statistical differences ($p < 0.05$) in the total GAG assays between the experimental groups. The proline incorporation data was not included here. Details of the data and analyses of the assays are found in Appendix C.

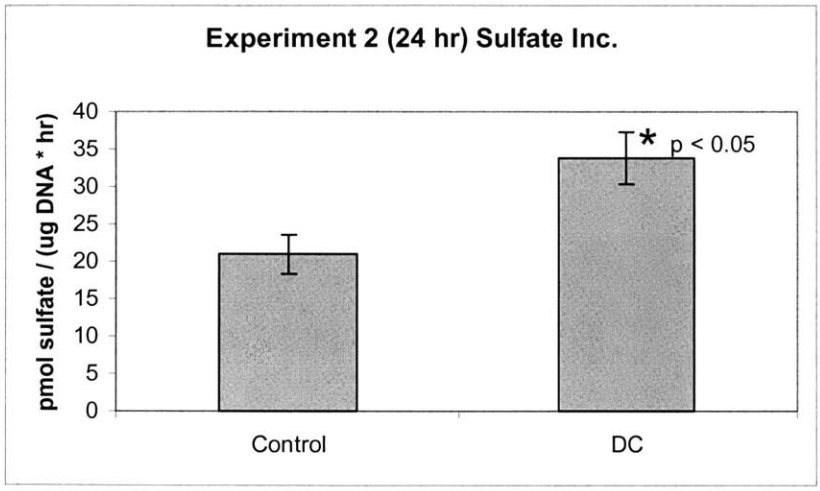

Figure 3.2a. Experiment 2 (24 hour): rate of ^{35}S-sulfate incorporation (mean ± SEM). (*: statistically different, $p < 0.05$).

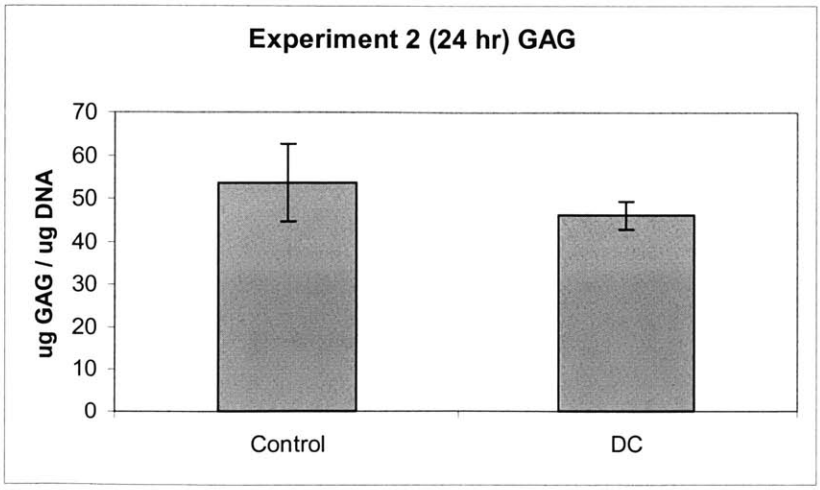

Figure 3.2b. Experiment 2 (24 hour): total GAG content (mean ± SEM).

3.4 Experiment 3

Design

During the explant procedure, 6 cartilage slices were obtained from one joint. The slices taken closer to the superficial core surface were chosen over those that were deeper and more vascularized. The cartilage plugs were all fed with the regular medium. Three groups of plugs (Control) were allowed to remain in free swell conditions. Another three groups of plugs (DC) were mechanically loaded using the dynamic compression protocol with 4 compression days. Two slices were used per Control and DC group, with two plugs per slice going to each group. Following the loading protocol, each set of the Control and DC groups was radiolabeled for 1, 2, or 4 hours. The groups at each time point were analyzed as 3 separate experiments of 2 experimental groups (Control, DC) with N = 4. The goal of this experiment was to repeat Experiments 1 and 2 with different radiolabel times. Details of the experimental design are found in Appendix C.

Assays

I. T_{label} = 1 hour

Figure 3.3a shows the results of the sulfate incorporation (mean ± SEM) assay. The sulfate incorporation in the DC group was 1.618 times that of the Control group indicating a trend towards increased synthesis caused by compression. However, T-test analysis indicated that this trend did not quite reach statistical significance ($p = 0.083$). Figure 3.3b shows the results of the total GAG (mean ± SEM) assay. While the total GAG in the DC group was 3.00 times that of the Control group, T-test analysis indicates that the difference in total GAG is not statistically significant. The proline incorporation

data was not included here. Details of the data and analyses of the assays are found in Appendix C.

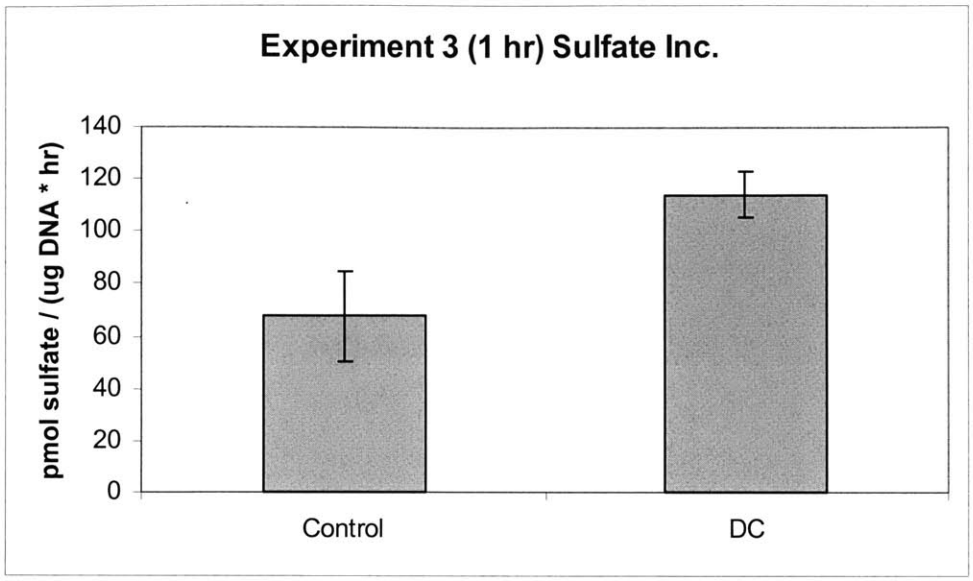

Figure 3.3a. Experiment 3 (1 hour): rate of ^{35}S-sulfate incorporation (mean ± SEM).

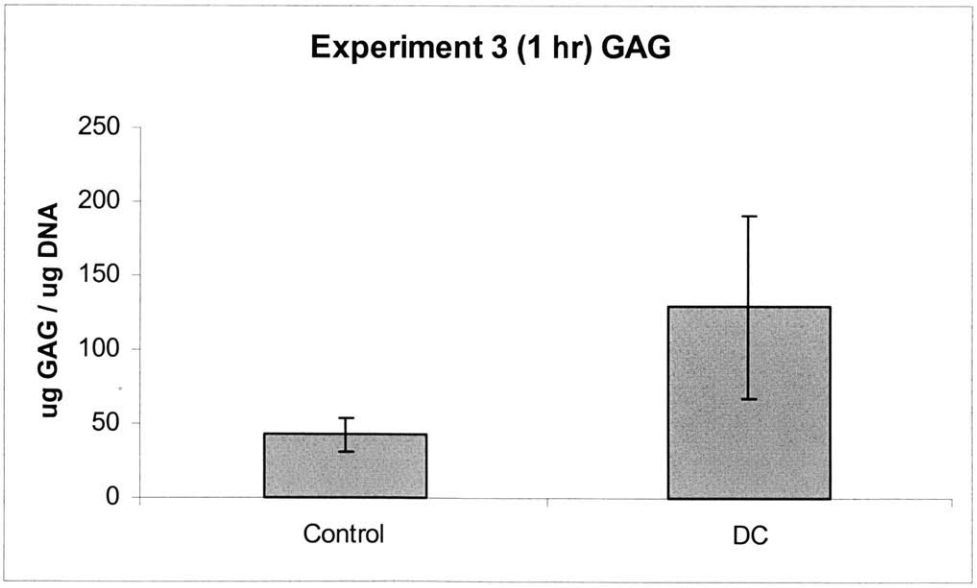

Figure 3.3b. Experiment 3 (1 hour): total GAG content (mean ± SEM). (*: statistically different, $p < 0.05$).

II. $T_{label} = 2$ hours

Figure 3.4a shows the results of the sulfate incorporation (mean ± SEM) assay.

The sulfate incorporation in the DC group was similar to that of the Control group and T-test analysis indicates that the difference is not statistically different ($p = 0.773$). Figure 3.4b shows the results of the total GAG (mean ± SEM) assay. Again, the T-test analysis indicates that the difference in total GAG between the DC and Control groups is not statistically significant. The proline incorporation data was not included here. Details of the data and analyses of the assays are found in Appendix C.

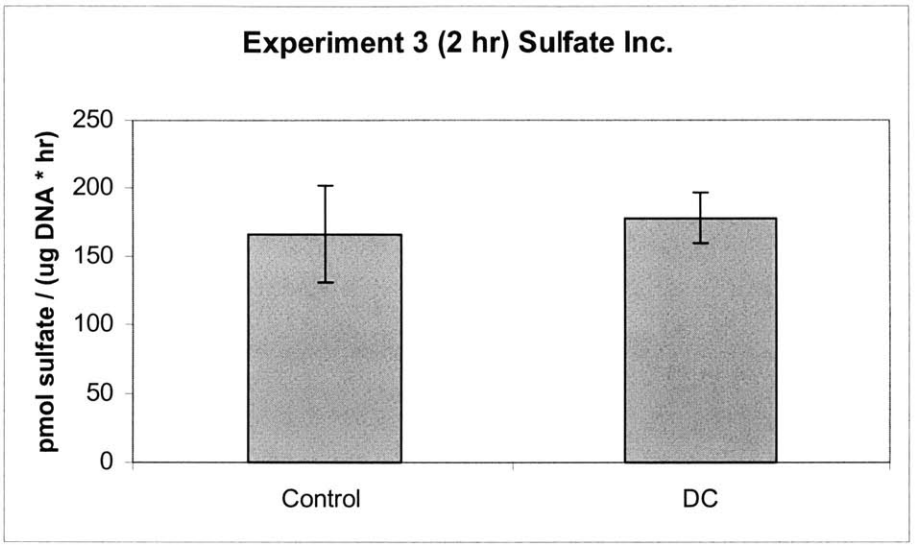

Figure 3.4a. Experiment 3 (2 hour): rate of ^{35}S-sulfate incorporation (mean ± SEM).

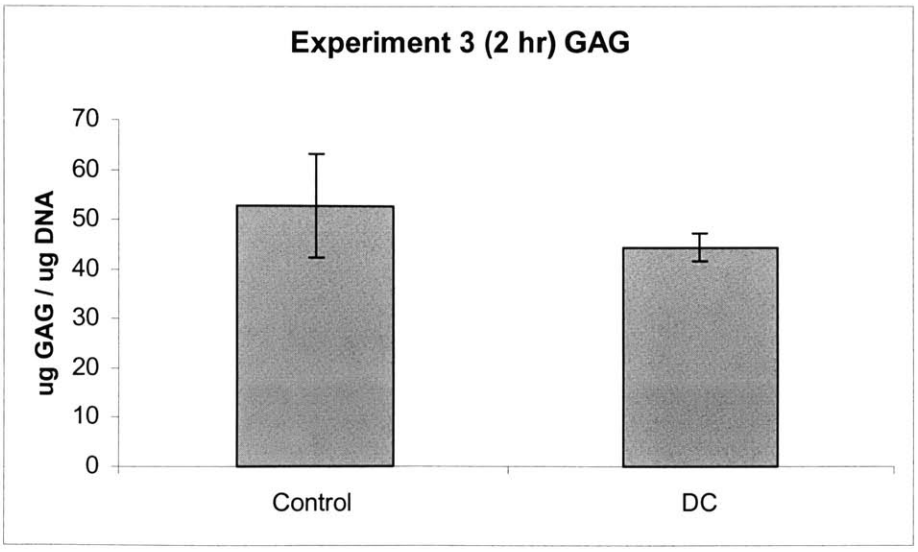

Figure 3.4b. Experiment 3 (2 hour): total GAG content (mean ± SEM).

III. T_{label} = 4 hours

Figure 3.5a shows the results of the sulfate incorporation (mean ± SEM) assay. The sulfate incorporation in the DC group was 3.11 times that of the Control group and T-test analysis indicates that the difference is statistically different ($p < 0.05$). Figure 3.5b shows the results of the total GAG (mean ± SEM) assay. The total GAG in the DC group was 2.97 times that of the Control group and T-test analysis again indicates that the difference is statistically different ($p < 0.05$). The proline incorporation data was not included here. Details of the data and analyses of the assays are found in Appendix C.

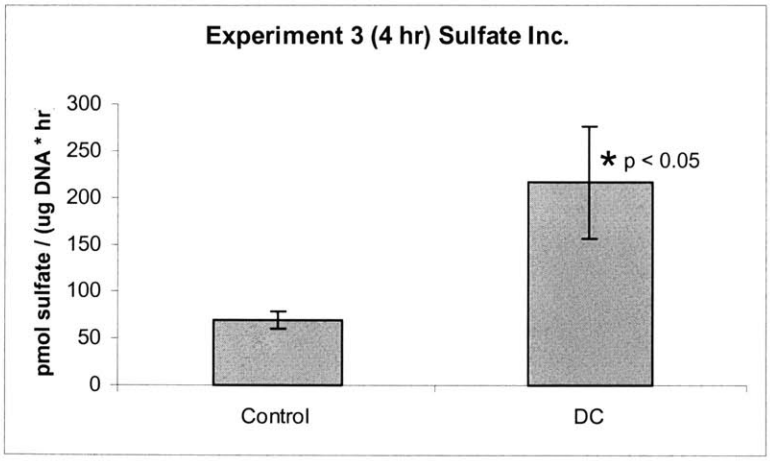

Figure 3.5a. Experiment 3 (4 hour): rate of ^{35}S-sulfate incorporation (mean ± SEM). (*: statistically different, $p < 0.05$).

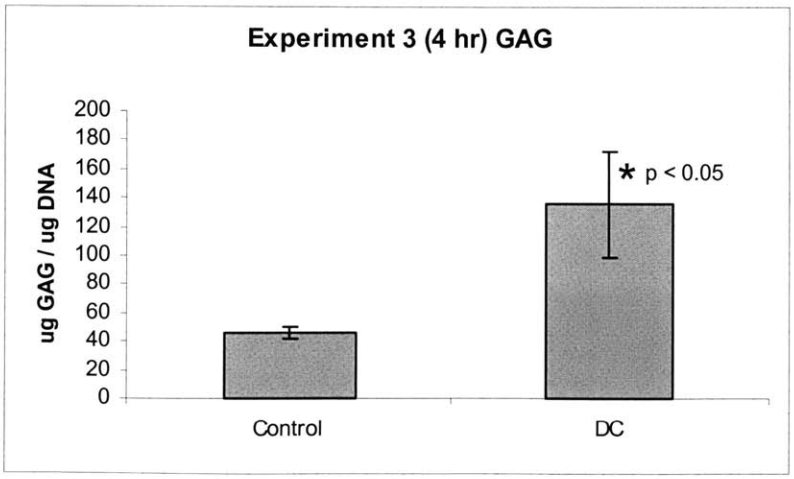

Figure 3.5b. Experiment 3 (4 hour): total GAG content (mean ± SEM). (*: statistically different, $p < 0.05$).

3.5 Experiment 4

Design

During the explant procedure, 4 cartilage slices were obtained from one joint. The slices taken closer to the superficial core surface were chosen over those that were deeper and more vascularized. The joint used in this experiment was fairly small and results in a very small yield of cartilage. The cartilage plugs were all fed with the regular medium. Two groups of plugs (Control) were allowed to remain in free swell conditions. Another two groups of plugs (DC) were mechanically loaded using the dynamic compression protocol with 4 compression days. In this experiment, two slices were used for each set of Control and DC groups. From each slice, one plug went into the Control group and the remaining three plugs went into the DC group. Following the loading protocol, each set of the Control and DC groups was radiolabeled for 1 or 2 hours using twice the typical concentration of radiolabels as before. During the experiment, some plugs were lost in handling. The groups at each time point were analyzed as 2 separate experiments of 2 experimental groups with N = 2 (Control) and N = 5 or 6 (DC). The goal of this experiment was to repeat a portion of Experiment 3 with twice the concentration of radioactive labels. Details of the experimental design are found in Appendix C.

Assays

I. T_{label} = 1 hour

Figure 3.6a shows the results of the sulfate incorporation (mean ± SEM) assay. Figure 3.6b shows the results of the proline incorporation (mean ± SEM) assay. Figure 3.6c shows the results of the total GAG (mean ± SEM) assay. While the mean of the DC

group was greater than those of the Control group for each assay, the experiment did not demonstrate significant statistical differences ($p > 0.05$) between the Control and DC groups in any of the three assays. Details of the data and analyses of the assays are found in Appendix C.

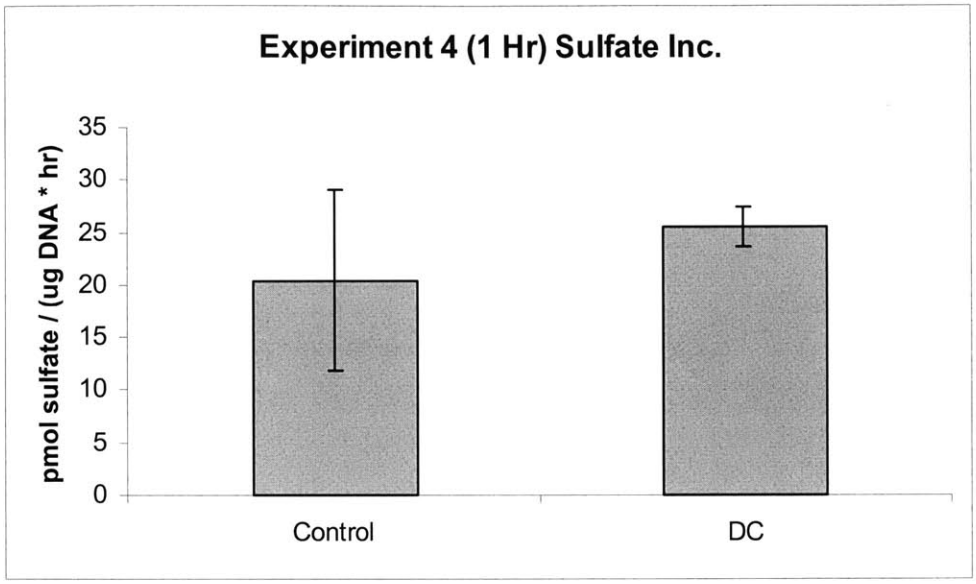

Figure 3.6a. Experiment 4 (1 hour): rate of ^{35}S-sulfate incorporation (mean ± SEM).

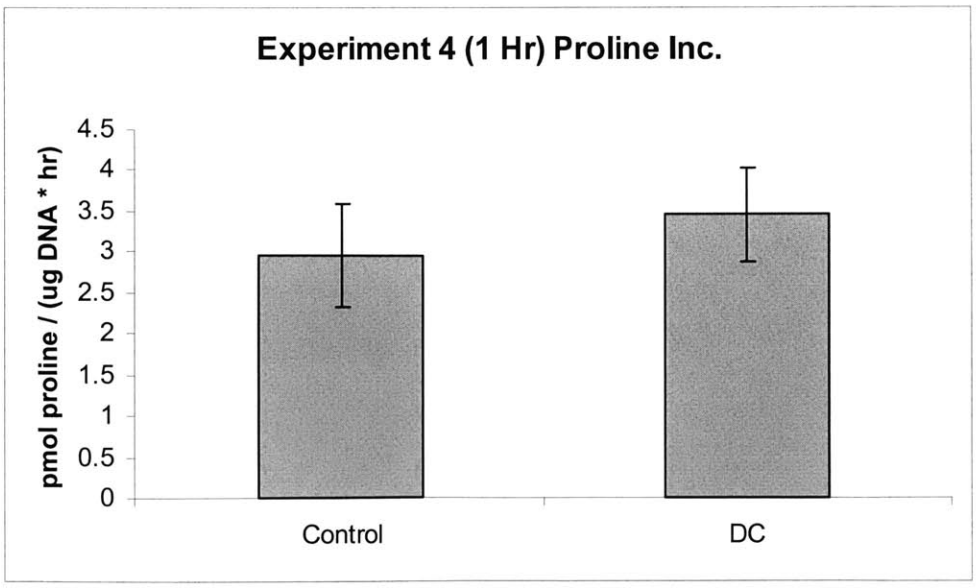

Figure 3.6b. Experiment 4 (1 hour): rate of ^{3}H-proline incorporation (mean ± SEM).

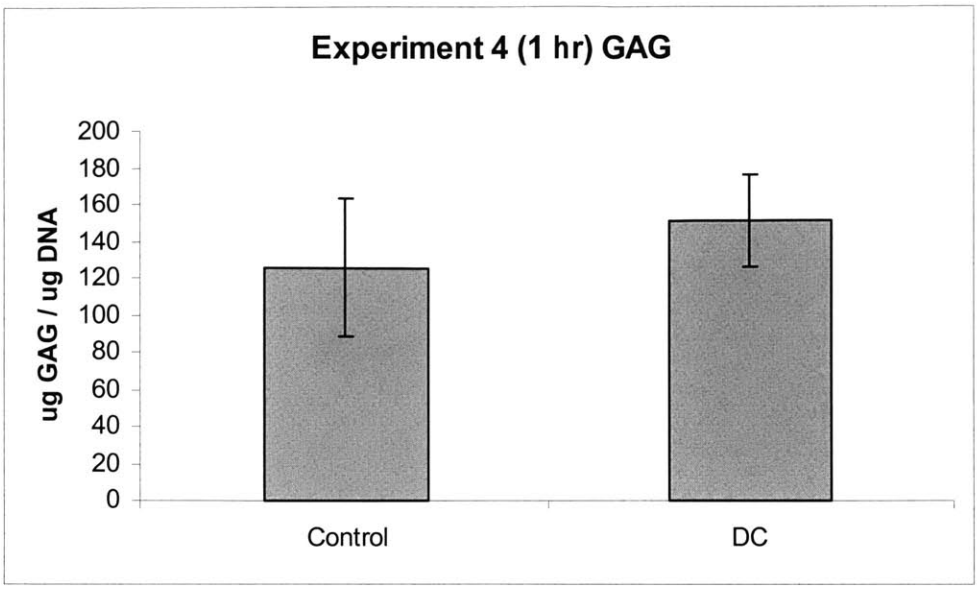

Figure 3.6c. Experiment 4 (1 hour): total GAG content (mean ± SEM).

II. T_{label} = 2 hour

Figure 3.7a shows the results of the sulfate incorporation (mean +/- SEM) assay. Figure 3.7b shows the results of the proline incorporation (mean +/- SEM) assay. Figure 3.7c shows the results of the total GAG (mean +/- SEM) assay. Again, while the mean of the DC group was greater than those of the Control group for each assay, the experiment did not demonstrate significant statistical differences ($p > 0.05$) between the Control and DC groups in any of the three assays. Details of the data and analyses of the assays are found in Appendix C.

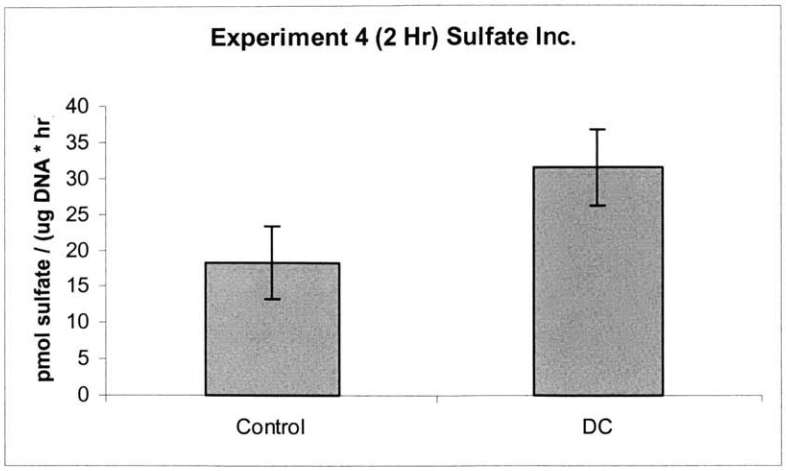

Figure 3.7a. Experiment 4 (2 hour): rate of ^{35}S-sulfate incorporation (mean ± SEM). (*: statistically different, $p < 0.05$).

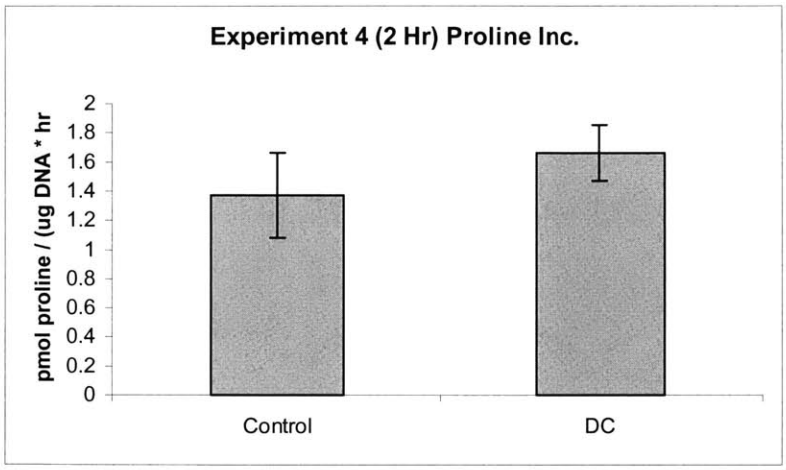

Figure 3.7b. Experiment 4 (2 hour): rate of ^{3}H-proline incorporation (mean ± SEM).

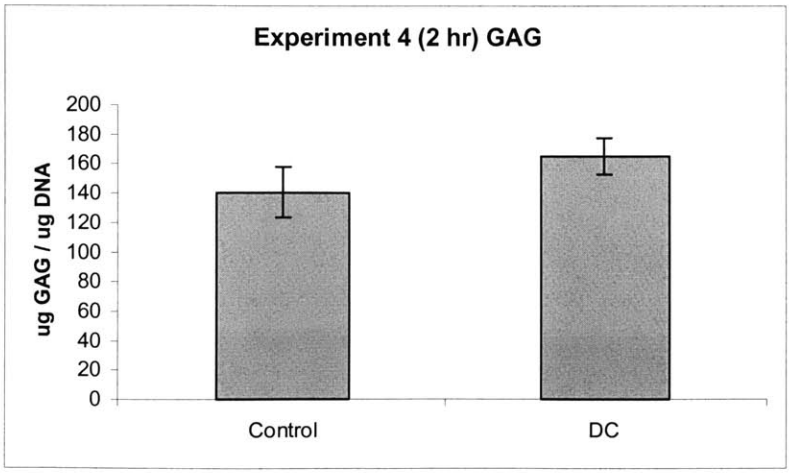

Figure 3.7c. Experiment 4 (2 hour): total GAG content (mean ± SEM).

3.6 Experiment 5

Design

During the explant procedure, 12 cartilage slices were obtained from two joints. The slices from both joints were pooled, measured, and sorted by height measured by a digital caliper. The 12 slices nearest to 1.00 mm were chosen for the experiment. The cartilage plugs were all fed with the regular medium. Four groups of plugs (Control) were allowed to remain in free swell conditions. Another Four groups of plugs (DC) were mechanically loaded using the dynamic compression protocol with 4 compression days. 6 slices were used per two sets of Control and DC group, with one plug per slice going to each group of each set. Following the loading protocol, each of the 4 sets of the Control and DC groups was radiolabeled for 1, 2, 4, or 24 hours using twice the typical concentration of radiolabels. The groups at each time point were analyzed as 4 separate experiments of 2 experimental groups (Control, DC) with N = 6. The goal of this experiment was to repeat Experiment 4 with a larger yield of cartilage. Details of the experimental design are found in Appendix C.

Assays

I. T_{label} = 1 hour

Figure 3.8a shows the results of the sulfate incorporation (mean ± SEM) assay. The sulfate incorporation in the DC group was 1.18 times that of the Control group indicating a trend towards increased synthesis caused by compression. However, T-test analysis indicated that this trend did not quite reach statistical significance ($p = 0.073$). Figure 3.8b shows the results of the proline incorporation (mean ± SEM) assay. The proline incorporation in the DC group was 1.47 times that of the Control group and T-test

analysis indicates that the difference is statistically different ($p < 0.05$). Figure 3.8c shows the results of the total GAG (mean ± SEM) assay. While the total GAG in the DC group was 1.14 times that of the Control group, ANOVA analysis indicates that the difference total in GAG is not statistically significant ($p > 0.05$). Details of the data and analyses of the assays are found in Appendix C.

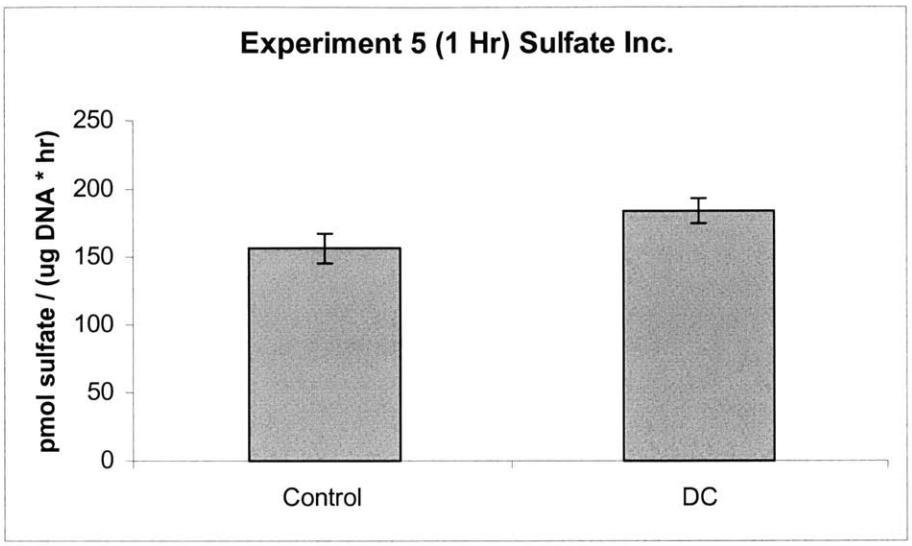

Figure 3.8a. Experiment 5 (1 hour): rate of ^{35}S-sulfate incorporation (mean ± SEM).

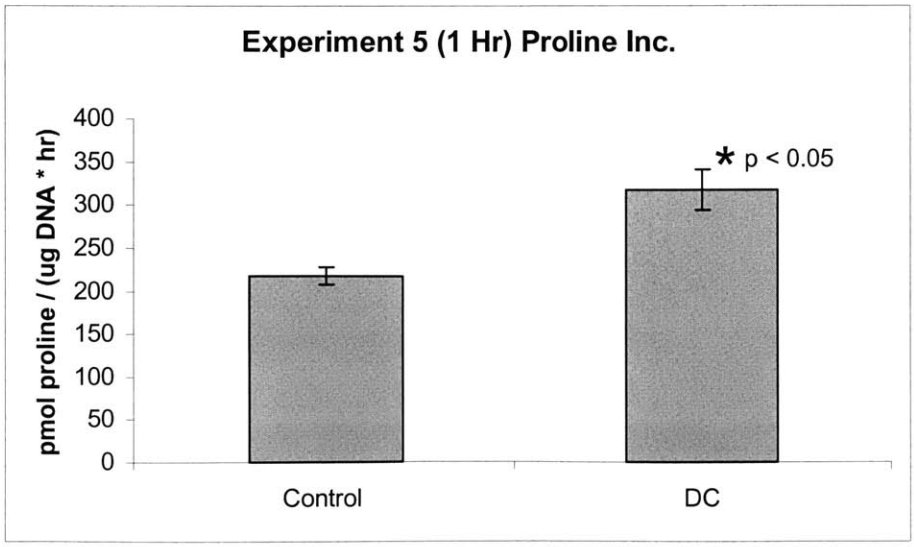

Figure 3.8b. Experiment 5 (1 hour): rate of ^{3}H-proline incorporation (mean ± SEM). (*, +: statistically different, $p < 0.05$).

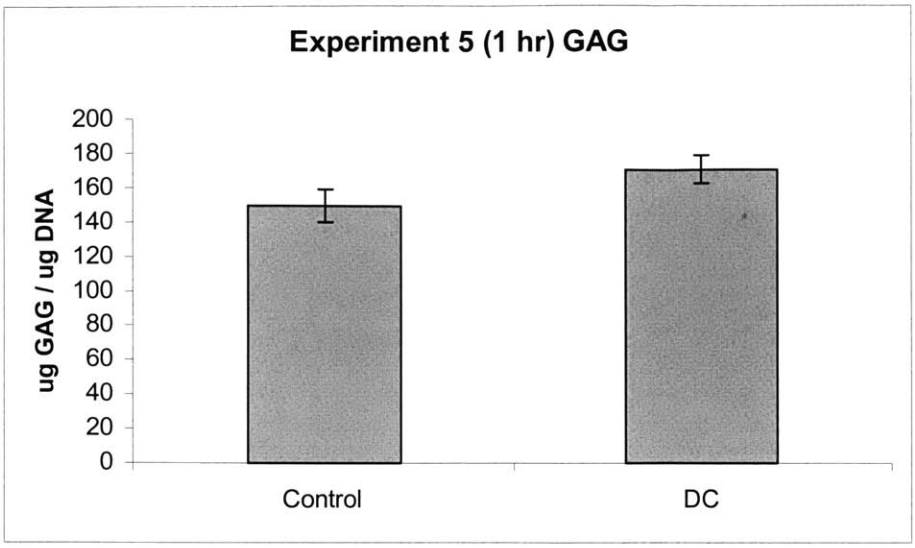

Figure 3.8c. Experiment 5 (1 hour): total GAG content (mean ± SEM).

II. T_{label} = 2 hour

Figure 3.9a shows the results of the sulfate incorporation (mean ± SEM) assay. While the sulfate incorporation in the DC group was 1.09 times that of the Control group, ANOVA analysis indicates that the difference is not statistically different ($p > 0.05$). Figure 3.9b shows the results of the proline incorporation (mean ± SEM) assay. The proline incorporation in the DC group was 1.28 times that of the Control group indicating a trend towards increased synthesis caused by compression. However, T-test analysis indicated that this trend did not quite reach statistical significance ($p = 0.093$). Figure 3.9c shows the results of the total GAG (mean ± SEM) assay. While the total GAG in the DC group was actually smaller than that of the Control group, T-test analysis indicates that the difference in total GAG is not statistically significant ($p > 0.05$). Details of the data and analyses of the assays are found in Appendix C.

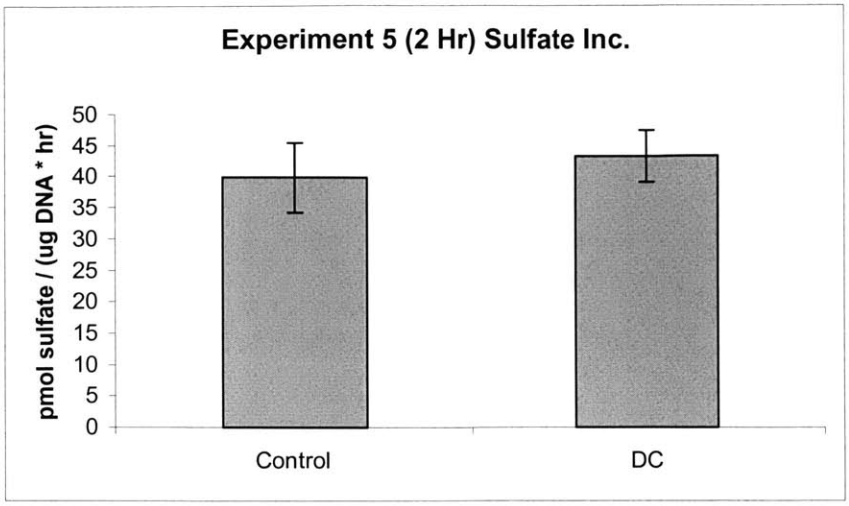

Figure 3.9a. Experiment 5 (2 hour): rate of ^{35}S-sulfate incorporation (mean ± SEM).

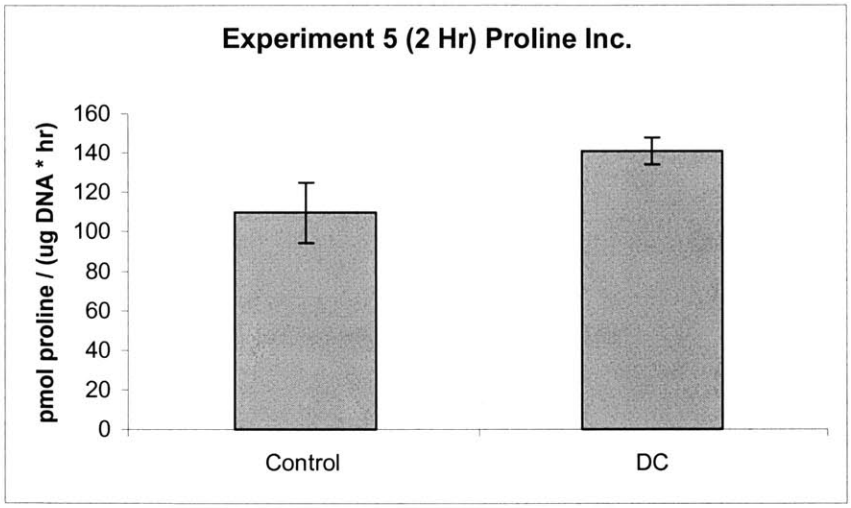

Figure 3.9b. Experiment 5 (2 hour): rate of ^{3}H-proline incorporation (mean ± SEM).

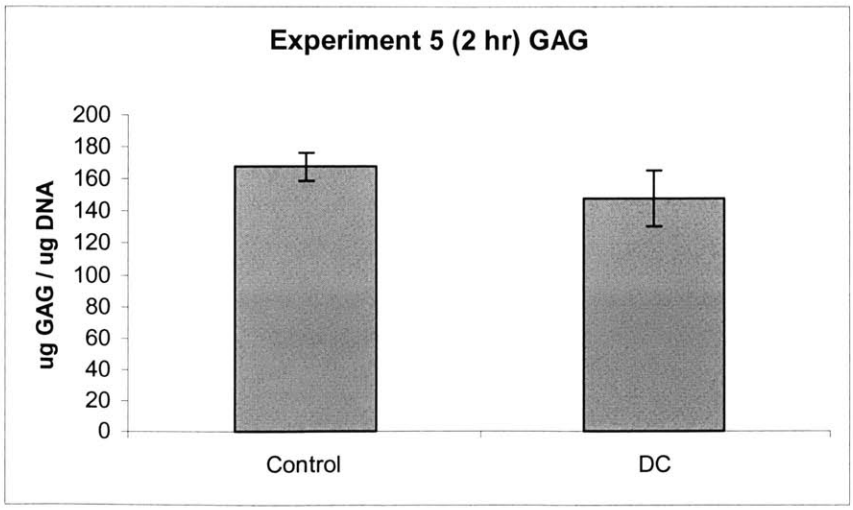

Figure 3.9c. Experiment 5 (2 hour): total GAG content (mean ± SEM).

III. T_{label} = 4 hour

The data for this experiment is not shown because of problems in the DNA content assay. The sulfate and proline incorporations and the total GAG content data could not be properly normalized to the DNA data.

IV. T_{label} = 24 hour

Figure 3.10a shows the results of the sulfate incorporation (mean ± SEM) assay. While the sulfate incorporation in the DC group was 1.20 times that of the Control group, T-test analysis indicates that the difference is not statistically different ($p > 0.05$). Figure 3.10b shows the results of the proline incorporation (mean ± SEM) assay. While the proline incorporation in the DC group was actually smaller than that of the Control group, T-test analysis indicates that the difference in the incorporation is not statistically significant ($p > 0.05$). Figure 3.10c shows the results of the total GAG (mean ± SEM) assay. The total GAG in the DC group was 1.23 times that of the Control group and T-test analysis indicates that the difference is statistically different ($p < 0.05$). Details of the data and analyses of the assays are found in Appendix C.

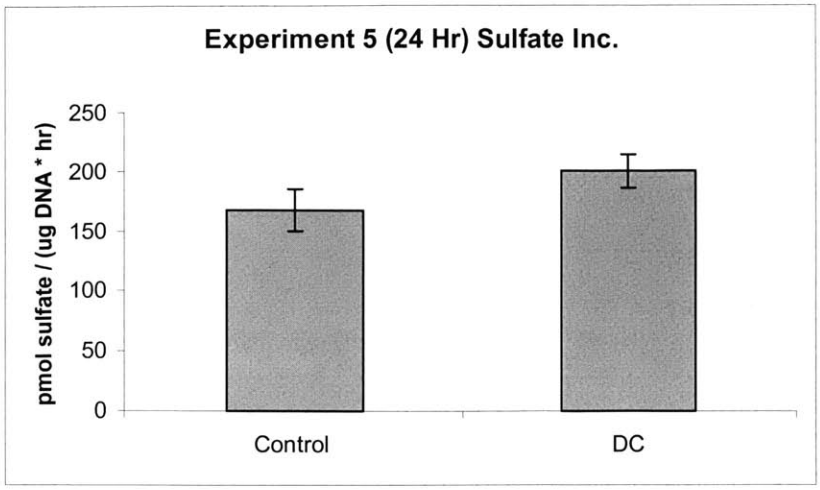

Figure 3.10a. Experiment 5 (24 hour): rate of ^{35}S-sulfate incorporation (mean ± SEM).

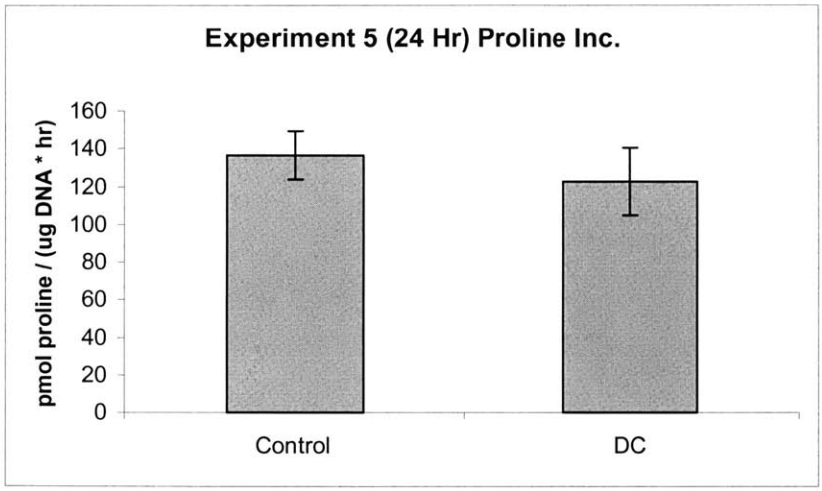

Figure 3.10b. Experiment 5 (24 hour): rate of ^{3}H-proline incorporation (mean ± SEM).

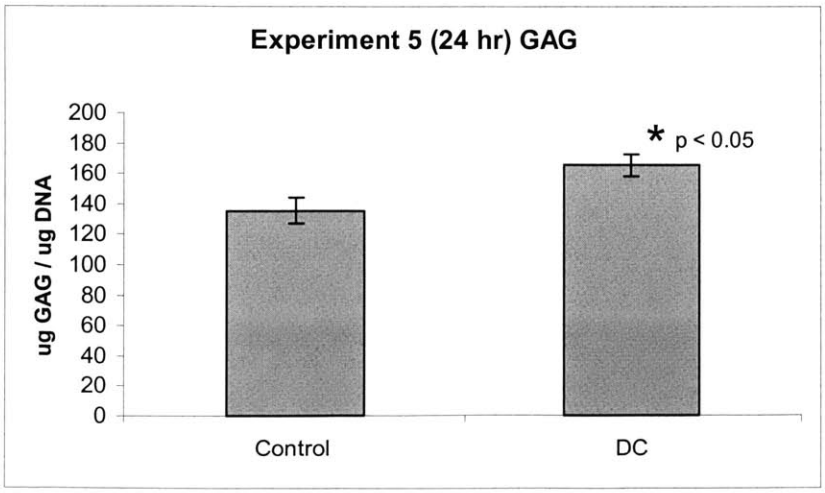

Figure 3.10c. Experiment 5 (24 hour): total GAG content (mean ± SEM). (*: statistically different, $p < 0.05$).

3.7 Experiment 6

Design

During the explant procedure, 12 cartilage slices were obtained from two joints. The slices from both joints were pooled, measured, and sorted by height measured by a digital caliper. The 12 slices nearest to 1.00 mm were chosen for the experiment. The cartilage plugs were fed with the regular medium (R), medium with ascorbate replaced by acorbyl-2-phosphate (A), or medium with FBS replaced by ITS (I). Half of the plugs within each medium group were allowed to remain in free swell conditions and the remaining plugs (DC) were mechanically loaded using the dynamic compression protocol with 6 compression days. 6 slices were used per set of free swell/DC/regular medium/experimental medium plugs (I or A), with one plug per slice going to each experimental condition. Following the loading protocol, the samples were radiolabeled for 2 hours using twice the typical concentration of radiolabels. The plugs were analyzed as 2 separate experiments of 4 experimental groups (R/R-DC/I/I-DC and R/R-DC/A/A-DC) with N = 6. The goal of this experiment was to examine the effects of replacing FBS and ascorbate in the media with ITS and A2P, respectively, as well as to continue to observe the effects of the compression protocol on the plugs. Details of the experimental design are found in Appendix C.

Assays

I. Regular vs. ITS Media

Figure 3.11a shows the results of the sulfate incorporation (mean ± SEM) assay for the experimental conditions. For the two groups in the regular medium (R, R-DC), the ratio of the DC group over the free swell group was 2.29. For the other two groups (I,

I-DC) in the ITS medium, the corresponding ratio was 0.94. For the two free swell groups (R, I), the ratio of the I group over the R group was 2.59. For the other two groups (R-DC, I-DC) the corresponding ratio was 1.07. The two factor ANOVA analysis (R vs. I and free swell vs. DC) indicated statistical differences ($p < 0.05$) for the data due to the two media, the compression protocol, and the interaction between both factors.

Figure 3.11b shows the results of the proline incorporation (mean ± SEM) assay for the experimental conditions. For the two groups in the regular medium (R, R-DC), the ratio of the DC group over the free swell group was 1.54. For the other two groups (I, I-DC) in the ITS medium, the corresponding ratio was 1.13. For the two free swell groups (R, I), the ratio of the I group over the R group was 1.42. For the other two groups (R-DC, I-DC) the corresponding ratio was 1.05. The two factor ANOVA analysis (R vs. I and free swell vs. DC) indicated a statistical difference ($p < 0.05$) for the data due to the compression protocol, but not due to the media or the interaction between both factors ($p > 0.05$).

Figure 3.11c shows the results of the total GAG (mean ± SEM) assay for the experimental conditions. For the two groups in the regular medium (R, R-DC), the ratio of the DC group over the free swell group was 1.15. For the other two groups (I, I-DC) in the ITS medium, the corresponding ratio was 0.95. For the two free swell groups (R, I), the ratio of the I group over the R group was 1.20. For the other two groups (R-DC, I-DC) the corresponding ratio was 0.98. The two factor ANOVA analysis (R vs. I and free swell vs. DC) indicated no statistical differences ($p > 0.05$) for the data due to the media, the compression protocol, or the interaction between both factors. Details of the data and analyses of the three assays are found in Appendix C.

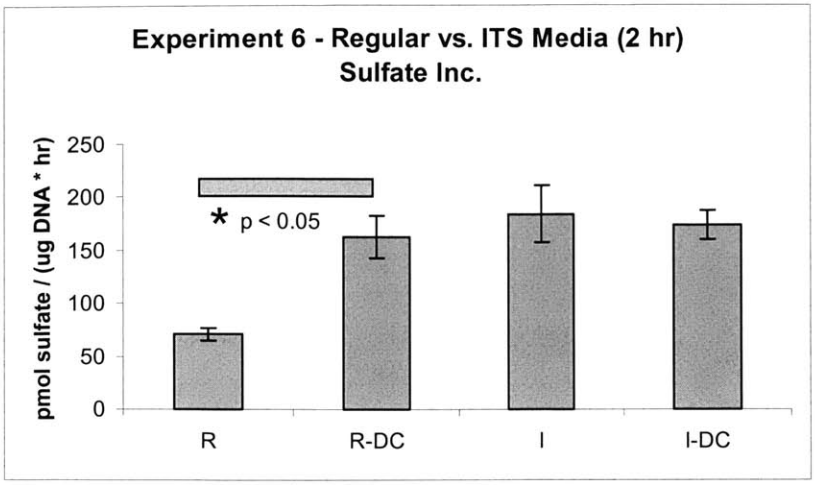

Figure 3.11a. Experiment 6 (2 hour, Regular vs. ITS media): rate of ^{35}S-sulfate incorporation (mean ± SEM). (*: statistically different, $p < 0.05$).

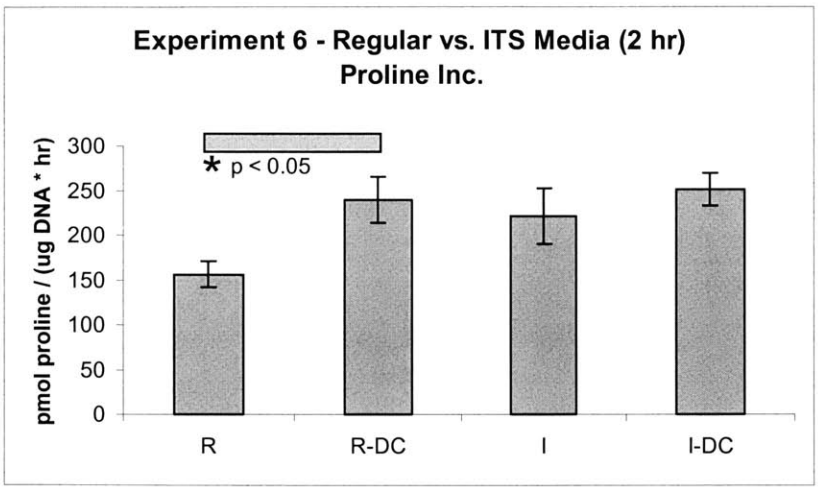

Figure 3.11b. Experiment 6 (2 hour, Regular vs. ITS media): rate of ^{3}H-proline incorporation (mean ± SEM). (*: statistically different, $p < 0.05$).

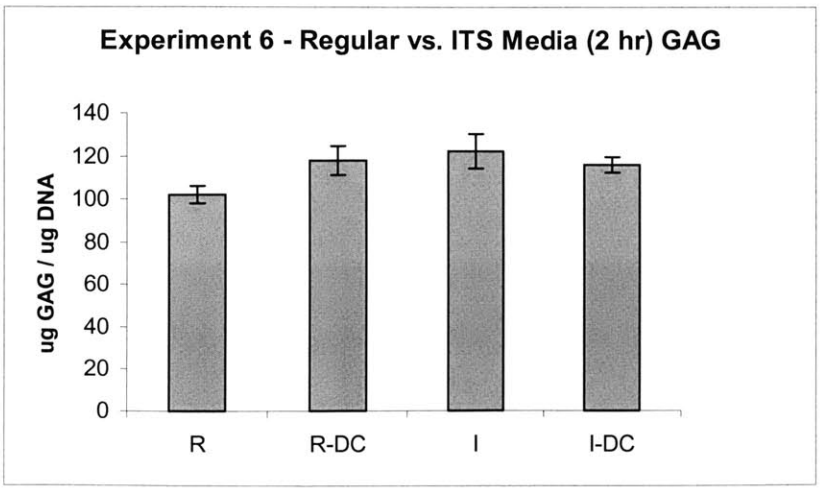

Figure 3.11c. Experiment 6 (2 hour, Regular vs. ITS media): total GAG content (mean ± SEM).

II. Regular vs. A2P Media

Figure 3.12a shows the results of the sulfate incorporation (mean ± SEM) assay for the experimental conditions. For the two groups in the regular medium (R, R-DC), the ratio of the DC group over the free swell group was 2.24. For the other two groups (A, A-DC) in the A2P medium, the corresponding ratio was 1.72. For the two free swell groups (R, A), the ratio of the A group over the R group was 1.22. For the other two groups (R-DC, A-DC) the corresponding ratio was 0.94. The two factor ANOVA analysis (R vs. A and free swell vs. DC) indicated a statistical difference ($p < 0.05$) for the data due to the compression protocol, but not due to the media or the interaction between both factors ($p > 0.05$).

Figure 3.12b shows the results of the proline incorporation (mean ± SEM) assay for the experimental conditions. For the two groups in the regular medium (R, R-DC), the ratio of the DC group over the free swell group was 1.54. For the other two groups (A, A-DC) in the A2P medium, the corresponding ratio was 1.20. For the two free swell groups (R, A), the ratio of the A group over the R group was 1.17. For the other two groups (R-DC, A-DC) the corresponding ratio was 0.91. The two factor ANOVA analysis (R vs. A and free swell vs. DC) indicated a statistical difference ($p < 0.05$) for the data due to the compression protocol, but not due to the media or the interaction between both factors ($p > 0.05$).

Figure 3.12c shows the results of the total GAG (mean ± SEM) assay for the experimental conditions. For the two groups in the regular medium (R, R-DC), the ratio of the DC group over the free swell group was 1.40. For the other two groups (A, A-DC) in the A2P medium, the corresponding ratio was 1.26. For the two free swell groups (R,

A), the ratio of the A group over the R group was 1.00. For the other two groups (R-DC, A-DC) the corresponding ratio was 0.90. The two factor ANOVA analysis (R vs. A and free swell vs. DC) indicated a statistical difference ($p < 0.05$) for the data due to the compression protocol, but not due to the media or the interaction between both factors ($p > 0.05$). Details of the data and analyses of the three assays are found in Appendix C.

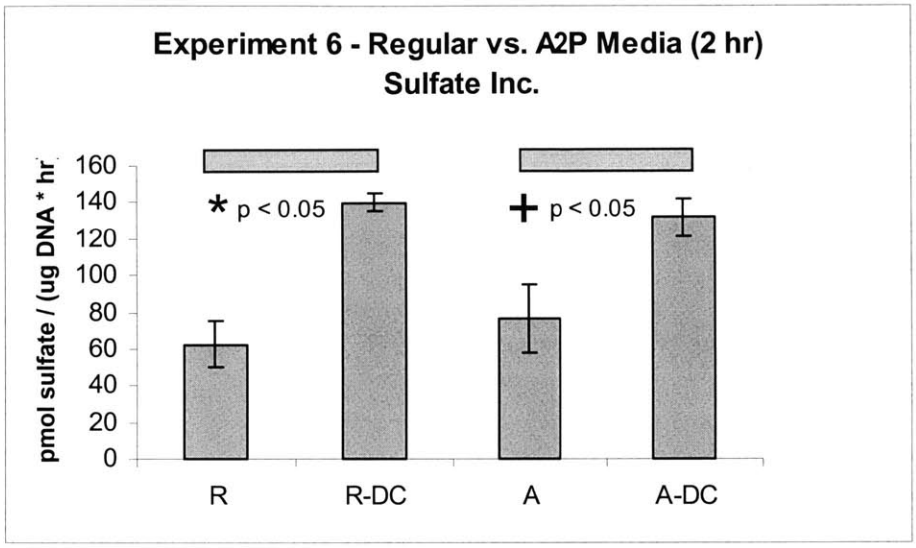

Figure 3.12a. Experiment 6 (2 hour, Regular vs. A2P media): rate of ^{35}S-sulfate incorporation (mean ± SEM). (*, +: statistically different, $p < 0.05$).

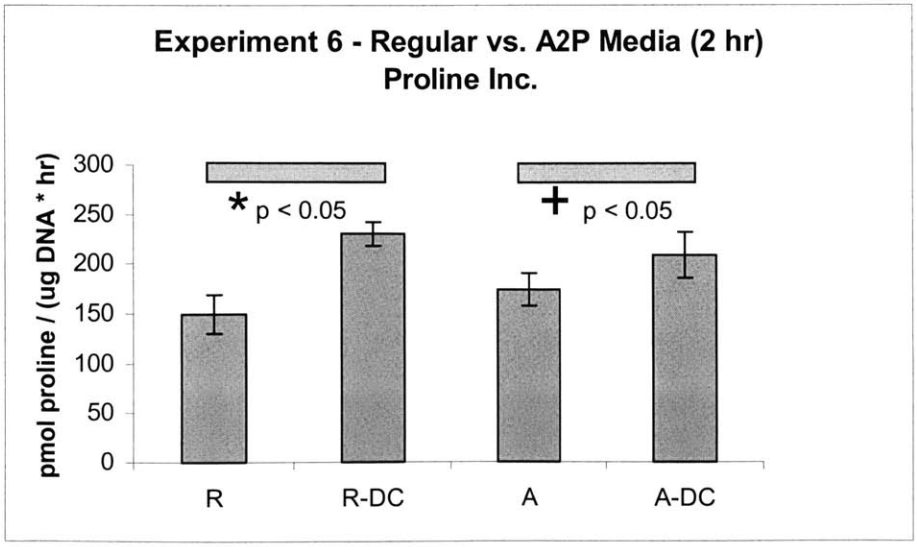

Figure 3.12b. Experiment 6 (2 hour, Regular vs. A2P media): rate of ^{3}H-proline incorporation (mean ± SEM). (*, +: statistically different, $p < 0.05$).

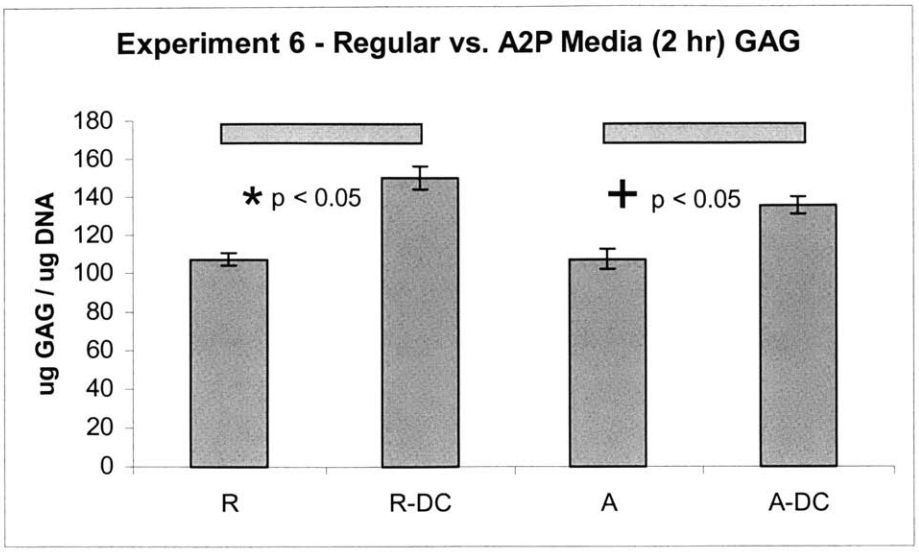

Figure 3.12c. Experiment 6 (2 hour, Regular vs. A2P media): total GAG content (mean ± SEM). (*, +: statistically different, $p < 0.05$).

3.8 Experiment 7

Design

During the explant procedure 12 cartilage slices were obtained from two joints. The slices from both joints were pooled, measured, and sorted by height measured by a digital caliper. The 12 slices nearest to 1.00 mm were chosen for the experiment. The cartilage plugs were fed with the regular medium (R) and medium with FBS replaced by ITS (I). Half of the plugs within each medium group were allowed to remain in free swell conditions and the remaining plugs (DC) were mechanically loaded using the dynamic compression protocol with 6 compression days. One plug per slice was used for each experimental condition. Following the loading protocol, the samples were radiolabeled for 2 hours using the typical concentration of radiolabels. One set of plugs was lost during the protocol, so the plugs were analyzed as one set of 4 experimental groups (R/R-DC/I/I-DC) with N = 11. The goal of this experiment was to repeat the regular vs. ITS media portion of Experiment 6. Details of the experimental design are found in Appendix C.

Assays

Figure 3.13a shows the results of the sulfate incorporation (mean ± SEM) assay for the experimental conditions. For the two groups in the regular medium (R, R-DC), the ratio of the DC group over the free swell group was 1.57. For the other two groups (I, I-DC) in the ITS medium, the corresponding ratio was 0.72. For the two free swell groups (R, I), the ratio of the I group over the R group was 2.08. For the other two groups (R-DC, I-DC) the corresponding ratio was 0.95. The two factor ANOVA analysis (R vs. I and free swell vs. DC) indicated statistical differences ($p < 0.05$) for the data due to the two media and the interaction between both factors, but not due to the compression protocol alone ($p > 0.05$).

Figure 3.13b shows the results of the proline incorporation (mean ± SEM) assay for the experimental conditions. For the two groups in the regular medium (R, R-DC), the ratio of the DC group over the free swell group was 1.37. For the other two groups (I, I-DC) in the ITS medium, the corresponding ratio was 0.75. For the two free swell groups (R, I), the ratio of the I group over the R group was 1.86. For the other two groups (R-DC, I-DC) the corresponding ratio was 1.01. Again, the two factor ANOVA analysis (R vs. I and free swell vs. DC) indicated statistical differences ($p < 0.05$) for the data due to the two media and the interaction between both factors, but not due to the compression protocol alone ($p > 0.05$).

The total GAG content data was not shown here. Details of the data and analyses of the three assays are found in Appendix C.

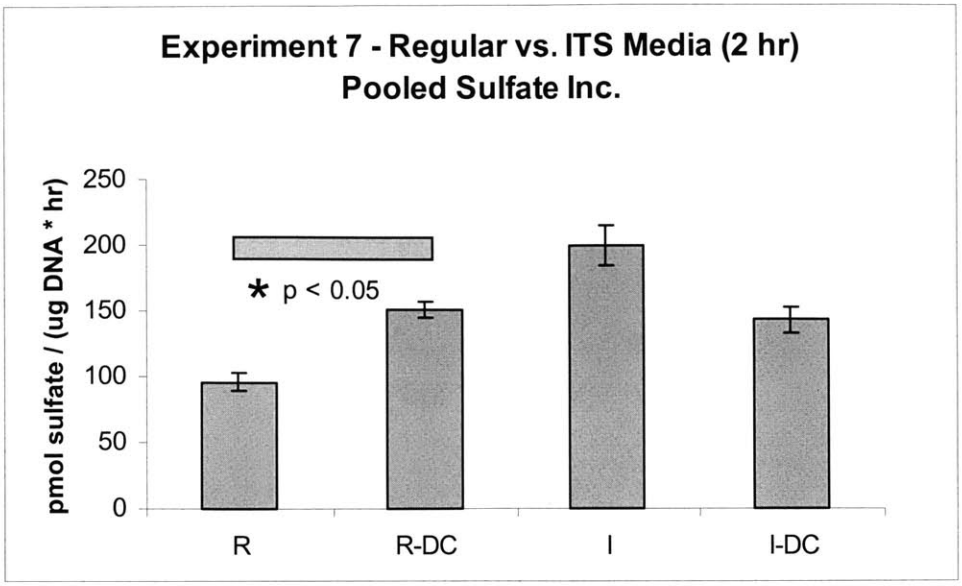

Figure 3.13a. Experiment 7 (2 hour, Regular vs. ITS media): rate of ^{35}S-sulfate incorporation (mean ± SEM). (*: statistically different, $p < 0.05$).

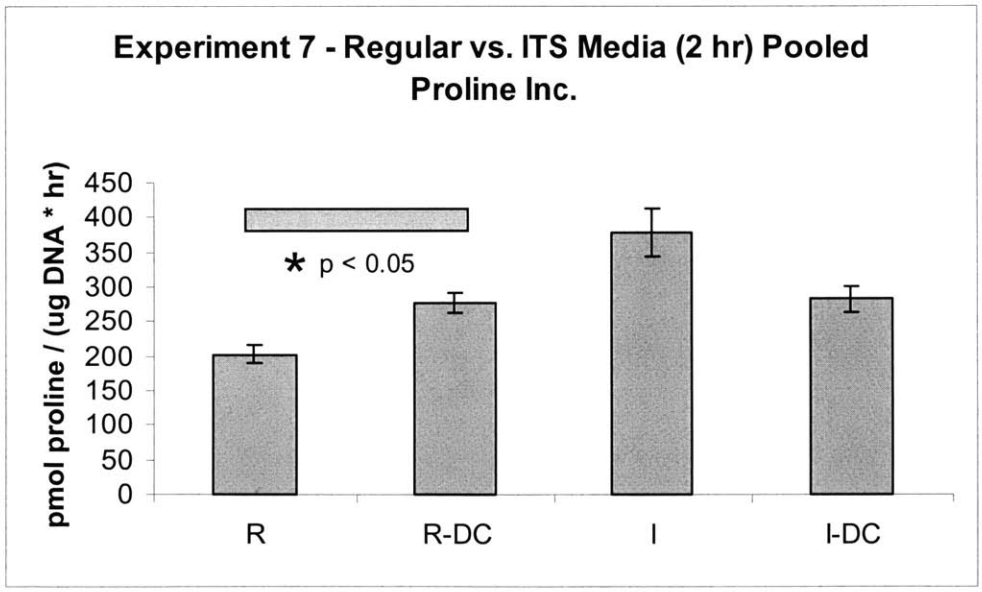

Figure 3.13b. Experiment 7 (2 hour, Regular vs. ITS media): rate of ^{3}H-proline incorporation (mean ± SEM). (*: statistically different, $p < 0.05$).

3.9 Experiment 8

Design

During the explant procedure, 12 cartilage slices were obtained from two joints. The slices from both joints were pooled, measured, and sorted by height measured by a digital caliper. The 12 slices nearest to 1.00 mm were chosen for the experiment. The

cartilage plugs were fed with the regular medium (R) and medium with both FBS replaced by ITS and ascorbate replaced by A2P (IA). Half of the plugs within each medium group were allowed to remain in free swell conditions and the remaining plugs (DC) were mechanically loaded using the dynamic compression protocol with 6 compression days. One plug per slice was used for each experimental condition. Following the loading protocol, the samples were radiolabeled for 2 hours using the typical concentration of radiolabels. The plugs were not labeled with proline in this experiment. The plugs were analyzed as two set of 4 experimental groups (R/R-DC/IA/IA-DC) with N = 6 for both sets. The goal of this experiment was to examine the effects of replacing both FBS and ascorbate in the media with both ITS and A2P, simultaneously, as well as to continue to observe the effects of the compression protocol on the plugs. Details of the experimental design are found in Appendix C.

Assays

I. Regular vs. ITS-A2P Media, Set 1

Figure 3.14a shows the results of the sulfate incorporation (mean ± SEM) assay for the experimental conditions. For the two groups in the regular medium (R, R-DC), the ratio of the DC group over the free swell group was 0.16. For the other two groups (IA, IA-DC) in the ITS-A2P medium, the corresponding ratio was 0.14. For the two free swell groups (R, IA), the ratio of the IA group over the R group was 1.22. For the other two groups (R-DC, IA-DC) the corresponding ratio was 1.06. The two factor ANOVA analysis (R vs. IA and free swell vs. DC) indicated statistical differences ($p < 0.05$) for the data due to the compression protocol, but not due to the two media or the interaction between both factors ($p > 0.05$).

Figure 3.14b shows the results of the total GAG (mean ± SEM) assay for the experimental conditions. For the two groups in the regular medium (R, R-DC), the ratio of the DC group over the free swell group was 1.10. For the other two groups (IA, IA-DC) in the ITS-A2P medium, the corresponding ratio was 1.02. For the two free swell groups (R, IA), the ratio of the IA group over the R group was 1.01. For the other two groups (R-DC, IA-DC) the corresponding ratio was 0.93. The two factor ANOVA analysis (R vs. I and free swell vs. DC) indicated no statistical differences ($p > 0.05$) for the data due to the two media, the compression protocol, or the interaction between both factors. Details of the data and analyses of the three assays are found in Appendix C.

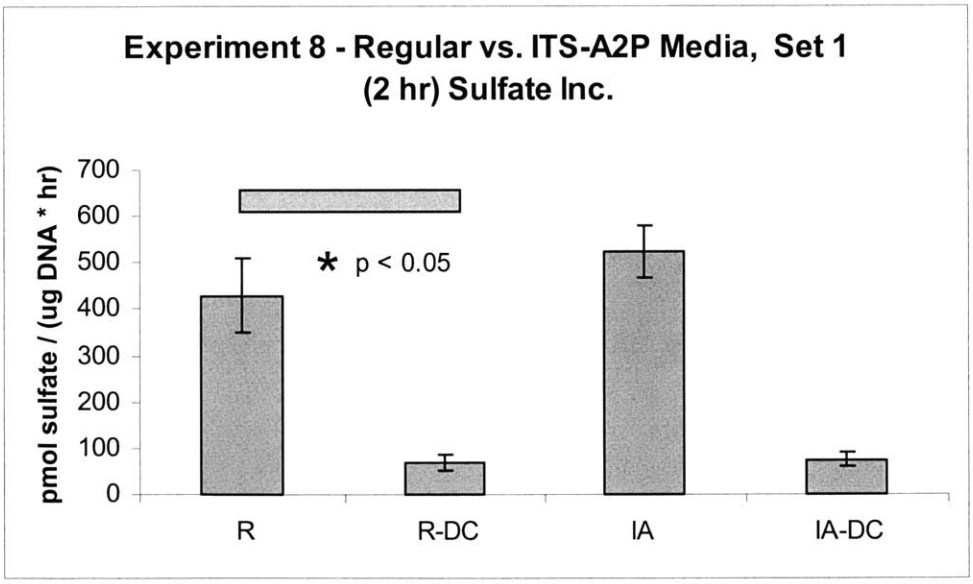

Figure 3.14a. Experiment 8 (2 hour, Regular vs. ITS-A2P media Set 1): rate of ^{35}S-sulfate incorporation (mean ± SEM). (*: statistically different, $p < 0.05$).

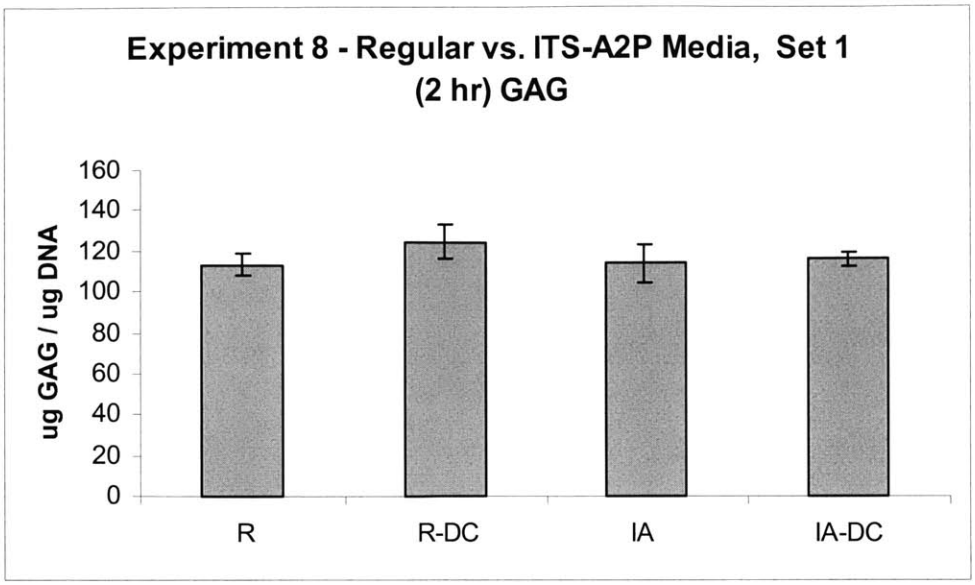

Figure 3.14b. Experiment 8 (2 hour, Regular vs. ITS-A2P media Set 1): total GAG content (mean ± SEM).

II. Regular vs. ITS-A2P Media, Set 2

Figure 3.15a shows the results of the sulfate incorporation (mean ± SEM) assay for the experimental conditions. For the two groups in the regular medium (R, R-DC), the ratio of the DC group over the free swell group was 0.97. For the other two groups (IA, IA-DC) in the ITS-A2P medium, the corresponding ratio was 0.98. For the two free swell groups (R, IA), the ratio of the IA group over the R group was 1.04. For the other two groups (R-DC, IA-DC) the corresponding ratio was 1.05. The two factor ANOVA analysis (R vs. IA and free swell vs. DC) indicated no statistical differences ($p > 0.05$) for the data due to the two media, the compression protocol, or the interaction between both factors.

Figure 3.15b shows the results of the total GAG (mean ± SEM) assay for the experimental conditions. For the two groups in the regular medium (R, R-DC), the ratio of the DC group over the free swell group was 0.84. For the other two groups (IA, IA-DC) in the ITS-A2P medium, the corresponding ratio was 0.95. For the two free swell

groups (R, IA), the ratio of the IA group over the R group was 0.72. For the other two groups (R-DC, IA-DC) the corresponding ratio was 0.81. The two factor ANOVA analysis (R vs. I and free swell vs. DC) indicated no statistical differences ($p > 0.05$) for the data due to the two media, the compression protocol, or the interaction between both factors.

Details of the data and analyses of the three assays are found in Appendix C.

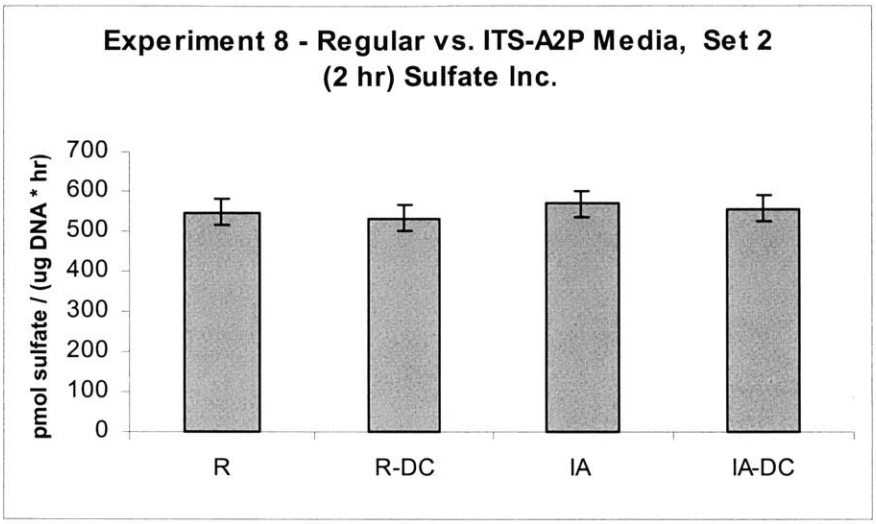

Figure 3.15a. Experiment 8 (2 hour, Regular vs. ITS-A2P media Set 2): rate of ^{35}S-sulfate incorporation (mean ± SEM).

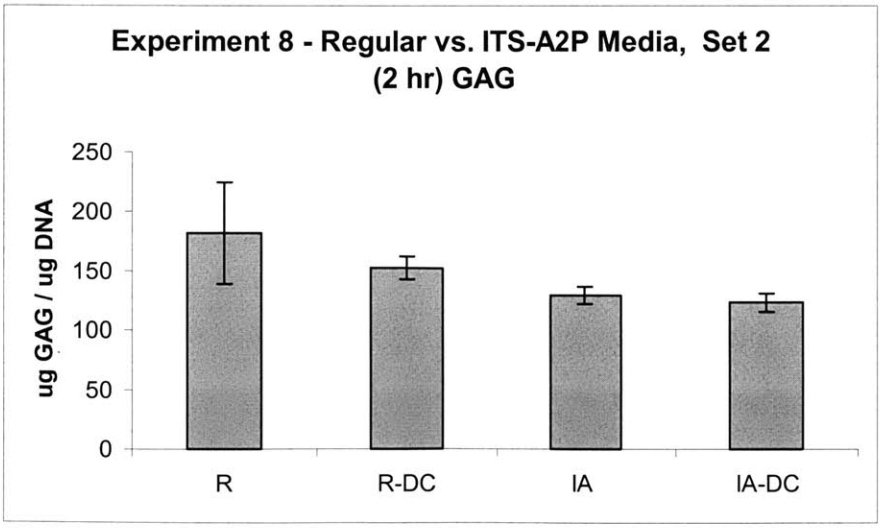

Figure 3.15b. Experiment 8 (2 hour, Regular vs. ITS-A2P media Set 2): total GAG content (mean ± SEM).

Chapter 4 – Discussion

4.1 Free swell vs. dynamic compression

The primary goal of the series of experiments was to demonstrate the effects of an extended mechanical loading protocol on articular cartilage explants. Experiments 1-8 all tested plugs in the regular medium in free swell versus loaded conditions. Throughout the course of the experiments, the depths from which the cartilage slices were obtained, the radiolabel time points following the compression protocol, and the number of compression/rest days in the protocol were varied.

In Experiments 1-4, the cartilage slices were taken as near to the femoropatellar groove surface as possible after slicing with the microtome. In Experiment 1, the T-test analyses of the assays indicated nothing conclusive could be made concerning the effects of the loading from the experiment, which led to a repeat of the experiment with a larger N size in Experiment 2. In Experiment 2, the T-test analysis of the sulfate incorporation assay indicated a significant effect ($p < 0.05$) due to the loading (Figure 3.2a). However a similar effect was not seen in the GAG assay (Figure 3.2b). Experiment 2 indicated that biosynthesis rates were elevated in the loaded samples in the 24 hours following the loading, but that the general total synthesis of matrix proteins was not significantly elevated over the course of the loading protocol. In Experiment 3, shorter radiolabel time points were chosen to further focus on the biosynthesis rates. Even though, the biosynthesis rates were not shown to be statistically elevated due to the loading, Figures 3.3a and 3.5a show that the biosynthesis rates for the loaded samples were higher than the free swell controls. Furthermore, Figures 3.3b and 3.5b also showed a similar increase in total GAG content for the loaded samples. Because a change in GAG content is a long-

term response of cartilage to stimuli, this was the first indication that the samples were responding to the extended loading as predicted. Experiment 4 was designed to be a repeat of Experiment 3 to verify the positive trends seen previously, but its usefulness was diminished by the very low yield of cartilage from the harvest. As expected from a smaller experiment size (N), a higher level of variability was observed in the results as compared to previous experiments. Although statistical differences due to loading were not revealed through T-test analyses of the assays, the biosynthetic rates and the GAG content (Figures 3.6a-3.6c, 3.7a-3.7c) all visually indicate the predicted trend of increased activity from the dynamic compression over free swell conditions. From the results of the first four experiments, attempting to obtain the most superficial slices from the cores seemed to cause an increased level of variability in the data. However, overall the first four experiments demonstrated encouraging evidence towards demonstrating the validity of the experimental goal and hypothesis.

In order to address the variability issues raised by taking the more superficial slices of cores, the harvesting protocol was adjusted to produce more uniform slices closer to 1 mm in thickness. The adjusted harvesting protocol was used in Experiments 5-8. Experiment 5 repeated Experiment 4 with a larger N size and more uniform plugs. Again, the results of the experiment demonstrated the positive trends in the increased biosynthesis rates due to the dynamic compression in three of the radiolabel time points (Figures 3.8a-3.8b, 3.9a-3.9b, and 3.10a). Figure 3.8b showed a statistical significance in the increased rates due to the compression protocol by T-test analysis ($p < 0.05$). Similarly, the positive trend in increased extracellular matrix growth due to loading was

seen in two of the time points (Figures 3.8c and 3.10c). Figure 3.10c showed a statistical significance in the GAG content due to the compression by T-test analysis ($p < 0.05$). In Experiment 6, the compression protocol was lengthened by two pairs of compression/rest days for laboratory logistical reasons. Figures 3.11a-3.11b and 3.12a-3.12b showed statistical differences calculated via T-test and ANOVA ($p < 0.05$) due to the compression protocol for biosynthesis rates in the plugs fed by the regular medium in the Regular vs. ITS media sub-experiment and by the plugs in both the regular and A2P media in the Regular vs. A2P media sub-experiment. A positive trend was also visually indicated for the mechanically loaded plugs fed by the ITS medium in the Regular vs. ITS media sub-experiment. Figure 3.12c showed the statistical differences calculated by T-test and ANOVA ($p < 0.05$) in the GAG content for compressed plugs in both regular and A2P media further indicating increased long-term growth in both media due to the loading protocol. In Experiment 7, statistical differences were again seen in the biosynthesis rates via ANOVA analysis ($p < 0.05$) (Figures 3.13a and 3.13b) indicating the expected level of increases synthesis following the extended compression protocol. In Experiment 8, the GAG contents of the plugs fed by regular media in free swell and compression conditions were statistically equivalent by T-test and ANOVA analysis. However, the biosynthesis rates for the compressed plugs actually fell significantly (4x) for one trial (Figure 3.14a) of the experiment while staying the same for the other trial (Figure 3.15a). Because the GAG contents of the plugs were virtually equivalent, it was possible that an error was made in the actual biosynthesis assay procedure for the first trial. It was also possible that during the final compression day in the protocol, the plugs in the first trial may have been injured (an inadvertently high loading offset prior to the

sinusoidal compression portion of the protocol) and that there was insufficient time during the radiolabel time for the GAG to be lost to the media.

4.2 Regular vs. A2P media

A second goal of the experiments was to investigate the effects of replacing the FBS in the regular feeding medium with A2P in an extended mechanical loading protocol for articular cartilage explants. Because the normal ascorbate in the regular medium breaks down within 2 days, the longer lasting A2P was investigated as a source of acorbate in the feeding medium. Experiment 6 tested plugs in the regular medium as well as A2P medium for free swell versus loaded conditions. Throughout the course of the experiments, the adjusted harvesting protocol was used to obtain plugs of thickness near 1 mm.

In Experiment 6, the data from the free swell plugs in both Regular and A2P media were compared via T-test and ANOVA analysis. The data for the compressed plugs were also compared in a similar fashion. The biosynthesis rates and the total GAG content of the free swell plugs were statistically the same for the plugs in both media (Figures 3.12a – 3.12c). The rates and GAG content were also the same for the dynamically compressed plugs, indicating any differences between compressed and free swell plugs were due to the loading protocol and not the media.

4.3 Regular vs. ITS media

A third goal of the experiments was to investigate the effects of replacing the FBS in the regular feeding medium with ITS in an extended mechanical loading protocol for articular cartilage explants. Because FBS contains, among other things, various growth factors, it must be replaced with ITS prior to the study of any other growth factors, such

as IGF-I. Experiments 6 and 7 tested plugs in both the regular medium and the ITS medium for free swell versus loaded conditions. Throughout the course of the experiments, the adjusted harvesting protocol was used to obtain plugs of thickness near 1 mm.

In Experiment 6, the data from the free swell plugs in both Regular and ITS media were compared via T-test and ANOVA analysis. The data for the compressed plugs were also compared in a similar fashion. The biosynthesis tates and the total GAG content of the dynamically compressed plugs were statistically the same for the plugs in both media (Figures 3.11a – 3.11c). However, the free swell plugs fed with ITS medium showed higher biosynthesis rates and GAG content than the free swell plugs in regular medium, which was surprising. The data indicates that there was some statistical effect from the interaction of the medium and the loading protocol in the experiment. Experiment 7 repeated the Regular vs. ITS media sub-experiment with a larger N size. The same results were seen in Experiment 7 (Figures 3.13a and 3.13b) as in Experiment 6. Again these were unexpected results because the compressed plugs showed no preference for feeding medium while the free swell plugs favored the ITS medium for biosynthesis and matrix growth.

4.4 Regular vs. ITS-A2P media

The final goal of the experiments was to investigate the effects of replacing both the FBS and the ascorbate in the regular feeding medium with ITS and A2P in an extended mechanical loading protocol for articular cartilage explants in preparation for a study of the synergistic effects of extended dynamic compression and IGF-I. Experiment 8 tested plugs in both the regular medium and the ITS-A2P medium for free swell versus

loaded conditions. Throughout the course of the experiments, the adjusted harvesting protocol was used to obtain plugs of thickness near 1 mm.

In Experiment 8, the data from the free swell plugs in both Regular and ITS-A2P media were compared via T-test and ANOVA analysis. The data for the compressed plugs were also compared in a similar fashion. The total GAG contents of the free swell plugs were statistically the same for the plugs in both media (Figures 3.14b and 3.15b). Similarly, the GAG contents were also the same for the dynamically compressed plugs in both media. The same similarities by medium were seen in the biosynthesis rates for the plugs (Figures 3.14a and 3.15a) even though the dynamically compressed plugs results in unexpectedly low synthesis values compared to the free swell plugs, as discussed previously. The results of Experiment 8 seemed to indicate no long-term differences in using ITS-A2P media in the extended compression protocol preparation for the addition of IGF-I for study.

4.5 Variation in assay results

An interesting observation across all the experimental results was that the results of the assays were consistent within a single experiment, but varied greatly across separate experiments, sometimes by orders of magnitude. This precluded pooling of data across separate experiments and led to each experiment being analyzed separately. Because all the assay data were normalized to the results of the DNA content assay, the precision of the DNA assay could play a factor in the variability between separate experiments. Another source of the variability might have arisen from the use of different lots of various chemicals and materials in the assays (i.e. stock GAG and DNA standards, radionuclides, dyes, scintillation fluid, et cetera). A further, less remote,

possibility may have arisen from machine error/malfunction (especially the DNA plate reader). However this is very unlikely because the results within each experiment were fairly self-consistent.

4.6 Conclusions

This study seems to confirm the hypothesis that an extended alternate day compression protocol can stimulate cartilage growth and repair in cartilage explants. Increased levels of total GAG content and increased rates of extracellular matrix protein synthesis were observed in the majority of experiments. Furthermore, the study also seems to confirm the hypothesis that replacing FBS and L-ascorbic acid with ITS and A2P, respectively did not significantly affect the results of free swell and dynamic compression conditions. However, future research is needed to confirm the repeatability of using the ITS-A2P feeding medium in the explant system.

4.7 Future Work

The first step in future work would be to repeat and validate the results of the later experiments for free swell vs. dynamic compression and Regular vs. ITS-A2P media conditions with larger N sizes for greater statistical confidence. It may also be interesting to further vary the number of compression/rest days in the protocol to discover any potential upper limits to the length of the explant viabilities. Further research would also be needed to confirm these results for human cartilage systems. Before proceeding with the study of the synergistic effects of IGF-I stimulation with the dynamic compression protocol, it would be useful to investigate the variability issues of the assays seen during the course of the experiments. Specifically, it would be useful to conduct a deeper study on the DNA content assay because the DNA data is used to normalize the other assay

results. Alternatively, other assays may be added to the protocol to observe other facets of cartilage growth and repair. These assays might include measurement of wet and dry weight or cell viability assays at various time points in the protocol.

Finally, experiments investigating the potential synergistic effects of IGF-I and extended dynamic compression in the bovine explant system should be conducted and studied to build on the data collected in this study. The data from that study should help improve the understanding of the cellular mechanisms between the two stimulatory pathways. For example, real-time polymerase chain reaction (PCR) assays may be performed on the cartilage samples to probe into the gene expression activity of the cartilage response to stimuli arising from two separate pathways. The results of that study might be used to construct and/or extend existing mathematical models of the mechanical and biochemical aspects of chondrocyte metabolism. The results of that study might also lead to novel treatment therapies for osteoarthritis and other traumatic knee injuries.

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Appendix A – GAG and DNA Content Assays

A.1 GAG Assay

A.1.1 Objectives

The goals of this experiment were to quantify the precision of the physical pipeting processes of the GAG content assay and to quantify a useful range of GAG standards for use in calculating actual GAG content from spectrophotometric GAG data.

A.1.2 Methods

Using a 2 mg/mL concentration of stock GAG solution, at least 200 μL of GAG standards from 2 mg/mL down to 0 ug/mL were made by serial dilutions in Tris buffer. Next, six other test GAG solutions were made, ranging between 15.38 and 66.67 ug/mL in concentration. Table A1 shows the concentrations of the standards and test solutions. Then using the previously described GAG content protocol, 2 sets of 20 μL of each the GAG standards and test solutions were aliquoted into a 96 well microplate in triplicate. For the first set, a new pipet tip was used for each of the three aliquots. For the second set, the same pipet tip was reused for the three aliquots. Finally, the DMMB dye was added as described previously and the photometric optical density (OD) results were recorded, analyzed, and converted to μg/mL of GAG.

Table A1. GAG standards and test solutions.

GAG Standards [μg/mL]					
2000.00	1000.00	500.00	200.00	100.00	50.00
25.00	12.50	6.25	3.13	1.56	0.00
GAG Test Solutions [μg/mL]					
66.67	40.00	28.57	22.22	18.18	15.38

A.1.3 Results

Table A2 shows the OD results of the assay for the first set of aliquots (new tips). The data in the first set indicates that the assay results saturate for concentrations above 100 μg/mL. The data also indicates that the standard deviation of each set of three aliquots is usually within 3% of the mean of the set.

Table A3 shows the OD results of the assay for the second set of aliquots (reused tips). The data in the second set again indicates that the assay results saturate for concentrations above 100.00 μg/mL. The data also indicates that the standard deviation of each set of three aliquots is usually within 4% of the mean of the set, with the exception of the 1000.00 μg/uL standard.

Table A2. GAG Standards assay data for individual tips.

Standards [μg/mL]	Individual Tips [OD]			Mean [OD]	Std. Dev. [OD]	Std. Dev/Mean [%]
2000.00	0.548	0.556	0.543	0.549	0.006557	1.19
1000.00	0.553	0.556	0.548	0.552	0.004041	0.73
500.00	0.547	0.565	0.537	0.550	0.014189	2.58
200.00	0.531	0.539	0.540	0.537	0.004933	0.92
100.00	0.493	0.487	0.486	0.489	0.003786	0.77
50.00	0.380	0.380	0.373	0.378	0.004041	1.07
25.00	0.295	0.297	0.290	0.294	0.003606	1.23
12.50	0.242	0.239	0.238	0.240	0.002082	0.87
6.25	0.214	0.210	0.211	0.212	0.002082	0.98
3.13	0.203	0.201	0.200	0.201	0.001528	0.76
1.56	0.192	0.188	0.191	0.190	0.002082	1.09
0.00	0.181	0.180	0.180	0.180	0.000577	0.32

Table A3. GAG Standards assay data for repeated tips.

Standards [μg/mL]	Repeated Tips [OD]			Mean [OD]	Std. Dev. [OD]	Std. Dev/Mean [%]
2000.00	0.543	0.546	0.555	0.548	0.006245	1.14
1000.00	0.548	0.543	0.454	0.515	0.052887	10.27
500.00	0.537	0.531	0.564	0.544	0.017578	3.23
200.00	0.540	0.521	0.541	0.534	0.011269	2.11
100.00	0.486	0.480	0.491	0.486	0.005508	1.13
50.00	0.373	0.372	0.378	0.374	0.003215	0.86
25.00	0.290	0.289	0.293	0.291	0.002082	0.72
12.50	0.238	0.238	0.239	0.238	0.000577	0.24
6.25	0.211	0.210	0.214	0.212	0.002082	0.98
3.13	0.200	0.197	0.200	0.199	0.001732	0.87
1.56	0.191	0.187	0.191	0.190	0.002309	1.22
0.00	0.180	0.182	0.185	0.182	0.002517	1.38

Figure A1 shows three plots of the GAG standards between 0 and 50 (Trend 1), 0 and 100 (Trend 2), or 0 and 200 μg/mL (Trend 3), plots of the trendlines calculated for each range of standards, and the corresponding equations for each trendline. The trendlines were calculated as best fit linear curves using Microsoft EXCEL. Table A4 shows the concentrations of the GAG standards and test solutions calculated from each of the three trendlines, as well as the error percentage.

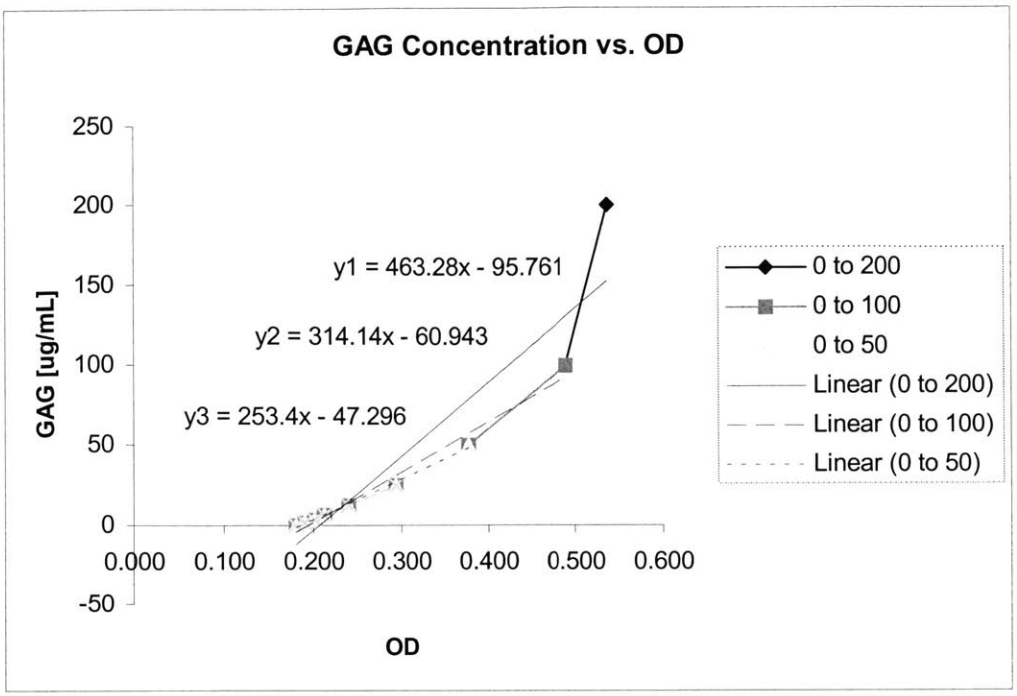

Figure A1. GAG standard data and fitted curves for various standard ranges.

Table A4. GAG standard curve fitting data for various standard ranges.

Standards	Mean OD	Trend 1 0 to 200	Trend 1 Error [%]	Trend 2 0 to 100	Trend 2 Error [%]	Trend 3 0 to 50	Trend 3 Error [%]
2000.00	0.549	158.58	92.07	111.52	94.42	91.82	95.41
1000.00	0.552	160.12	83.99	112.57	88.74	92.67	90.73
500.00	0.550	158.89	68.22	111.73	77.65	91.99	81.60
200.00	0.537	152.87	23.57	107.65	46.18	88.70	55.65
100.00	0.489	130.63	30.63	92.57	7.43	76.53	23.47
50.00	0.378	79.20	58.41	57.70	15.39	48.40	3.19
25.00	0.294	40.44	61.77	31.41	25.66	27.20	8.81
12.50	0.240	15.27	22.17	14.35	14.77	13.44	7.48
6.25	0.212	2.30	63.20	5.55	11.20	6.34	1.45
3.13	0.201	-2.49	179.59	2.30	26.28	3.72	19.10
1.56	0.190	-7.58	585.34	-1.15	173.71	0.93	40.19
0.00	0.180	-12.22		-4.29	N/A	-1.60	

Tests	Mean OD	Trend 1 0 to 200	Trend 1 Error [%]	Trend 2 0 to 100	Trend 2 Error [%]	Trend 3 0 to 50	Trend 3 Error [%]
66.67	0.412	95.26	42.90	68.59	2.88	57.19	14.22
40.00	0.309	47.39	18.48	36.13	9.68	31.00	22.49
28.57	0.303	44.46	55.60	34.14	19.48	29.40	2.90
22.22	0.280	33.80	52.11	26.91	21.10	23.57	6.07
18.18	0.263	26.24	44.30	21.78	19.79	19.43	6.88
15.38	0.252	21.14	37.41	18.32	19.11	16.65	8.19

A.1.4 Discussion and conclusions

The results in Tables A2 and A3 indicate that the OD results from a GAG assay are sufficiently precise and repeatable. The standard deviation of a set of triplicate sample aliquots typically falls within 3-4 percent of the mean of the set. Furthermore, the results also indicate no appreciable increase in precision from changing tips between each of the triplicate aliquots of a sample. Therefore, there should be negligible bias between reusing tips (dry tip vs. "wet" tip) within a set of replicated aliquots, and pipet tips can be reused for each GAG standard or test solution, saving a large number of tips.

Figure A1 indicates that the trendline for a given range of GAG standards becomes more accurate visually as the upper bound of the standard range decreases to 50 μg/mL. The results of Table A4 also indicate that a GAG standard of 50 μg/mL leads to lower error values of calculated concentrations for both the standard and test solutions that fall in the correct range. Table A4 also indicates a lower bound of 3.13 μg/mL (not including the 0 μg/mL of pure Tris buffer) for the serially diluted GAG standards. Therefore, the GAG standard range of 0 to 50 μg/mL leads to the best standard curve while still providing a reasonably large data range.

A.2 DNA assay

A.2.1 Objectives

The goals of this experiment were to quantify the precision of the physical pipeting processes of the DNA content assay and to quantify a useful range of DNA standards for use in calculating actual DNA content from flurometric DNA data.

A.2.2 Methods

Using a 10 μg/mL concentration of stock DNA solution, at least 200 μL of DNA standards from 10 μg/mL down to 0 μg/mL were made by serial dilutions in Tris buffer. Next, six other test DNA test solutions were made, ranging between 5.00 and 1.43 ug/mL in concentration. Table A5 shows the concentrations of the standards and test solutions. Then using the previously described DNA content protocol, 2 sets of 20 μL of each the DNA standards and test solutions were aliquoted into a 96 well microplate in triplicate. For the first set, a new pipet tip was used for each of the three aliquots. For the second set, the same pipet tip was reused for the three aliquots. Finally, the Hoechst dye was added as described previously and the fluorometric count results were recorded, analyzed, and converted to ug/mL of DNA.

Table A5. DNA standards and test solutions.

DNA Standards [μg/mL]					
10.00	8.00	6.00	5.00	2.50	1.25
0.63	0.31	0.16	0.08	0.04	0.00
DNA Test Solutions [μg/mL]					
5.00	3.33	2.50	2.00	1.67	1.43

A.2.3 Results

Tables A6 and A7 show the results of the assay for the first set of aliquots (new tips). The data in the first set indicate that the assay results do not saturate for concentrations in question. However, the data indicate that the results become less reliable if the concentration falls below 0.10 μg/mL. The data also indicate that the standard deviation of each set of three aliquots is usually within 7% of the mean of the set.

Tables A8 and A9 show the results of the assay for the second set of aliquots (reused tips). The data in the second set again indicate that the assay results do not saturate for concentrations in question, and still indicate that the results become less reliable if the concentration falls below 0.10 μg/mL. The data also indicate that the standard deviation of each set of three aliquots is usually within 8% of the mean of the set.

Table A6. DNA Standards assay data for individual tips.

Standards [μg/mL]	Individual Tips [Counts]			Mean [Counts]	Std. Dev. [Counts]	Std. Dev./Mean [%]
10.00	12931	12753	12498	12727.33	217.64	1.71
8.00	10283	10074	9855	10070.67	214.02	2.13
6.00	8365	7936	7670	7990.33	350.67	4.39
5.00	6793	6600	6811	6734.67	116.97	1.74
2.50	3905	3983	3673	3853.67	161.25	4.18
1.25	2334	2463	2196	2331.00	133.53	5.73
0.63	1629	1686	1715	1676.67	43.75	2.61
0.31	1207	1201	1223	1210.33	11.37	0.94
0.16	1032	1009	987	1009.33	22.50	2.23
0.08	903	951	1404	1086.00	276.44	25.45
0.04	928	1024	804	918.67	110.30	12.01
0.00	883	808	919	870.00	56.63	6.51

Table A7. DNA test dilutions assay data for individual tips.

Test Dilutions [μg/mL]	Individual Tips [Counts]			Mean [Counts]	Std. Dev. [Counts]	Std. Dev./Mean [%]
5.00	5935	6372	6117	6141.33	219.51	3.57
3.33	4795	4576	4552	4641.00	133.91	2.89
2.50	3920	3666	3722	3769.33	133.45	3.54
2.00	4506	4704	4618	4609.33	99.28	2.15
1.67	2604	2730	2697	2677.00	65.34	2.44
1.43	2544	2484	2523	2517.00	30.45	1.21

Table A8. DNA standards assay data for repeated tips.

Standards [μg/mL]	Repeated Tips [Counts]			Mean [Counts]	Std. Dev. [Counts]	Std. Dev./Mean [%]
10.00	12498	12487	11972	12319.00	300.56	2.44
8.00	9855	9465	9593	9637.67	198.80	2.06
6.00	7670	7536	7270	7492.00	203.60	2.72
5.00	6811	6887	6854	6850.67	38.11	0.56
2.50	3673	3606	3631	3636.67	33.86	0.93
1.25	2196	2393	2341	2310.00	102.09	4.42
0.63	1715	1576	1535	1608.67	94.34	5.86
0.31	1223	1157	1094	1158.00	64.51	5.57
0.16	987	1129	961	1025.67	90.43	8.82
0.08	1404	894	792	1030.00	327.88	31.83
0.04	804	892	986	894.00	91.02	10.18
0.00	919	952	968	946.33	24.99	2.64

Table A9. DNA test dilutions assay data for repeated tips.

Test Dilutions [μg/mL]	Repeated Tips [Counts]			Mean [Counts]	Std. Dev. [Counts]	Std. Dev./Mean [%]
5.00	5935	6201	6257	6131.00	172.03	2.81
3.33	4795	4278	4445	4506.00	263.84	5.86
2.50	3920	3741	3580	3747.00	170.08	4.54
2.00	4506	4426	4756	4562.67	172.14	3.77
1.67	2604	2639	2721	2654.67	60.05	2.26
1.43	2544	2478	2450	2490.67	48.26	1.94

Figure A2 shows a plot of the DNA standards between 0 and 10 μg/mL. The plot visually corroborates the fact that the DNA concentration does not saturate within the range of standards used.

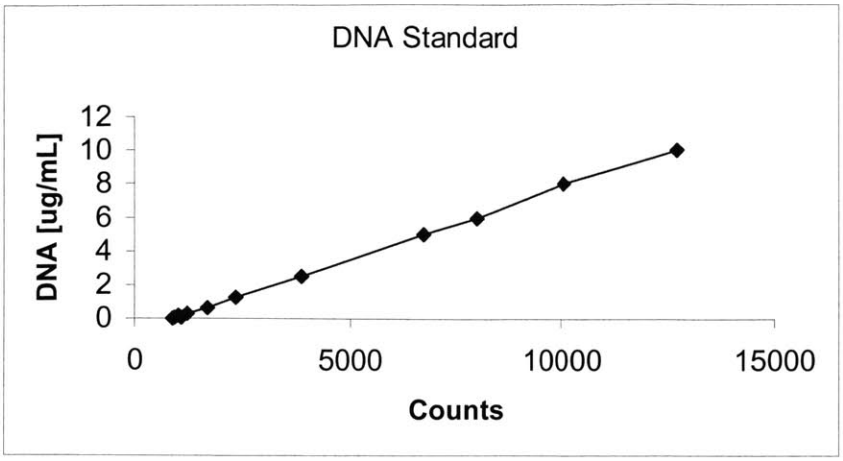

Figure A2. DNA standard curve.

Trendlines were calculated for two ranges of standards for 0.16 to 5.00 µg/mL (Trend 1) and 0 to 10.00 µg/mL (Trend 2), with concentrations < 0.10 omitted. Table A10 shows the concentrations of the DNA standards and test solutions calculated from Trend 1, as well as the error percentage. Table A11 shows the concentrations of the DNA standards and test solutions calculated from Trend 1, as well as the error percentage. The error percentages for both trends are similar and sufficiently low.

Table A10. DNA standard curve fitting data for Trend 1 (0.16 to 5.00 µg/mL).

DNA Standards [µg/mL]	Mean [Counts]	Trend 1	Error [%]
10.00	12727.33	10.07	0.66
8.00	10070.67	7.81	2.37
6.00	7990.33	6.04	0.74
5.00	6734.67	4.98	0.43
2.50	3853.67	2.53	1.30
1.25	2331.00	1.24	0.83
0.63	1676.67	0.68	9.46
0.31	1210.33	0.29	7.77
0.16	1009.33	0.12	24.75
0.08	1086.00	0.18	133.82
0.04	918.67	0.04	3.96
0.00	870.00	0.00	
DNA Test Dilutions [µg/mL]	Mean [Counts]	Trend 1	Error [%]
5.00	6141.33	4.47	10.51
3.33	4641.00	3.20	3.97
2.50	3769.33	2.46	1.57
2.00	4609.33	3.17	58.70
1.67	2677.00	1.53	7.99
1.43	2517.00	1.40	2.17

Table A11. DNA standard curve fitting data for Trend 2 (0 an 0.16 to 10.00 µg/mL).

DNA Standards [μg/mL]	Mean [Counts]	Trend 2	Error [%]
10.00	12727.33	10.01	0.14
5.00	6734.67	4.96	0.88
2.50	3853.67	2.52	0.98
1.25	2331.00	1.24	0.86
0.63	1676.67	0.69	9.93
0.31	1210.33	0.29	6.10
0.16	1009.33	0.12	20.76
0.00	870.00	0.01	
8.00	10070.67	7.77	2.86
6.00	7990.33	6.02	0.26
DNA Test Dilutions [μg/mL]	Mean [Counts]	Trend 2	Error [%]
5.00	6141.33	4.46	10.90
3.33	4641.00	3.19	4.33
2.50	3769.33	2.45	1.87
2.00	4609.33	3.16	58.11
1.67	2677.00	1.53	8.12
1.43	2517.00	1.40	2.26

A.2.4 Discussion and conclusions

The results in Tables A6, A7, A8, and A9 indicate that the results from a DNA assay are sufficiently precise and repeatable. The standard deviation of a set of triplicate sample aliquots typically falls within 8-10 percent of the mean of the set. While this number is not as low as the value determined for the GAG assay, it should be sufficient for the purposes of the assay. Also, the results show that DNA concentrations that fall below 0.10 μg/mL become difficult to assay. Furthermore, the results also indicate no appreciable increase in precision from changing tips between each of the triplicate aliquots of a sample. Therefore, there should be negligible bias between reusing tips (dry tip vs. "wet" tip) within a set of replicated aliquots, and pipet tips can be reused for each DNA standard or test solution, saving a large number of tips.

Figure A2 indicates that the DNA concentration data does not saturate in the range of 0 to 10 μg/mL. The results of Tables J and K indicate that a DNA standard range including 0 and 0.16 to 5.00 (or 10.00) μg/mL leads to an adequate standard curve while still providing a reasonable data range.

Appendix B – Experimental protocols

B.1 Cartilage explant and harvest

Materials

2 scalpel handles (sterile)
2 #10 scalpel blades
3 Tissue forceps (sterile)
2 Flat forceps (sterile)
Squirt bottle (sterile)
3 mm dermal punch (sterile)
Metal spatula (sterile)
Knife or Hacksaw
9 mm hollow drill bit
12 well culture plate(s)
24 or 48 well culture plate(s)
Sterile PBS with PenStrep
Feeding Medium

Coring

1. Using a scalpel, cut away enough muscle on the proximal end of the femur of the calf joint to be able to mount the joint to the drilling apparatus (Figure B1).
2. Expose the femoropatellar groove by carefully cutting open the join capsule and severing the medial, lateral, and cruciate ligaments.
3. Remove the tibia, patella, and surrounding tissues with scalpel and forceps.
4. Constantly wash down the articular cartilage with the sterile PBS supplemented with PenStrep in squirt bottle.
5. Adjust the drilling apparatus to place a roughly horizontal surface of the groove under the drill press.
6. Using a 9mm hollow drill bit, penetrate at least 2 mm into the groove to obtain a core. Up to 5 cores from the medial side and up to 4 cores from the lateral side can be harvested. Wash down the cores with the PBS solution throughout the coring process.
7. After drilling, use a knife or hacksaw to cut through the side of the joint to release the cores. The cores must be long enough to have sufficient bone to mount into the microtome.
8. Place cores in a 12 well culture plate and cover with PBS solution.

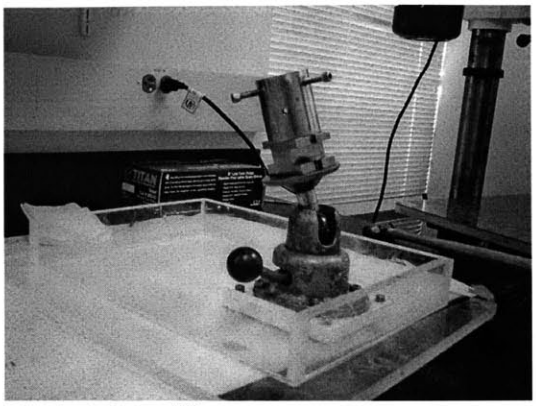

Figure B1. Drilling apparatus.

Slicing

1. Place a core into the polysulfone holder for the microtome (Figure B2).
2. Rotate the microtome height dial counterclockwise until the blade is well above theheight of the mounted core.
3. Rotate the dial clockwise to successive 6 o'clock positions (bottom) until the most superficial layer of the core can be removed to leave a flat surface (Figure B3). Remove the uneven layer.
4. For the first 1 mm slice, rotate the dial clockwise to roughly the second instance of the 5 o'clock position (690 degrees clockwise form previous position) (Figure B3).
5. Place a sterile petri dish in front of the microtome to catch potentially falling slices, and slice with the blade.
6. Holding the slice with forceps, measure the slice thickness with the digital caliper and place in a labeled culture dish well with enough PBS solution to cover.
7. For the second 1 mm slice, rotate the dial clockwise to roughly the second instance of the 4 o'clock position (690 degrees clockwise from previous position) (Figure B3). The exact position may need to be adjusted by experience if the previous slice was too thin/thick.
8. Slice, measure, and store the slice as before.
9. Repeat for the remainder of the cores.

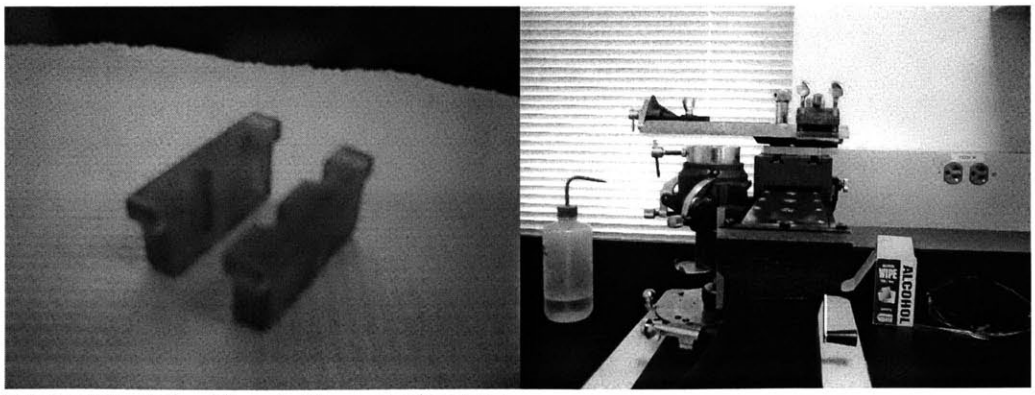

Figure B2. Polysulfone holder and microtome.

Figure B3. Dial at successive slice positions: uneven superficial slice (6 o'clock), first 1 mm slice (5 o'clock), and second 1 mm slice (4 o'clock).

Punching

1. Under the hood, transfer a slice using forceps and spatula into a sterile petri dish with enough of the PBS solution to cover.
2. Using a sterile 3 mm dermal punch, take four plugs from each slice.
3. Transfer the plugs into labeled wells of a culture plate.
4. Aliquot 0.5 mL of feeding medium into each well.
5. Incubate ~48 hours to allow plugs to reach metabolic steady state before beginning experiments. Change the feeding media every 2 days if necessary.

B.2 Mechanical loading (dynamic compression)

Sample preparation

1. Under the hood, transfer and center the plugs into the wells of the polysulfone loading chamber (Delta series) (Figure B4).
2. Gently aliquot 0.5 mL of the appropriate feeding medium into each well so as not to move the plugs within the wells.
3. Align the chamber cover to the chamber bottom and carefully put in place.
4. Transfer the chamber from the hood into the custom build compression apparatus (Figure B4) in the incubator.

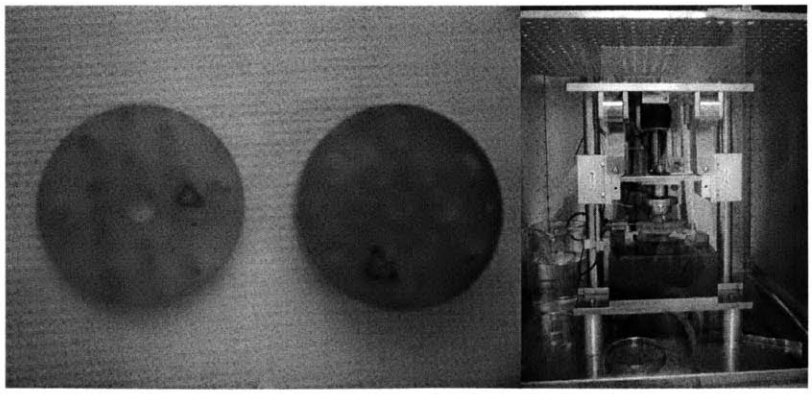

Figure B4. Polysulfone load chamber (Delta series) and custom built compression apparatus.

Compression protocol

1. Click the Dynamic Acquisition Software icon to start the compression program.
2. In the "File" drop down menu on the main screen click on "Change User" (Figure B5).
3. Select the folder where data will be saved and click <OK> to return to the main screen (Figure B5).
4. In the "Control" drop down menu and click on the "Axial Position" tab (Figure B6).
5. Click on the "Load" text field and enter "-50 g" (Figure B6).
6. Click on the <Update> button to calibrate the chamber in the compression apparatus (the platen should just make contact with the top of the chamber). Click <OK> to return to the main screen (Figure B6).
7. In the "File" drop down menu on the main screen click on "Select Protocol". Choose the appropriate protocol for the experiment. The protocol should be displayed in the main screen (Figure B6).
8. The sample protocol in Figure B6 was used for the loading protocol.

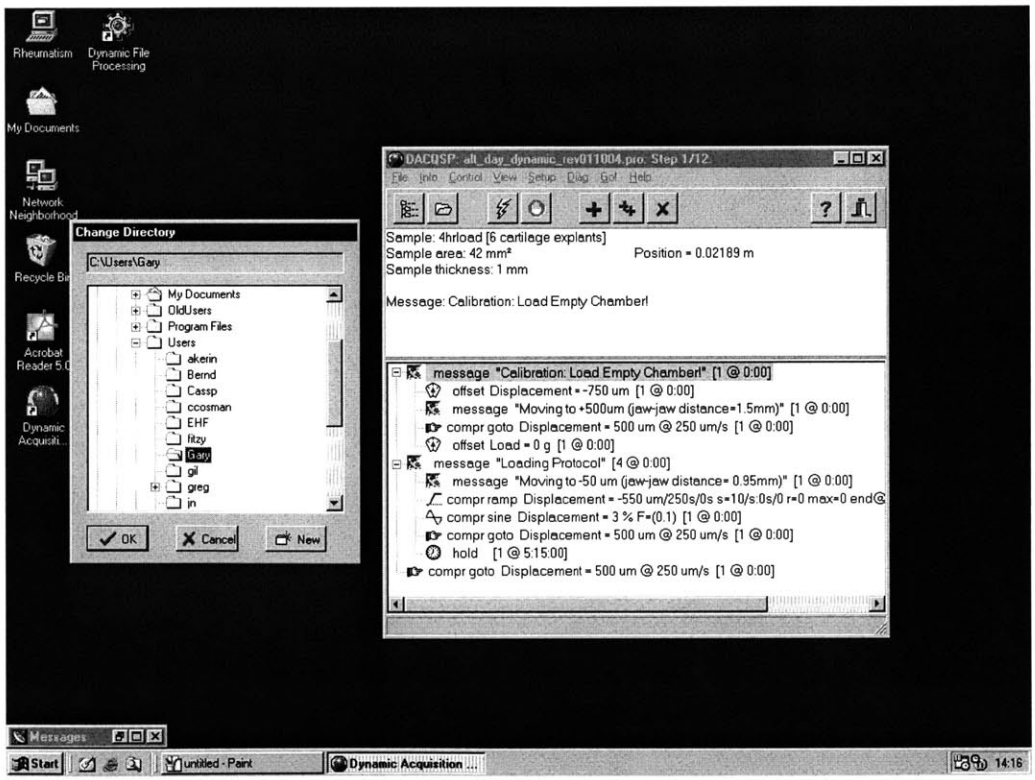

Figure B5. Compression protocol step #2 through #3 screenshot.

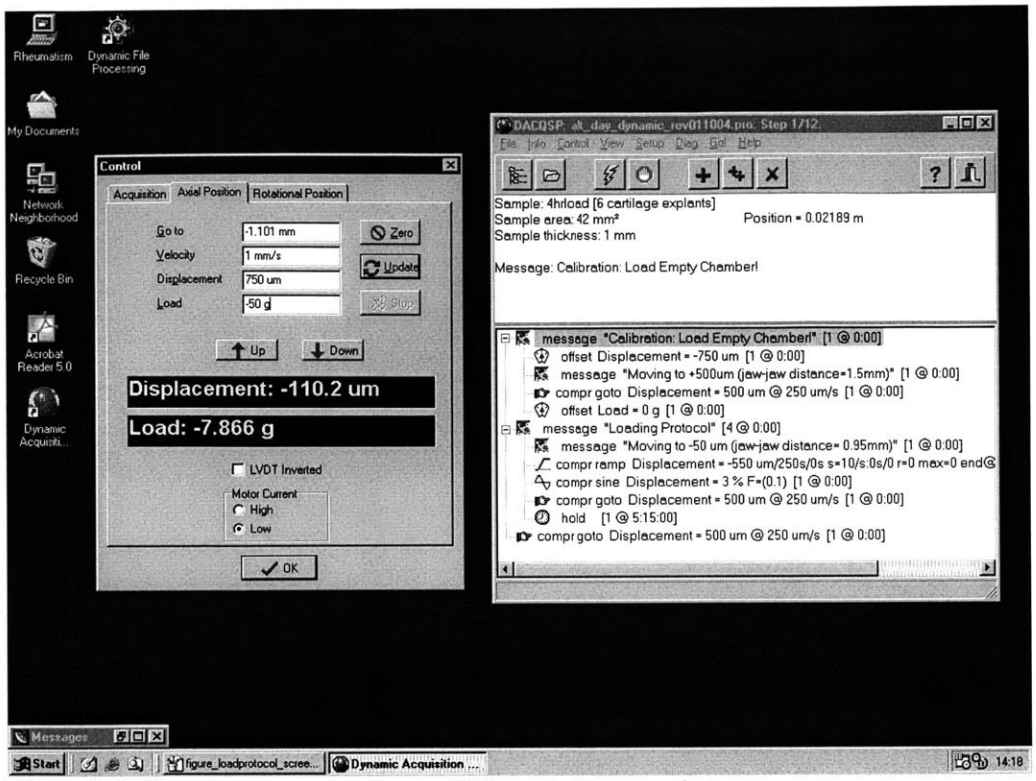

Figure B6. Compression protocol steps #4 through #8 screenshot.

B.3 GAG content assay

Materials

Stock GAG standard solution (2 mg/mL)
Tris buffer
DMMB Dye
96 well microplate (Clear Bacti Plate, NUNC, #269620)

GAG standards preparation

Tube	Standard [μg/mL]	
1	200	(Stock GAG solution diluted 10x)
2	100	
3	50	
4	25	
5	12.5	
6	6.25	
7	3.125	
8	0	(Tris Buffer)

1. Thaw at least 100 μL of frozen stock GAG solution (2 mg/mL) at room temperature.
2. Prepare the Tris buffer solution.
3. Label 7 test tubes #1-8 for the serial dilution.

4. Add 900 μL of Tris buffer to tube #1.
5. Add 100 μL of Tris buffer to tube #2-8.
6. Add 100 μL of GAG stock solution to tube #1.
7. Vortex tube #1 and take a 100 μL aliquot to tube #2.
8. Vortex tube #2 and take a 100 μL aliquot to tube #3.
9. Continue serial dilution through tube #7. Save tube #8 with just Tris buffer.

Sample preparation

1. Check that there is enough DMMB dye and that it is not expired.
2. For each digested cartilage plug sample, add 100 μL of sample solution and 900 μL of Tris buffer into test tube and vortex for a 10x dilution.
3. Aliquot 20 μL of GAG standards in triplicate into the 96 well microplate
4. Aliquot 20 μL of GAG test samples in triplicate into the 96 well microplate.
5. Using a multichannel pipet, aliquot 200 μL of DMMB dye into each well.
6. Gently tap microplate to remove any air bubbles.

Using MAXY microplate reader/software (Vmax, Molecular Devices)

Figure B7. MAXY.

1. Power on MAXY and adjust the wavelength to 520 nm (on left side of machine).
2. Click on the MAXY icon to start the software.
3. Adjust wavelength to 520 nm in MAXY software (Figure B8).
4. Adjust options:
    a. Click the "Shake (AUTOMIX)" option is OFF. This option should only be used if the plate is specially sealed for mixing.
    b. Click the "Calibrate before reading" option ON.
    c. Click the "Eject plate after reading" option ON.
5. Read a blank plate to verify correct operation of machine by clicking <Read Plate>.
6. Open data file for saving data by clicking on folder icon in the upper left corner.
7. Read the microplate at 520 nm by clicking <Read Plate>.
8. Record data.

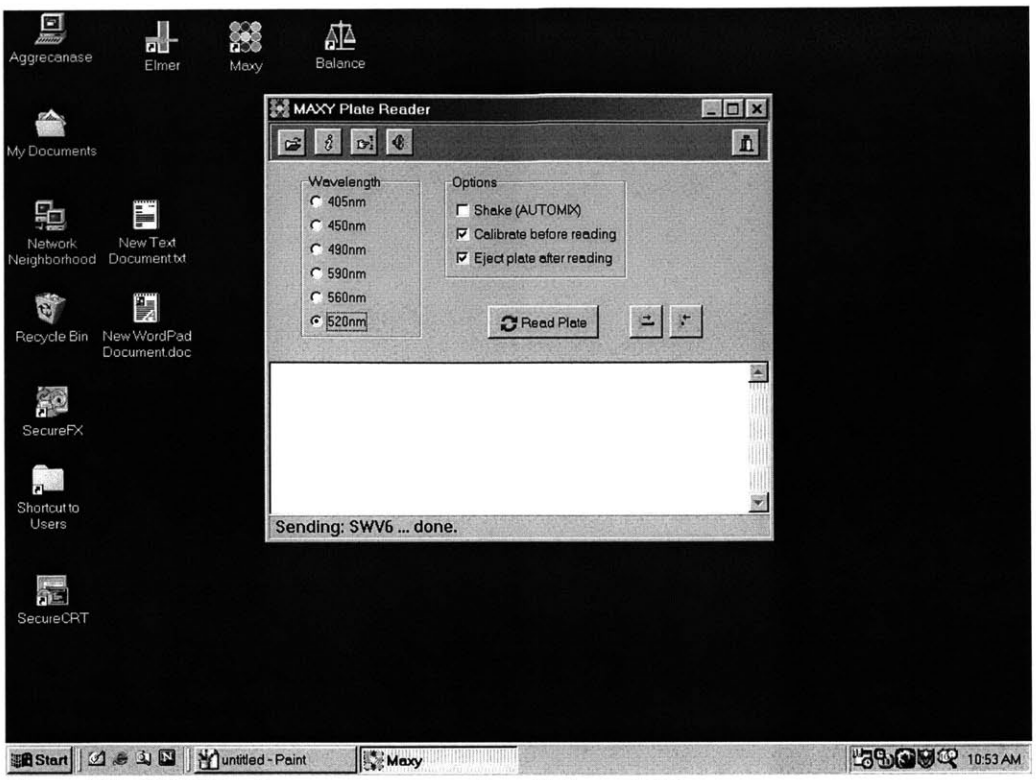

Figure B8. MAXY software main screen, steps #3 through #7.

B.4 DNA content assay

Materials

Stock DNA standard solution (10 μg/mL)
Tris buffer
TEN Buffer
Hoechst Dye
96 well microplate (Microfluor 2 Black, ThermoLab Systems, #7805)

DNA standards preparation

Tube	Standard [μg/mL]
1	10 (Stock DNA solution)
2	5
3	2.5
4	1.25
5	0.625
6	0.312
7	0.156
8	0 (Tris Buffer)

1. Thaw at least 100 μL of frozen stock DNA solution (10 μg/mL) at room temperature.
2. Prepare the Tris buffer solution.

3. Label 7 test tubes #1-8 for the serial dilution.
4. Add 100 µL of Tris buffer to tube #1-8.
5. Add 100 µL of DNA stock solution to tube #1.
6. Vortex tube #1 and take a 100 µL aliquot to tube #2.
7. Vortex tube #2 and take a 100 µL aliquot to tube #3.
8. Continue serial dilution through tube #7. Save tube #8 with just Tris buffer.

Sample preparation

1. Check that there is enough Hoechst dye and that it is not expired.
2. Dilute Hoechst dye 10,000x in TEN buffer (5 µL Hoechst dye in 50 mL TEN buffer.
3. Aliquot 20 µL of DNA standards in triplicate into the 96 well microplate
4. Aliquot 20 µL of DNA test samples in triplicate into the 96 well microplate.
5. Using a multichannel pipet, aliquot 200 µL of Hoechst dye into each well.
6. Gently tap microplate to remove any air bubbles.

Using Victor Microplate Reader (Wallac 1420 Multilabel Counter, ThermoLab Systems)

Figure B9. Victor.

1. Restart computer, and power on Victor (switch above power plug at rear of machine).
2. Click on the Victor icon to start the software and wait for initialization.
3. In the main screen, click the second button in the row of icons/buttons under the drop down menus to start the new assay wizard (Figure B10).
4. In the wizard screen, select the appropriate protocol and click <Next> (Figure B11).
5. Specify wells for measurement (Dark blue = measure, Clear = no measure) and click <Next> (Figure B12).
6. Add any notes or information and click <Next> (Figure B13).
7. Insert microplate into Victor and click <Next> to begin assay (Figure B14).
8. In the main screen, click the "Live display" tab to observe assay (Figure B15).
9. Upon the conclusion of the protocol, click on the third button in the row of icons/buttons under the drop down menus to bring up the data. Export the data from the File menu of the data.

Notes

1. The "DNA-Quant" protocol was used for these experiments.

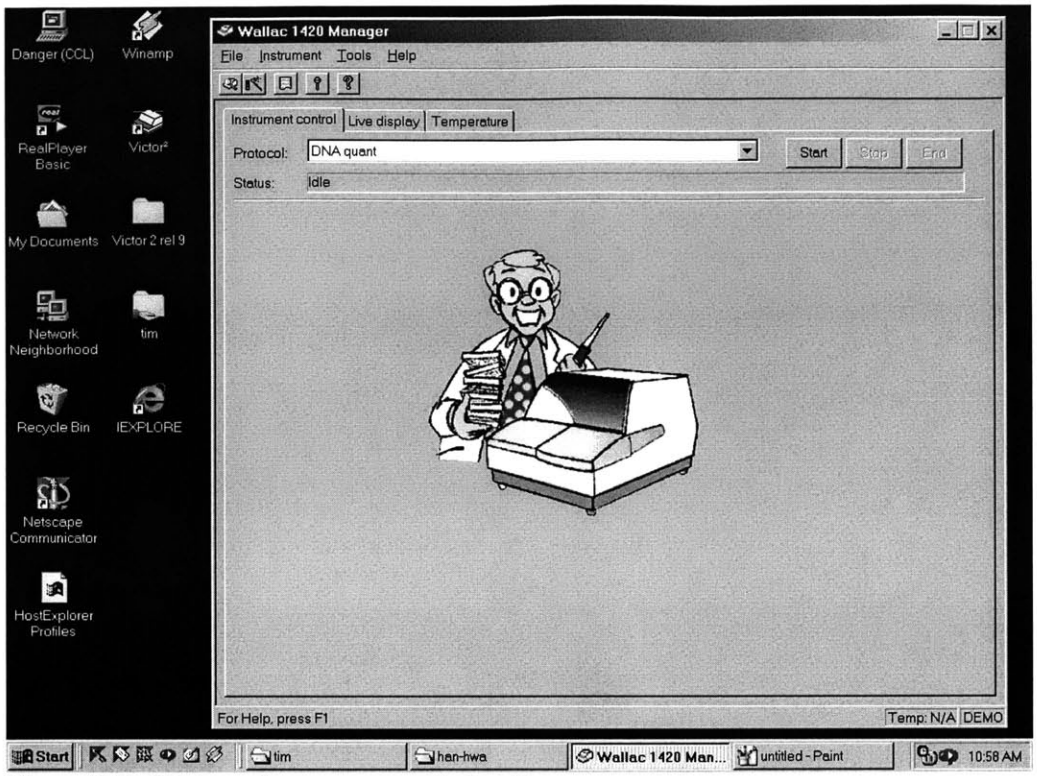

Figure B10. Victor software screenshot, step #3.

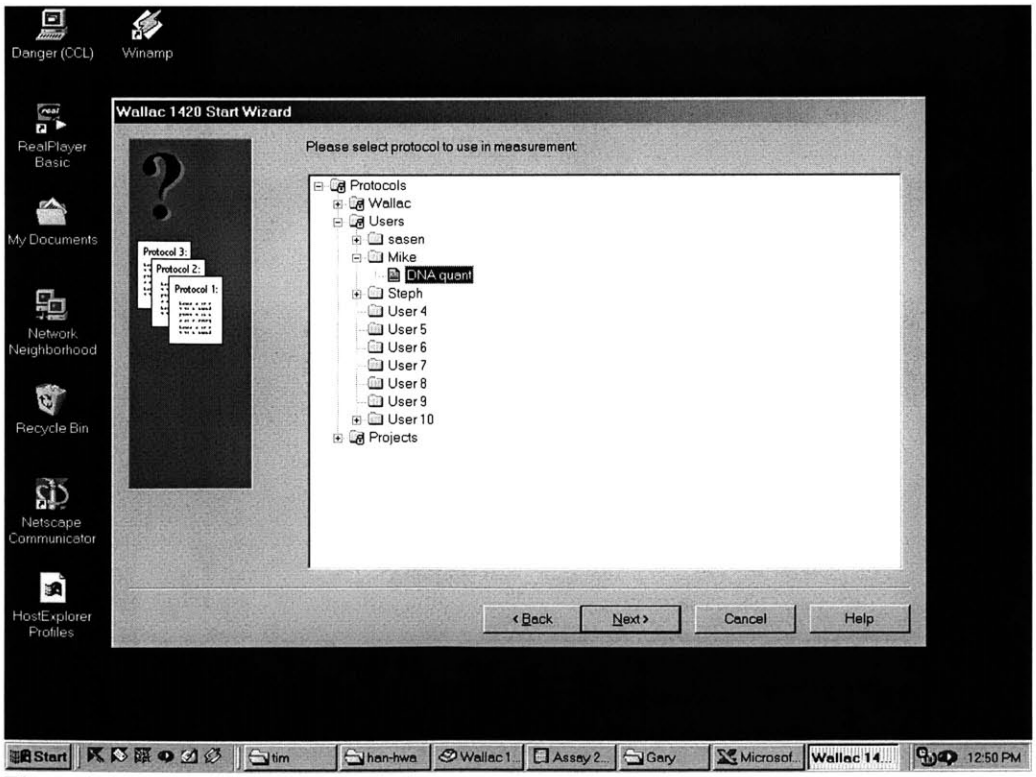

Figure B11. Victor software screenshot, step #4.

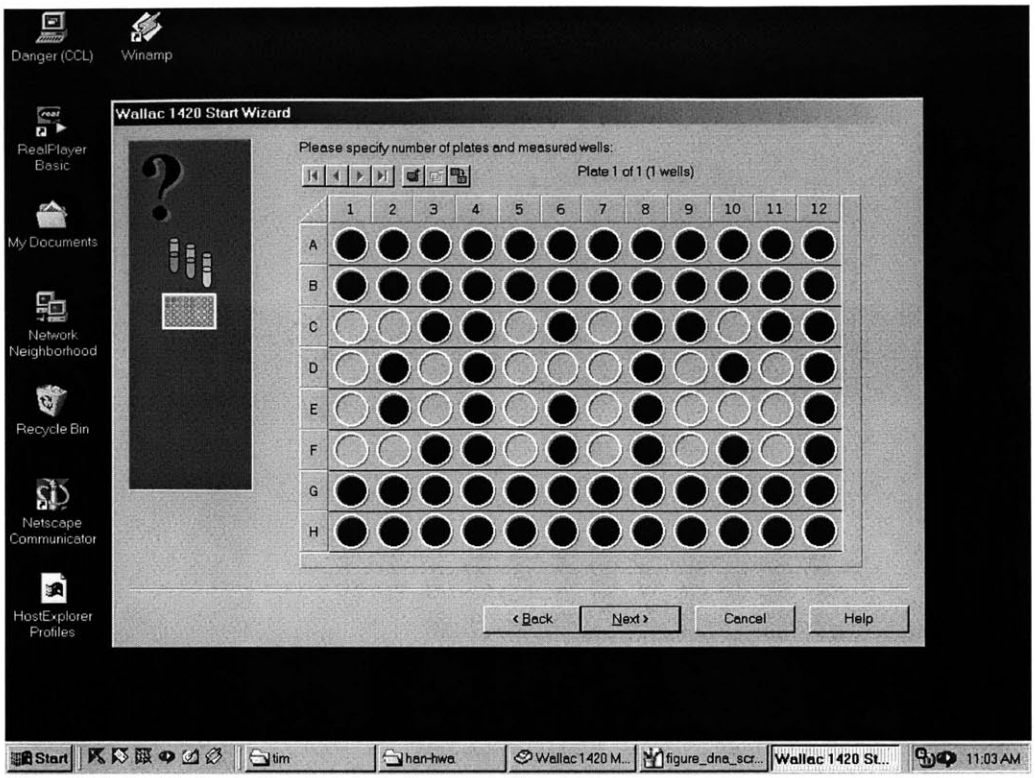

Figure B12. Victor software screenshot, step #5.

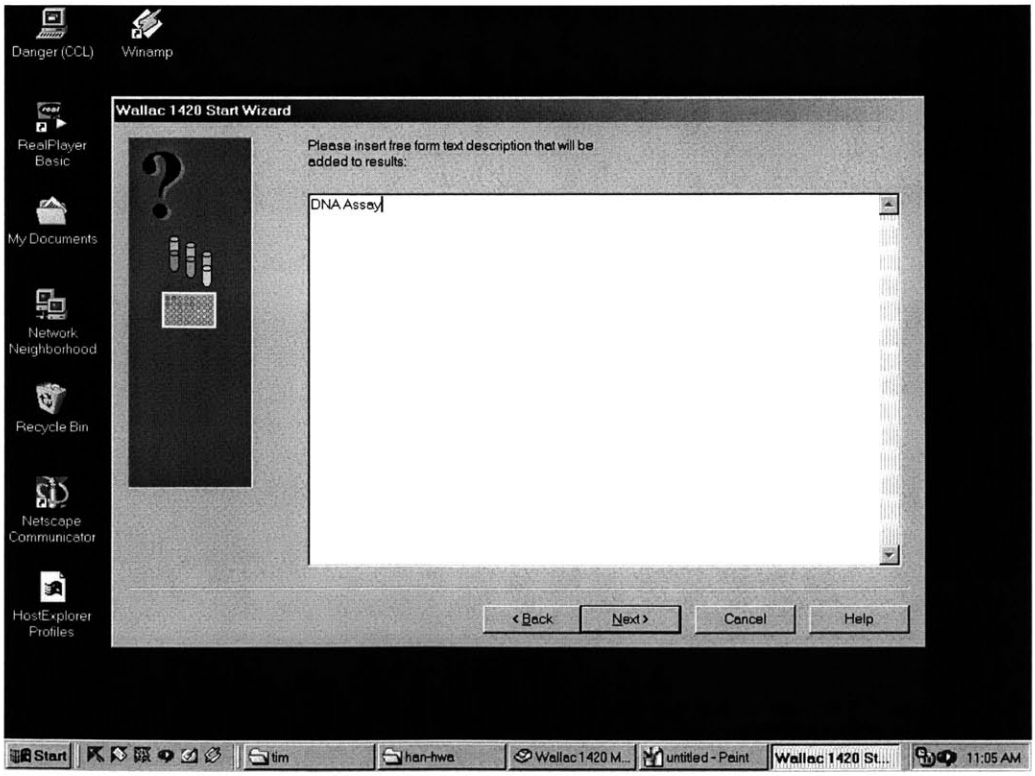

Figure B13. Victor software screenshot, step #6.

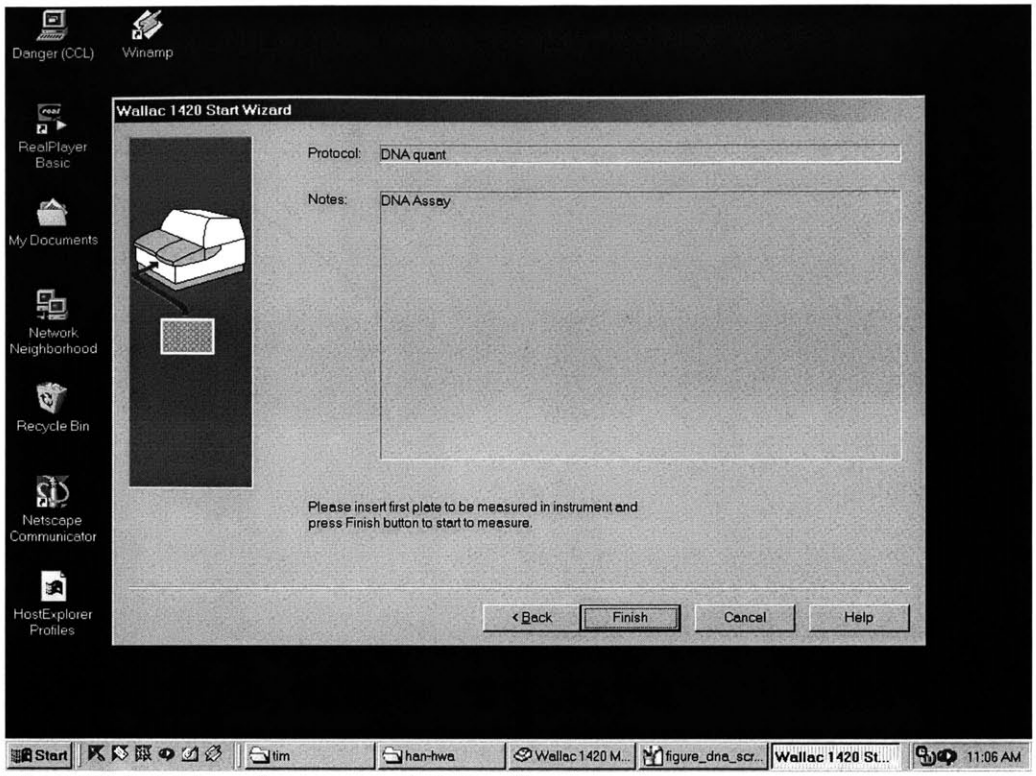

Figure B14. Victor software screenshot, step #7.

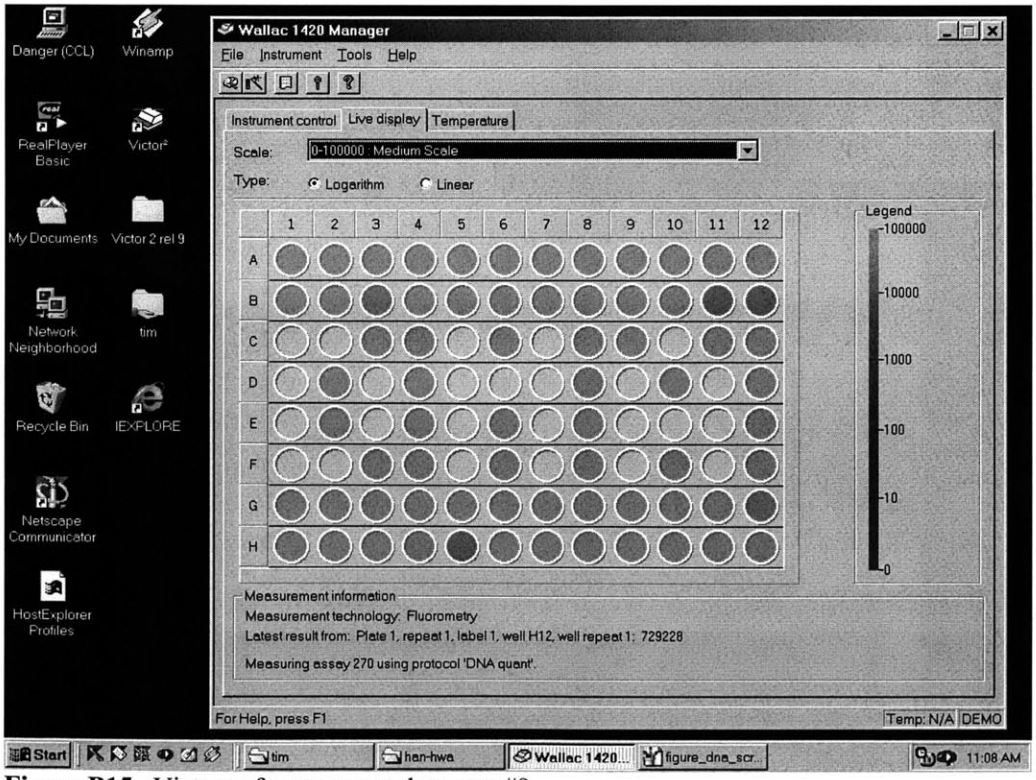

Figure B15. Victor software screenshot, step #8.

B.5 Radiolabel incorporation

Materials

Media to be labeled.
20 mL and 200 mL pipetman
Sterile pipette tips.
Aluminum foil
Latex gloves
Radionuclides
Sterile spatulas
Cold PBS with 0.8 mM Na_2SO_4 and 1.0 mM proline

Preparation of radioactive media

1. Calculate volume of isotope needed:

$$^{35}S_{vol} \equiv total\ volume\ ^{35}S\ label\ needed$$
$$^{3}H_{vol} \equiv total\ volume\ ^{3}H\ label\ needed$$
$$V \equiv total\ volume\ media\ needed\ [mL]$$
$$A \equiv radioactive\ activity\ \left[\frac{\mu Ci}{mL}\right]$$
$$C_S \equiv sulfate\ concentration\ \left[\frac{\mu Ci}{mL}\right]$$
$$C_H \equiv sulfate\ concentration\ \left[\frac{\mu Ci}{mL}\right]$$
$$T = time\ past\ calibration\ date\ [day]$$

For ^{35}S, the half-life is 87.4 days:

$$A = 10{,}000 \cdot 2^{-T/87.4}\ \left[\frac{\mu Ci}{mL}\right]$$
$$^{35}S_{vol} = \frac{V \cdot C_S}{C}\ [mL]$$

For ^{3}H, the half-life is 8-9 years so the activity remains 1000 [μCi/mL]:

$$^{3}H_{vol} = \frac{V \cdot C_H}{1000}\ [mL]$$

2. Wipe hood with 70% EtOH and line work area with aluminum foil.
3. Double glove and tape lab coat sleeves.
4. Carefully place radionuclides in hood on foil and loosen caps.

5. Aliquot calculated volume of ^{35}S radionuclide using sterile technique into medium to be labeled. Return pipette tip to paper wrapper.
6. Save 1 mL media for calibration. Return pipette tip to paper wrapper.
7. Aliquot calculated volume of ^{3}H radionuclide using sterile technique into medium to be labeled. Return pipette tip to paper wrapper.
8. Save 1 mL media for calibration. Return pipette tip to paper wrapper.
6. Return radionuclides into containers, return to storage, and note amounts used in logbook.
7. Dispose of all material used in cleaning hood and preparing media into radioactive waste containers.

Labeling

1. Wipe hood with 70% EtOH and line work area with aluminum foil.
2. Aspirate plug media from wells in culture plate.
3. Aliquot 0.5 mL radiolabeled media per well using sterile technique. Return pipet tip to paper wrapper.
4. Return plugs to incubator for desired radiolabel time.
5. Dispose of all material used in aliquoting media into radioactive waste containers.

Washing

1. Complete PBS solution with 0.8 mM Na_2SO_4 and 1.0 mM proline.
2. Wipe hood with 70% EtOH and line work area with aluminum foil.
3. Remove radioactive media with a pipette and aliquot into a waste container.
4. Aliquot 1 mL of PBS per well and refrigerate for 15 minutes.
5. Repeat media removal, wash, and refrigeration 3x for a total of 4 washes of 15 minutes each.
6. Place each sample in a vial. Samples are now ready for proteinase K digestion.
7. Dispose of radioactive media in sink and note amount of radioactivity disposed.
8. Dispose of all materials used in aliquoting media into radioactive waste containers.

B.6 Liquid scintillation counting

Standard and sample preparation

1. Aliquot 20 μL of radiolabel standards ([media + ^{35}S] and [media + ^{3}H and ^{35}S]) in duplicate into scintillation vials.
2. Aliquot 100 μL of radiolabeled cartilage samples in duplicate into scintillation vials.
3. Dispense 2 mL of scintillation fluid into each vial.
4. Cap vial, close tightly, and vortex to mix well.
5. Label top of vials (NOT the sides) with sample numbers.

Using the Liquid scintillation counter (RackBeta 1211, LKB)

Figure B16. RackBeta liquid scintillation counter.

1. Power on the counter.
2. Click on the LSC icon to start the software.
3. Enter ID into the "User" pull down field. If an email address is entered (e.g. garylee@mit.edu), the program will output an email when the job is finished (Figure B17).
4. Screen for radioactive contamination:
    a. Place the LSC racks (with empty glass vials) into the loading area of the counter. Place a "stop" rack (a rack with no glass vials) as the last rack.
    b. Select "3H, 35S" from the "Isoptope" pull down field.
    c. Select "0:30" (30 seconds) from the "Time" pull down field.
    d. Select "1" from the "Repeats" pull down field.
    e. Click the "Chemiluminescence" option OFF. This option adds chemiluminscence (CLM) and percent error to the output, listed as CPM5 and CLM%, respectively. The CPM5 count is the total count out of the photomultiplier tubes, while CLM% is interpreted as the fraction of channel 1 counts due to the CPM5 counts.
    f. Click the "Percent Error" option ON. This option adds the channel 1 and channel 2 percent errors to the output. This is always printed if only one or two channels are specified by the isotope.
    g. Click the "Ratio" option ON. This option adds the ration of channel 4 to channel 3 counts to the output. The CPM3 and CPM4 channels are automatically set to the CPM1 and CPM2 windows if two channels are specified by the isotope.
    h. Click the "High Count Reject" option OFF. This option lists samples with counts greater than 3 million cpm as "HCR" in the output. By default, this option is off, but counts greater than 3 million cpm may not be linear.
    i. Click on the <Add to Queue> button. This will prompt for an output file, which can be a new or existing file to which to append data.
    j. When the job is finished, open the data file and check for radioactively contaminated vials. Replace any contaminated glass vials and save them for cleaning.

5. Place the scintillation vials into the glass tubes in the liquid scintillation counter racks (up to 10 per rack). Place a "stop" rack as the last rack as before.
6. Start the LSC:
    a. Select "3H, 35S" from the "Isoptope" pull down field.
    b. Select "3:00" (3 minutes) from the "Time" pull down field.
    c. Select "1" from the "Repeats" pull down field.
    d. Click the other options as before.
    e. Click on the <Add to Queue> button and input the output file name as before.
7. Record the results upon the completion of the LSC.

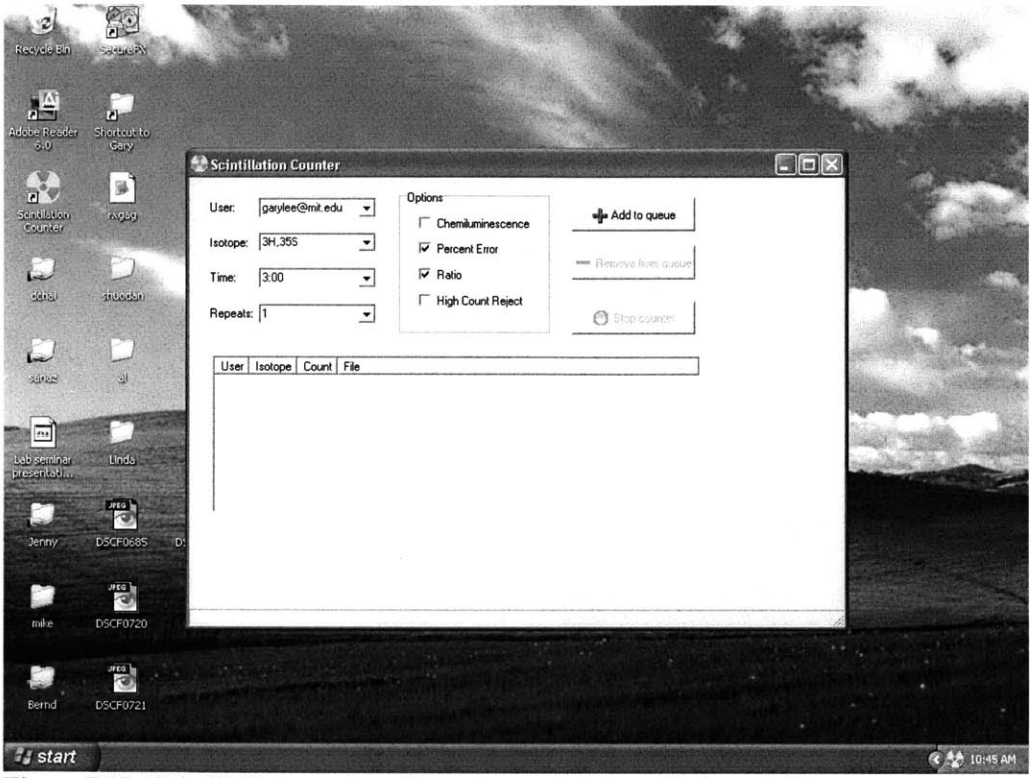

Figure B17. Scintillation counter software screenshot, steps #3 through #7.

B.7 Solutions

Regular Feeding Medium

For 50 mL:
45 mL DMEM (high glucose)
5 mL fetal bovine serum (FBS)
500 μL 1M HEPES buffer
500 μL non-essential amino acid solution
500 μL sodium pyruvate solution
500 μL PenStrep
200 μL stock proline
200 μL L-ascorbic acid

ITS Feeding Medium

For 50 mL:
49 mL DMEM (high glucose)
500 µL ITS
500 µL 1M HEPES buffer
500 µL non-essential amino acid solution
500 µL sodium pyruvate solution
500 µL PenStrep
200 µL stock proline
200 µL L-ascorbic acid

A2P Feeding Medium

For 50 mL:
45 mL DMEM (high glucose)
5 mL fetal bovine serum (FBS)
500 µL 1M HEPES buffer
500 µL non-essential amino acid solution
500 µL sodium pyruvate solution
500 µL PenStrep
200 µL stock proline
200 µL ascorbyl-2-phosphate (A2P)

ITS-A2P Feeding Medium

For 50 mL:
49 mL DMEM (high glucose)
500 µL ITS
500 µL 1M HEPES buffer
500 µL non-essential amino acid solution
500 µL sodium pyruvate solution
500 µL PenStrep
200 µL stock proline
200 µL ascorbyl-2-phosphate

Appendix C – Experimental Results and Details

Experiment 1

Table C1. Experiment 1: Cartilage harvest data.

Slice	Plug A	B	C	D
1	Control	DC	N/A	N/A
2	Control	DC	N/A	N/A
3	Control	DC	N/A	N/A
4	Control	DC	N/A	N/A
5	Control	DC	N/A	N/A
6	Control	DC	N/A	N/A

Table C2. Experiment 1: Individual cartilage plug assay data.

Sample	pmol Proline/ (ug DNA / hr)	pmol Sulfate/ (ug DNA / hr)	Normalized GAG Conc.
Control 1	25.95	1.62	209.43
Control 2	20.51	1.83	169.09
Control 3	17.94	1.22	201.08
Control 4	16.36	1.26	186.78
Control 5	22.58	2.02	144.66
Control 6	13.11	0.97	250.49
Dynamic 1	19.21	1.57	180.12
Dynamic 2	21.26	1.39	191.77
Dynamic 3	22.94	2.65	124.69
Dynamic 4	20.15	1.37	199.18
Dynamic 5	19.18	1.26	175.92
Dynamic 6	12.99	1.24	188.72

Table C3. Experiment 1: Radiolabel protocol parameters.

Media	Total Volume [mL]	^{35}S Activity Concentration [μCi/mL]	^{3}H Activity Concentration [μCi/mL]	Total ^{35}S Volume [μL]	Total ^{35}S Volume [μL]
Regular	18.00	10.00	20.00	23.80	340.00

Table C4. Experiment 1: 24 hour sulfate incorporation ANOVA analysis.

Experiment 1 - 24 Hr Sulfate
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	6	8.925668	1.487611	0.162167	0.164402
DC	6	9.483358	1.58056	0.290397	0.219999

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.025918	1	0.025918	0.114539	0.742033	4.964591
Within Groups	2.262821	10	0.226282			
Total	2.288739	11				

Table C5. Experiment 1: 24 hour proline incorporation ANOVA analysis.

Experiment 1 - 24 Hr Proline
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	6	116.4459	19.40766	21.03784	1.872513
DC	6	115.7345	19.28908	11.52634	1.386022

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.042183	1	0.042183	0.002591	0.960408	4.964591
Within Groups	162.8209	10	16.28209			
Total	162.8631	11				

Table C6. Experiment 1: 24 hour GAG ANOVA analysis.

Experiment 1 - 24 Hr GAG
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	6	1161.544	193.5907	1317.014	14.81561
DC	6	1060.397	176.7328	718.919	10.94622

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	852.5665	1	852.5665	0.837519	0.381645	4.964591
Within Groups	10179.67	10	1017.967			
Total	11032.23	11				

Experiment 2

Table C7. Experiment 2: Cartilage harvest data.

Slice	Plug A	B	C	D
1	Control A	DC A	Control B	DC B
2	Control A	DC A	Control B	DC B
3	Control A	DC A	Control B	DC B
4	Control A	DC A	Control B	DC B
5	Control A	DC A	Control B	DC B
6	Control C	DC C	N/A	N/A
7	Control C	DC C	N/A	N/A
8	Control C	DC C	N/A	N/A
9	Control C	DC C	N/A	N/A

Table C8. Experiment 2: Individual cartilage plug assay data.

Sample	pmol Proline/	pmol Sulfate/	Normalized

	(ug DNA / hr)	(ug DNA / hr)	GAG Conc.
Control A_1	0.53	17.95	38.07
Control A_2	0.40	13.91	45.20
Control A_3	0.40	13.71	43.41
Control A_4	0.16	14.82	48.19
Control A_5	1.09	26.09	53.80
DC A_1	2.90	69.24	82.80
DC A_2	1.35	31.90	41.99
DC A_3	1.47	46.81	42.31
DC A_4	1.41	29.52	52.41
DC A_5	0.71	31.54	50.82
Control B_1	0.60	22.84	37.06
Control B_2	-0.34	47.57	110.65
Control B_3	0.18	12.16	30.75
Control B_4	0.14	21.99	38.32
Control B_5	0.43	21.52	53.10
DC B_1	0.81	22.64	29.21
DC B_2	0.09	24.49	38.12
DC B_3	0.45	32.56	43.48
DC B_4	1.28	23.48	44.84
DC B_5	2.24	45.99	47.11
Control C_1	0.76	24.68	37.44
Control C_2	0.25	17.94	33.12
Control C_3	0.21	8.14	33.46
Control C_4	0.48	30.31	147.62
DC C_1	1.25	30.75	38.70
DC C_2	0.20	36.23	38.59
DC C_3	0.35	26.09	44.41
DC C_4	1.11	22.32	51.07

Table C9. Experiment 2: Radiolabel protocol parameters.

Media	Total Volume [mL]	^{35}S Activity Concentration [μCi/mL]	^{3}H Activity Concentration [μCi/mL]	Total ^{35}S Volume [μL]	Total ^{35}S Volume [μL]
Regular	23.00	10.00	20.00	35.60	440.00

Table C10. Experiment 2: 24 hour sulfate incorporation ANOVA analysis.

Experiment 2 - Sulfate 24 Hr
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	14	293.6228	20.97306	95.42516	2.610763
DC	14	473.5821	33.82729	163.5943	3.418378

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1156.62	1	1156.62	8.930754	0.006053	4.2252
Within Groups	3367.253	26	129.5097			
Total	4523.873	27				

Table C11. Experiment 2: 24 hour GAG ANOVA analysis.

Experiment 2 - 24 Hr GAG
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	14	750.1786	53.58419	1127.273	8.973265
DC	14	645.8728	46.13377	149.0482	3.262867

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	388.5612	1	388.5612	0.608877	0.442257	4.2252
Within Groups	16592.17	26	638.1605			
Total	16980.73	27				

Experiment 3

Table C12. Experiment 3: Cartilage harvest data.

Slice	Plug A	B	C	D
1	Control 1	Control 1	DC 1	DC 1
2	Control 1	Contorl 1	DC 1	DC 1
3	Control 2	Control 2	DC 2	DC 2
4	Control 2	Control 2	DC 2	DC 2
5	Control 3	Control 3	DC 3	DC 3
6	Control 3	Control 3	DC 3	DC 3

Table C13. Experiment 3: Individual cartilage plug assay data.

Sample	pmol Proline/ (ug DNA / hr)	pmol Sulfate/ (ug DNA / hr)	Normalized GAG Conc.
Con 1_1	N/A	80.05	38.86
Con 1_2	N/A	41.06	21.82
Con 1_3	N/A	110.26	76.00
Con 1_4	N/A	39.90	35.56
DC 1_1	N/A	106.78	63.28
DC 1_2	N/A	103.78	73.22
DC 1_3	N/A	0.00	0.00
DC 1_4	N/A	131.85	251.57
Con 2_1	N/A	121.37	0.00
Con 2_2	N/A	97.65	35.82
Con 2_3	N/A	190.82	50.32
Con 2_4	N/A	254.71	71.89
DC 2_1	N/A	219.87	49.43
DC 2_2	N/A	196.11	42.28
DC 2_3	N/A	158.98	37.63
DC 2_4	N/A	137.90	48.55
Con 4_1	N/A	78.39	48.31
Con 4_2	N/A	43.44	43.16
Con 4_3	N/A	73.62	35.23
Con 4_4	N/A	83.20	55.79
DC 4_1	N/A	106.08	56.39
DC 4_2	N/A	168.50	136.12
DC 4_3	N/A	384.79	234.56
DC 4_4	N/A	207.79	114.95

Table C14. Experiment 3: Radiolabel protocol parameters.

Media	Total Volume [mL]	^{35}S Activity Concentration [μCi/mL]	^{3}H Activity Concentration [μCi/mL]	Total ^{35}S Volume [μL]	Total ^{35}S Volume [μL]
Regular	15.00	10.00	20.00	12.80	280.00

Table C15. Experiment 3: 1 hour sulfate incorporation ANOVA analysis.

Experiment 3 - 1 Hr Sulfate
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	4	271.2697	67.81744	1148.628	16.94571
DC	3	342.4022	114.1341	237.585	8.899156

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3677.539	1	3677.539	4.689477	0.082609	6.607877
Within Groups	3921.055	5	784.211			
Total	7598.594	6				

Table C16. Experiment 3: 1 hour GAG ANOVA analysis.

Experiment 3 - 1 Hr GAG
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	4	172.2415	43.06038	536.7972	11.58444
DC	3	388.0739	129.358	11226.28	61.17266

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	12766.75	1	12766.75	2.652781	0.164298	6.607877
Within Groups	24062.96	5	4812.591			
Total	36829.71	6				

Table C17. Experiment 3: 2 hour sulfate incorporation ANOVA analysis.

Experiment 3 - 2 Hr Sulfate
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	4	664.5434	166.1359	5050.053	35.53186
DC	4	712.8621	178.2155	1350.342	18.3735

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	291.8364	1	291.8364	0.091193	0.772859	5.987374
Within Groups	19201.18	6	3200.197			
Total	19493.02	7				

Table C18. Experiment 3: 2 hour GAG ANOVA analysis.

Experiment 3 - 2 Hr GAG
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	3	158.0265	52.67548	329.5437	10.48083
DC	4	177.8857	44.47143	30.93761	2.781079

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	115.3826	1	115.3826	0.767273	0.421143	6.607877
Within Groups	751.9001	5	150.38			
Total	867.2827	6				

Table C19. Experiment 3: 4 hour sulfate incorporation ANOVA analysis.

Experiment 3 - 4 Hr Sulfate
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	4	278.6422	69.66055	320.9262	8.957207
DC	4	867.1547	216.7887	14298.48	59.78812

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	43293.37	1	43293.37	5.922728	0.050908	5.987374
Within Groups	43858.21	6	7309.701			
Total	87151.58	7				

Table C20. Experiment 3: 4 hour GAG ANOVA analysis.

Experiment 3 - 4 Hr GAG
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	4	182.4875	45.62188	74.87655	4.326562
DC	4	542.0219	135.5055	5498.111	37.07462

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	16158.12	1	16158.12	5.798727	0.052713	5.987374
Within Groups	16718.96	6	2786.494			
Total	32877.08	7				

Experiment 4

Table C21. Experiment 4 cartilage harvest data.

Slice	Plug A	B	C	D
1	Control 1	DC 1	DC 1	Lost
2	Control 1	DC 1	DC 1	DC 1
3	Control 2	DC 2	DC 2	DC 2
4	Control 2	DC 2	Lost	DC 2

Table C22. Experiment 4 individual cartilage plug assay data.

Sample	pmol Proline/ (ug DNA / hr)	pmol Sulfate/ (ug DNA / hr)	Normalized GAG Conc.
Con 1_1	3.59	29.08	88.34
Con 1_2	2.33	11.72	163.50
DC 1_1	3.42	27.96	102.93
DC 1_2	3.16	31.09	118.02
DC 1_4	5.63	22.50	238.37
DC 1_5	2.90	25.35	125.62
DC 1_6	2.23	20.64	173.37
Con 2_1	1.08	13.19	122.91
Con 2_2	1.66	23.23	157.50
DC 2_1	2.26	41.81	180.27
DC 2_2	1.61	20.78	163.27
DC 2_3	1.69	45.91	203.44
DC 2_4	1.04	24.50	139.24
DC 2_6	1.71	23.83	141.17

Table C23. Experiment 4 radiolabel protocol parameters.

Media	Total Volume [mL]	^{35}S Activity Concentration [μCi/mL]	^{3}H Activity Concentration [μCi/mL]	Total ^{35}S Volume [μL]	Total ^{35}S Volume [μL]
Regular	10.00	20.00	40.00	18.00	360.00

Table C24. Experiment 4: 1 hour sulfate incorporation ANOVA analysis.

Experiment 4 - 1 Hr - Sulfate
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	2	40.79672	20.39836	150.7553	8.682029
DC	5	127.5395	25.5079	17.47246	1.869356

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	37.29627	1	37.29627	0.845164	0.400102	6.607877
Within Groups	220.6451	5	44.12902			
Total	257.9414	6				

Table C25. Experiment 4: 1 hour proline incorporation ANOVA analysis.

Experiment 4 - 1 Hr - Proline
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	2	5.918368	2.959184	0.801653	0.633108
DC	5	17.35273	3.470545	1.656131	0.575523

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.373558	1	0.373558	0.251514	0.637308	6.607877
Within Groups	7.426178	5	1.485236			
Total	7.799736	6				

Table C26. Experiment 4: 1 hour GAG ANOVA analysis.

Experiment 4 - 1 Hr - GAG
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	2	251.8405	125.9203	2824.814	37.58201
DC	5	758.3071	151.6614	3043.362	24.67129

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	946.5818	1	946.5818	0.315564	0.598532	6.607877
Within Groups	14998.26	5	2999.653			
Total	15944.85	6				

Table C27. Experiment 4: 2 hour sulfate incorporation ANOVA analysis.

Experiment 4 - 2 Hr - Sulfate
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	2	36.41205	18.20602	50.39163	5.019543
DC	5	156.8242	31.36484	134.0912	5.178633

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	247.3636	1	247.3636	2.10789	0.206257	6.607877
Within Groups	586.7565	5	117.3513			
Total	834.1201	6				

Table C28. Experiment 4: 2 hour proline incorporation ANOVA analysis.

Experiment 4 - 2 Hr - Proline
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	2	2.742411	1.371205	0.168247	0.290041
DC	5	8.315184	1.663037	0.188173	0.193997

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.121665	1	0.121665	0.660549	0.453311	6.607877
Within Groups	0.92094	5	0.184188			
Total	1.042605	6				

Table C29. Experiment 4: 2 hour GAG ANOVA analysis.

Experiment 4 - 2 Hr - GAG
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	2	280.4139	140.2069	598.0776	17.29274
DC	5	827.3832	165.4766	736.107	12.13348

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	912.2259	1	912.2259	1.287543	0.307952	6.607877
Within Groups	3542.506	5	708.5011			
Total	4454.731	6				

Experiment 5

Table C30. Experiment 5 cartilage harvest data.

Slice	Plug A	B	C	D	Thickness [mm]
1	Control 1	DC 1	Control 2	DC 2	0.98
2	Control 1	DC 1	Control 2	DC 2	0.97
3	Control 1	DC 1	Control 2	DC 2	0.99
4	Control 1	DC 1	Control 2	DC 2	0.98
5	Control 1	DC 1	Control 2	DC 2	0.99
6	Control 1	DC 1	Control 2	DC 2	1.00
7	Control 4	DC 4	Control 24	DC 24	1.00
8	Control 4	Lost	Control 24	DC 24	1.04
9	Control 4	DC 4	Control 24	DC 24	1.05
10	Control 4	DC 4	Control 24	DC 24	1.00
11	Control 4	DC 4	Control 24	DC 24	1.02
12	Control 4	DC 4	Control 24	DC 24	1.03

Table C31. Experiment 5 individual cartilage plug assay data.

Sample	pmol Proline/ (ug DNA / hr)	pmol Sulfate/ (ug DNA / hr)	Normalized GAG Conc.	Sample	pmol Proline/ (ug DNA / hr)	pmol Sulfate/ (ug DNA / hr)	Normalized GAG Conc.
Control 1_1	229.50	168.13	150.02	Control 4_1	1319.00	1029.98	933.01
Control 1_2	188.29	143.09	108.11	Control 4_2	2206.55	1579.09	1457.32
Control 1_3	229.94	126.89	146.62	Control 4_3	457.54	413.59	321.12
Control 1_4	231.67	174.10	177.62	Control 4_4	839.28	811.08	507.90
Control 1_5	183.65	131.87	154.97	Control 4_5	599.22	680.29	422.62
Control 1_6	239.30	192.03	157.73	Control 4_6	629.50	553.69	331.03
DC 1_1	409.57	215.88	196.63	DC 4_1	159.32	124.61	106.18
DC 1_2	342.66	184.20	169.26	DC 4_2	N/A	N/A	N/A
DC 1_3	333.62	180.18	147.20	DC 4_3	575.99	554.14	501.13
DC 1_4	304.41	163.98	184.57	DC 4_4	695.57	545.98	457.43
DC 1_5	245.29	157.58	149.39	DC 4_5	94.10	113.87	124.68
DC 1_6	276.09	201.83	176.93	DC 4_6	508.38	427.23	309.74
Control 2_1	111.80	42.32	181.52	Control 24_1	194.54	207.58	160.96
Control 2_2	73.27	23.76	138.08	Control 24_2	133.29	107.00	112.00
Control 2_3	174.75	60.96	189.76	Control 24_3	107.28	152.41	122.09
Control 2_4	125.32	45.52	183.65	Control 24_4	125.60	141.97	115.80
Control 2_5	77.10	26.51	146.67	Control 24_5	114.06	223.75	154.09
Control 2_6	96.09	39.13	163.59	Control 24_6	145.12	171.75	145.16
DC 2_1	134.87	33.69	175.09	DC 24_1	187.92	221.17	175.60
DC 2_2	132.95	37.52	139.46	DC 24_2	140.83	192.88	150.97
DC 2_3	158.24	49.64	158.59	DC 24_3	99.75	187.80	182.18
DC 2_4	165.62	60.24	186.53	DC 24_4	104.14	197.62	182.85
DC 2_5	125.08	35.28	65.61	DC 24_5	61.18	151.22	163.33
DC 2_6	128.74	43.24	159.57	DC 24_6	142.61	254.25	138.25

Table C32. Experiment 5 radiolabel protocol parameters.

Media	Total Volume [mL]	^{35}S Activity Concentration [μCi/mL]	^{3}H Activity Concentration [μCi/mL]	Total ^{35}S Volume [μL]	Total ^{35}S Volume [μL]
Regular	27.00	20.00	40.00	63.80	1040.00

Table C33. Experiment 5: 1 hour sulfate incorporation ANOVA analysis.

Experiment 5 - 1 Hr Sulfate
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	6	936.1192	156.0199	673.9036	10.59798
DC	6	1103.652	183.942	489.4754	9.032123

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2338.929	1	2338.929	4.020924	0.072752	4.964591
Within Groups	5816.895	10	581.6895			
Total	8155.825	11				

Table C34. Experiment 5: 1 hour proline incorporation ANOVA analysis.

Experiment 5 - 1 Hr Proline
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	6	1302.347	217.0578	594.5216	9.954242
DC	6	1911.633	318.6056	3292.338	23.42484

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	30935.83	1	30935.83	15.91816	0.00256	4.964591
Within Groups	19434.3	10	1943.43			
Total	50370.13	11				

Table C35. Experiment 5: 1 hour GAG ANOVA analysis.

Experiment 5 - 1 Hr GAG
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	6	895.0659	149.1777	521.919	9.326655
DC	6	1023.98	170.6633	382.3845	7.983154

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1384.898	1	1384.898	3.062906	0.11066	4.964591
Within Groups	4521.517	10	452.1517			
Total	5906.415	11				

Table C36. Experiment 5: 2 hour sulfate incorporation ANOVA analysis.

Experiment 5 - 2 Hr Sulfate
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	6	238.2125	39.70208	184.1828	5.540499
DC	6	259.6129	43.26882	103.4542	4.152393

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	38.16493	1	38.16493	0.265369	0.61765	4.964591
Within Groups	1438.185	10	143.8185			
Total	1476.35	11				

Table C37. Experiment 5: 2 hour proline incorporation ANOVA analysis.

Experiment 5 - 2 Hr Proline
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	6	658.3257	109.7209	1411.155	15.33599
DC	6	845.4917	140.9153	281.9	6.854439

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2919.261	1	2919.261	3.448512	0.092969	4.964591
Within Groups	8465.276	10	846.5276			
Total	11384.54	11				

Table C38. Experiment 5: 2 hour GAG ANOVA analysis.

Experiment 5 - 2 Hr GAG
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	6	1003.273	167.2121	453.4164	8.693066
DC	6	884.8452	147.4742	1864.83	17.62966

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1168.757	1	1168.757	1.008312	0.338986	4.964591
Within Groups	11591.23	10	1159.123			
Total	12759.99	11				

Table C39. Experiment 5: 24 hour sulfate incorporation ANOVA analysis.

Experiment 5 - 24 Hr Sulfate
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	6	1004.463	167.4106	1865.609	17.63334
DC	6	1204.933	200.8221	1194.46	14.10946

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3348.998	1	3348.998	2.188838	0.169812	4.964591
Within Groups	15300.34	10	1530.034			
Total	18649.34	11				

Table C40. Experiment 5: 24 hour proline incorporation ANOVA analysis.

Experiment 5 - 24 Hr Proline
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	6	819.8906	136.6484	985.9474	12.81891
DC	6	736.4277	122.7379	1926.988	17.92107

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	580.5046	1	580.5046	0.39857	0.541989	4.964591
Within Groups	14564.68	10	1456.468			
Total	15145.18	11				

Table C41. Experiment 5: 24 hour GAG ANOVA analysis.

Experiment 5 - 24 Hr GAG
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*Std. Error*
Control	6	810.0939	135.0157	441.0982	8.574168
DC	6	993.1862	165.531	327.9334	7.392941

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2793.567	1	2793.567	7.265154	0.02249	4.964591
Within Groups	3845.158	10	384.5158			
Total	6638.725	11				

Experiment 6

Table C42. Experiment 6 cartilage harvest data.

Slice	Plug A	B	C	D	Thickness [mm]
1	Regular	Regular - DC	ITS	ITS-DC	0.95
2	Regular	Regular - DC	ITS	ITS-DC	0.95
3	Regular	Regular - DC	ITS	ITS-DC	0.96
4	Regular	Regular - DC	ITS	ITS-DC	0.98
5	Regular	Regular - DC	ITS	ITS-DC	0.98
6	Regular	Regular - DC	ITS	ITS-DC	0.98
7	Regular 2	Regular 2- DC	A2P	A2P-DC	0.99
8	Regular 2	Regular 2- DC	A2P	A2P-DC	1.02
9	Regular 2	Regular 2- DC	A2P	A2P-DC	1.02
10	Regular 2	Regular 2- DC	A2P	A2P-DC	1.03
11	Regular 2	Regular 2- DC	A2P	A2P-DC	0.97
12	Regular 2	Regular 2- DC	A2P	A2P-DC	0.98

Table C43. Experiment 6 individual cartilage plug assay data.

Sample	pmol Proline/ (ug DNA / hr)	pmol Sulfate/ (ug DNA / hr)	Normalized GAG Conc.	Sample	pmol Proline/ (ug DNA / hr)	pmol Sulfate/ (ug DNA / hr)	Normalized GAG Conc.
R_1	148.76	75.00	93.61	R_1	129.70	41.51	117.41
R_2	136.80	78.45	94.26	R_2	109.63	36.39	106.68
R_3	212.13	89.79	108.76	R_3	203.61	121.08	103.13
R_4	157.78	58.78	111.36	R_4	143.78	65.04	105.08
R_5	113.74	54.54	93.06	R_5	99.52	46.38	97.05
R_6	168.32	70.29	109.56	R_6	207.55	63.96	115.28
I_1	279.40	236.45	99.20	A_1	219.63	69.87	119.65
I_2	184.99	147.96	112.91	A_2	141.83	38.78	122.18
I_3	338.27	282.14	152.35	A_3	211.58	117.60	102.56
I_4	178.37	153.18	131.75	A_4	132.99	56.37	109.67
I_5	175.83	148.46	117.83	A_5	199.97	144.75	85.12
I_6	174.01	138.39	118.54	A_6	138.39	30.14	108.36
R-DC_1	326.53	239.72	123.57	R-DC_1	235.84	129.23	156.59
R-DC_2	209.59	148.30	102.47	R-DC_2	248.46	160.24	153.05
R-DC_3	279.52	188.44	142.70	R-DC_3	182.81	129.14	136.63
R-DC_4	242.80	141.00	118.74	R-DC_4	224.67	142.58	128.23
R-DC_5	162.20	117.93	101.21	R-DC_5	270.28	148.11	164.73
R-DC_6	218.83	140.72	116.06	R-DC_6	217.19	129.61	161.04
I-DC_1	259.32	180.51	111.06	A-DC_1	217.11	128.90	138.12
I-DC_2	176.91	113.52	111.85	A-DC_2	295.24	171.08	142.73
I-DC_3	247.34	178.92	118.95	A-DC_3	165.87	106.15	122.69
I-DC_4	295.51	181.05	130.37	A-DC_4	247.81	147.76	152.18
I-DC_5	255.42	194.18	111.22	A-DC_5	135.33	105.86	123.52
I-DC_6	273.90	196.01	110.42	A-DC_6	192.41	128.29	134.74

Table C44. Experiment 6 radiolabel protocol parameters.

Media	Total Volume [mL]	^{35}S Activity Concentration [μCi/mL]	^{3}H Activity Concentration [μCi/mL]	Total ^{35}S Volume [μL]	Total ^{35}S Volume [μL]
Regular	14.50	20.00	40.00	37.40	540.00
ITS	8.50	20.00	40.00	21.90	300.00
A2P	8.50	20.00	40.00	21.90	300.00

Table C45. Experiment 6, Regular vs. ITS Media: 2 hour sulfate incorporation ANOVA analysis.

Experiment 6 - Regular vs. ITS - Sulfate
Anova: Two-Factor With Replication

SUMMARY	Control	DC	Total
Regular			
Count	6	6	12
Sum	426.8442	976.1063	1402.95
Average	71.14069	162.6844	116.9125
Variance	169.008	1951.862	3249.554
ITS			
Count	6	6	12
Sum	1106.566	1044.204	2150.771
Average	184.4277	174.034	179.2309
Variance	3594.587	933.1101	2087.506
Total			
Count	12	12	
Sum	1533.411	2020.311	
Average	127.7842	168.3592	
Variance	5210.894	1346.483	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	23301.46	1	23301.46	14.01894	0.001279	4.35125
Columns	9877.983	1	9877.983	5.942924	0.024235	4.35125
Interaction	15586.84	1	15586.84	9.377564	0.006148	4.35125
Within	33242.84	20	1662.142			
Total	82009.13	23				

Table C46. Experiment 6, Regular vs. ITS Media: 2 hour proline incorporation ANOVA analysis.

Experiment 6 - Regular vs. ITS - Proline
Anova: Two-Factor With Replication

SUMMARY	Control	DC	Total
Regular			
Count	6	6	12
Sum	937.5419	1439.467	2377.009
Average	156.257	239.9111	198.0841
Variance	1102.491	3296.403	3908.048
ITS			
Count	6	6	12
Sum	1330.867	1508.401	2839.268
Average	221.8111	251.4002	236.6057
Variance	4904.075	1619.218	3203.91
Total			
Count	12	12	
Sum	2268.409	2947.868	
Average	189.034	245.6557	
Variance	3902.261	2270.373	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	8903.481	1	8903.481	3.260695	0.086034	4.35125
Columns	19236.04	1	19236.04	7.044759	0.015227	4.35125
Interaction	4384.551	1	4384.551	1.605741	0.219649	4.35125
Within	54610.93	20	2730.547			
Total	87135.01	23				

Table C47. Experiment 6, Regular vs. ITS Media: 2 hour GAGANOVA analysis.

Experiment 6 - Regular vs. ITS -
GAG
Anova: Two-Factor With Replication

SUMMARY	Control	DC	Total
Regular			
Count	6	6	12
Sum	610.6075	704.7565	1315.364
Average	101.7679	117.4594	109.6137
Variance	80.10222	233.3252	209.6188
ITS			
Count	6	6	12
Sum	732.5844	693.8517	1426.436
Average	122.0974	115.6419	118.8697
Variance	329.5284	62.01401	189.3391
Total			
Count	12	12	
Sum	1343.192	1398.608	
Average	111.9327	116.5507	
Variance	298.9106	135.146	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	514.0424	1	514.0424	2.916677	0.103145	4.35125
Columns	127.9569	1	127.9569	0.726028	0.404262	4.35125
Interaction	735.7313	1	735.7313	4.174541	0.054436	4.35125
Within	3524.849	20	176.2425			
Total	4902.58	23				

Table C48. Experiment 6, Regular vs. A2P Media: 2 hour sulfate incorporation ANOVA analysis.

Experiment 6 - Regular vs. A2P - Sulfate
Anova: Two-Factor With Replication

SUMMARY	Control	DC	Total
Regular			
Count	6	6	12
Sum	374.3642	838.9093	1213.273
Average	62.39403	139.8182	101.1061
Variance	964.4741	164.8051	2148.173
A2P			
Count	6	6	12
Sum	457.5063	788.0574	1245.564
Average	76.25105	131.3429	103.797
Variance	2073.657	629.5511	2056.489
Total			
Count	12	12	
Sum	831.8705	1626.967	
Average	69.32254	135.5806	
Variance	1433.337	380.6612	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	43.44425	1	43.44425	0.045343	0.83353	4.35125
Columns	26340.75	1	26340.75	27.49206	3.94E-05	4.35125
Interaction	748.0993	1	748.0993	0.780798	0.387396	4.35125
Within	19162.44	20	958.122			
Total	46294.73	23				

Table C49. Experiment 6, Regular vs. A2P Media: 2 hour proline incorporation ANOVA analysis.

Experiment 6 - Regular vs. A2P - Proline
Anova: Two-Factor With Replication

SUMMARY	Control	DC	Total
Regular			
Count	6	6	12
Sum	893.7886	1379.238	2273.027
Average	148.9648	229.873	189.4189
Variance	2161.744	883.3334	3169.439
A2P			
Count	6	6	12
Sum	1044.388	1253.774	2298.162
Average	174.0646	208.9623	191.5135
Variance	1630.675	3314.428	2579.915
Total			
Count	12	12	
Sum	1938.176	2633.012	
Average	161.5147	219.4177	
Variance	1895.646	2027.325	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	26.32376	1	26.32376	0.013178	0.909752	4.35125
Columns	20116.54	1	20116.54	10.07063	0.004777	4.35125
Interaction	3175.455	1	3175.455	1.589679	0.221883	4.35125
Within	39950.9	20	1997.545			
Total	63269.22	23				

Table C50. Experiment 6, Regular vs. A2P Media: 2 hour GAG ANOVA analysis.

Experiment 6 - Regular vs. A2P -
GAG
Anova: Two-Factor With Replication

SUMMARY	Control	DC	Total
Regular			
Count	6	6	12
Sum	644.6433	900.2668	1544.91
Average	107.4406	150.0445	128.7425
Variance	58.6924	208.8719	616.6455
A2P			
Count	6	6	12
Sum	647.5472	813.9828	1461.53
Average	107.9245	135.6638	121.7942
Variance	178.5044	129.0874	349.6693
Total			
Count	12	12	
Sum	1292.19	1714.25	
Average	107.6825	142.8541	
Variance	107.8806	210.0187	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	289.6766	1	289.6766	2.014595	0.171192	4.35125
Columns	7422.247	1	7422.247	51.61901	5.89E-07	4.35125
Interaction	331.4359	1	331.4359	2.305015	0.144607	4.35125
Within	2875.781	20	143.789			
Total	10919.14	23				

Experiment 7

Table C51. Experiment 7 cartilage harvest data.

Slice	Plug A	B	C	D	Thickness [mm]
1	Regular 1	Regular 1- DC	ITS 1	ITS 1-DC	0.99
2	Regular 1	Regular 1- DC	ITS 1	ITS 1-DC	1.00
3	Regular 1	Regular 1- DC	ITS 1	ITS 1-DC	1.00
4	Regular 1	Regular 1- DC	ITS 1	ITS 1-DC	0.99
5	Regular 1	Regular 1- DC	ITS 1	ITS 1-DC	1.02
6	Regular 1	Regular 1- DC	ITS 1	ITS 1-DC	1.02
7	Regular 2	Regular 2- DC	ITS 2	ITS 2-DC	1.03
8	Regular 2	Regular 2- DC	ITS 2	ITS 2-DC	1.03
9	Regular 2	Regular 2- DC	ITS 2	ITS 2-DC	1.03
10	Regular 2	Regular 2- DC	ITS 2	ITS 2-DC	1.04
11	Regular 2	Regular 2- DC	ITS 2	ITS 2-DC	1.04
12	Regular 2	Regular 2- DC	ITS 2	ITS 2-DC	1.05

Table C52. Experiment 7 individual cartilage plug assay data.

Sample	pmol Proline/ (ug DNA / hr)	pmol Sulfate/ (ug DNA / hr)	Normalized GAG Conc.	Sample	pmol Proline/ (ug DNA / hr)	pmol Sulfate/ (ug DNA / hr)	Normalized GAG Conc.
R_1	267.45	131.32	Saturated	R_1	291.65	123.41	Saturated
R_2	209.59	89.48	Saturated	R_2	175.52	92.44	Saturated
R_3	179.34	66.80	Saturated	R_3	216.32	130.13	Saturated
R_4	131.85	59.97	Saturated	R_4	194.95	107.49	Saturated
R_5	184.40	75.25	Saturated	R_5	164.00	83.36	Saturated
R_6	194.19	116.57	Saturated	R_6	220.66	98.13	Saturated
I_1	357.39	182.63	Saturated	I_1	647.14	210.10	Saturated
I_2	293.98	129.58	Saturated	I_2	305.15	151.45	Saturated
I_3	357.05	186.65	Saturated	I_3	332.84	214.85	Saturated
I_4	302.90	176.33	Saturated	I_4	316.72	232.21	Saturated
I_5	530.19	231.55	Saturated	I_5	281.59	172.36	Saturated
I_6	304.63	248.64	Saturated	I_6	426.38	316.08	Saturated
R-DC_1	307.15	144.07	Saturated	R-DC_1	392.86	196.59	Saturated
R-DC_2	310.46	132.01	Saturated	R-DC_2	289.59	151.83	Saturated
R-DC_3	256.12	154.00	Saturated	R-DC_3	250.57	122.73	Saturated
R-DC_4	229.65	132.00	Saturated	R-DC_4	237.40	167.28	Saturated
R-DC_5	245.53	153.40	Saturated	R-DC_5	244.75	154.85	Saturated
R-DC_6	263.71	152.88	Saturated	R-DC_6	291.51	155.33	Saturated
I-DC_1	275.79	134.09	Saturated	I-DC_1	378.56	219.63	Saturated
I-DC_2	270.56	118.24	Saturated	I-DC_2	247.65	161.77	Saturated
I-DC_3	331.88	151.99	Saturated	I-DC_3	231.76	135.48	Saturated
I-DC_4	300.12	154.61	Saturated	I-DC_4	212.34	121.60	Saturated
I-DC_5	387.73	159.07	Saturated	I-DC_5	239.16	98.83	Saturated
I-DC_6	95.24	12.62	Saturated	I-DC_6	220.30	123.21	Saturated

Table C53. Experiment 7 radiolabel protocol parameters.

Media	Total Volume [mL]	^{35}S Activity Concentration [μCi/mL]	^{3}H Activity Concentration [μCi/mL]	Total ^{35}S Volume [μL]	Total ^{35}S Volume [μL]
Regular	14.50	10.00	20.00	19.10	270.00
ITS	14.50	10.00	20.00	19.10	270.00

Table C54. Experiment 7, Trial 1: 2 hour sulfate incorporation ANOVA analysis.

Experiment 7 - Trial 1 - Sulfate
Anova: Two-Factor With Replication

SUMMARY	Control	DC	Total
Regular			
Count	5	5	10
Sum	422.821	715.4834	1138.304
Average	84.5642	143.0967	113.8304
Variance	804.3892	118.0405	1361.65
ITS			
Count	5	5	10
Sum	906.7468	717.9942	1624.741
Average	181.3494	143.5988	162.4741
Variance	1313.637	291.1936	1109.12
Total			
Count	10	10	
Sum	1329.568	1433.478	
Average	132.9568	143.3478	
Variance	3543.391	181.9519	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	11831.02	1	11831.02	18.72546	0.00052	4.493998
Columns	539.8625	1	539.8625	0.854463	0.369025	4.493998
Interaction	11588.02	1	11588.02	18.34084	0.000571	4.493998
Within	10109.04	16	631.815			
Total	34067.95	19				

Table C55. Experiment 7, Trial 1: 2 hour proline incorporation ANOVA analysis.

Experiment 7 - Trial 1 - Proline
Anova: Two-Factor With Replication

SUMMARY	Control	DC	Total
Regular			
Count	5	5	10
Sum	972.6248	1348.902	2321.527
Average	194.525	269.7803	232.1527
Variance	2451.463	1359.203	3266.788
ITS			
Count	5	5	10
Sum	1841.511	1566.084	3407.595
Average	368.3022	313.2168	340.7595
Variance	9064.185	2322.966	5903.844
Total			
Count	10	10	
Sum	2814.136	2914.986	
Average	281.4136	291.4986	
Variance	13506.55	2160.611	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	58977.26	1	58977.26	15.52256	0.001171	4.493998
Columns	508.5358	1	508.5358	0.133844	0.719271	4.493998
Interaction	21235.88	1	21235.88	5.589193	0.031054	4.493998
Within	60791.27	16	3799.454			
Total	141512.9	19				

Table C56. Experiment 7, Trial 2: 2 hour sulfate incorporation ANOVA analysis.

Experiment 7 - Trial 2 - Sulfate
Anova: Two-Factor With Replication

SUMMARY	Control	DC	Total
Regular			
Count	6	6	12
Sum	634.9547	948.6127	1583.567
Average	105.8258	158.1021	131.964
Variance	329.1727	574.8825	1156.247
ITS			
Count	6	6	12
Sum	1297.043	860.5269	2157.57
Average	216.1739	143.4211	179.7975
Variance	3277.366	1816.267	3758.823
Total			
Count	12	12	
Sum	1931.998	1809.14	
Average	160.9998	150.7616	
Variance	4960.255	1145.667	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	13728.3	1	13728.3	9.155728	0.006676	4.35125
Columns	628.9254	1	628.9254	0.419445	0.524579	4.35125
Interaction	23448.4	1	23448.4	15.63829	0.000782	4.35125
Within	29988.44	20	1499.422			
Total	67794.07	23				

Table C57. Experiment 7, Trial 2: 2 hour proline incorporation ANOVA analysis.

Experiment 7 - Trial 2 - Proline
Anova: Two-Factor With Replication

SUMMARY	Control	DC	Total
Regular			
Count	6	6	12
Sum	1263.095	1706.689	2969.784
Average	210.5158	284.4482	247.482
Variance	2070.216	3353.168	3955.901
ITS			
Count	6	6	12
Sum	2309.82	1529.771	3839.592
Average	384.9701	254.9619	319.966
Variance	18976.65	3827.435	14975.16
Total			
Count	12	12	
Sum	3572.915	3236.461	
Average	297.7429	269.7051	
Variance	17867.02	3501.031	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	31523.57	1	31523.57	4.467077	0.047316	4.35125
Columns	4716.739	1	4716.739	0.66839	0.423247	4.35125
Interaction	62387.63	1	62387.63	8.840697	0.007513	4.35125
Within	141137.4	20	7056.868			
Total	239765.3	23				

Experiment 8

Table C58. Experiment 8 cartilage harvest data.

Slice	Plug A	B	C	D	Thickness [mm]
1	Regular 1	Regular 1- DC	ITS-A2P 1	ITS-A2P 1-DC	0.99
2	Regular 1	Regular 1- DC	ITS-A2P 1	ITS-A2P 1-DC	1.00
3	Regular 1	Regular 1- DC	ITS-A2P 1	ITS-A2P 1-DC	1.00
4	Regular 1	Regular 1- DC	ITS-A2P 1	ITS-A2P 1-DC	0.99
5	Regular 1	Regular 1- DC	ITS-A2P 1	ITS-A2P 1-DC	1.02
6	Regular 1	Regular 1- DC	ITS-A2P 1	ITS-A2P 1-DC	1.02
7	Regular 2	Regular 2- DC	ITS-A2P 2	ITS-A2P 2-DC	1.03
8	Regular 2	Regular 2- DC	ITS-A2P 2	ITS-A2P 2-DC	1.03
9	Regular 2	Regular 2- DC	ITS-A2P 2	ITS-A2P 2-DC	1.03
10	Regular 2	Regular 2- DC	ITS-A2P 2	ITS-A2P 2-DC	1.04
11	Regular 2	Regular 2- DC	ITS-A2P 2	ITS-A2P 2-DC	1.04
12	Regular 2	Regular 2- DC	ITS-A2P 2	ITS-A2P 2-DC	1.05

Table C59. Experiment 8 individual cartilage plug assay data.

Sample	pmol Proline/ (ug DNA / hr)	pmol Sulfate/ (ug DNA / hr)	Normalized GAG Conc.	Sample	pmol Proline/ (ug DNA / hr)	pmol Sulfate/ (ug DNA / hr)	Normalized GAG Conc.
R1_1	795.08	N/A	101.01	R2_1	353.57	N/A	135.22
R1_2	380.83	N/A	105.92	R2_2	444.95	N/A	133.23
R1_3	271.43	N/A	109.73	R2_3	677.13	N/A	85.30
R1_4	360.70	N/A	105.97	R2_4	376.35	N/A	176.64
R1_5	293.00	N/A	126.17	R2_5	931.16	N/A	384.86
R1_6	478.53	N/A	131.37	R2_6	495.14	N/A	172.04
R1-DC_1	25.72	N/A	113.54	R2-DC_1	480.37	N/A	164.95
R1-DC_2	60.19	N/A	104.73	R2-DC_2	590.69	N/A	183.54
R1-DC_3	125.11	N/A	161.26	R2-DC_3	429.20	N/A	121.92
R1-DC_4	29.03	N/A	136.08	R2-DC_4	508.46	N/A	145.43
R1-DC_5	63.66	N/A	109.55	R2-DC_5	672.03	N/A	169.37
R1-DC_6	117.06	N/A	125.28	R2-DC_6	511.75	N/A	127.58
IA1_1	576.71	N/A	100.63	IA2_1	450.12	N/A	118.02
IA1_2	445.76	N/A	90.07	IA2_2	555.06	N/A	145.13
IA1_3	417.49	N/A	104.30	IA2_3	447.84	N/A	102.18
IA1_4	500.50	N/A	115.64	IA2_4	589.54	N/A	148.27
IA1_5	767.46	N/A	158.14	IA2_5	575.54	N/A	141.26
IA1_6	429.25	N/A	117.45	IA2_6	787.85	N/A	122.69
IA1-DC_1	20.39	N/A	109.45	IA2-DC_1	556.18	N/A	130.67
IA1-DC_2	80.72	N/A	108.72	IA2-DC_2	407.69	N/A	102.70
IA1-DC_3	57.85	N/A	130.08	IA2-DC_3	518.64	N/A	102.09
IA1-DC_4	109.23	N/A	110.12	IA2-DC_4	665.86	N/A	148.00
IA1-DC_5	57.14	N/A	121.73	IA2-DC_5	600.74	N/A	138.87
IA1-DC_6	118.94	N/A	117.94	IA2-DC_6	595.88	N/A	119.42

Table C60. Experiment 8 radiolabel protocol parameters.

Media	Total Volume [mL]	^{35}S Activity Concentration [μCi/mL]	^{3}H Activity Concentration [μCi/mL]	Total ^{35}S Volume [μL]	Total ^{35}S Volume [μL]
Regular	14.50	10.00	20.00	20.10	270.00
ITS-A2P	14.50	10.00	20.00	20.10	270.00

Table C61. Experiment 8, Trial 1: 2 hour sulfate incorporation ANOVA analysis.

Experiment 8 - Trial 1 - Sulfate
Anova: Two-Factor With Replication

SUMMARY	Control	DC	Total
Regular			
Count	6	6	12
Sum	2579.581	420.7755	3000.356
Average	429.9301	70.12926	250.0297
Variance	37354.79	1805.562	53106.53
IA			
Count	6	6	12
Sum	3137.167	444.2589	3581.426
Average	522.8612	74.04315	298.4522
Variance	17807.58	1344.987	63643.25
Total			
Count	12	12	
Sum	5716.748	865.0344	
Average	476.3957	72.0862	
Variance	27429.13	1436.245	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	14068.41	1	14068.41	0.965029	0.337658	4.35125
Columns	980796.8	1	980796.8	67.27818	7.91E-08	4.35125
Interaction	11886.08	1	11886.08	0.815331	0.377296	4.35125
Within	291564.6	20	14578.23			
Total	1298316	23				

Table C62. Experiment 8, Trial 1: 2 hour GAG ANOVA analysis.

Experiment 8 - Trial 1 - GAG
Anova: Two-Factor With Replication

SUMMARY	Control	DC	Total
Regular			
Count	6	6	12
Sum	680.1749	750.4517	1430.627
Average	113.3625	125.0753	119.2189
Variance	152.8226	443.6511	308.5398
IA			
Count	6	6	12
Sum	686.242	698.0288	1384.271
Average	114.3737	116.3381	115.3559
Variance	561.5761	72.93088	289.4647
Total			
Count	12	12	
Sum	1366.417	1448.48	
Average	113.8681	120.7067	
Variance	325.0055	255.6294	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	89.5357	1	89.5357	0.290941	0.595573	4.35125
Columns	280.6014	1	280.6014	0.911798	0.351038	4.35125
Interaction	142.5452	1	142.5452	0.463192	0.503939	4.35125
Within	6154.903	20	307.7452			
Total	6667.585	23				

Table C63. Experiment 8, Trial 2: 2 hour sulfate incorporation ANOVA analysis.

Experiment 8 - Trial 2 - Sulfate
Anova: Two-Factor With Replication

SUMMARY	Control	DC	Total
Regular			
Count	6	6	12
Sum	3278.3	3192.508	6470.809
Average	546.3834	532.0847	539.2341
Variance	48831.15	7449.941	25638.07
IA			
Count	6	6	12
Sum	3405.955	3344.994	6750.949
Average	567.6592	557.4989	562.5791
Variance	15470.44	7808.269	10609.39
Total			
Count	12	12	
Sum	6684.256	6537.502	
Average	557.0213	544.7918	
Variance	29351.45	7111.7	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	3269.941	1	3269.941	0.164402	0.689441	4.35125
Columns	897.3627	1	897.3627	0.045116	0.83394	4.35125
Interaction	25.68925	1	25.68925	0.001292	0.971688	4.35125
Within	397799	20	19889.95			
Total	401992	23				

Table C64. Experiment 8, Trial 2: 2 hour GAG ANOVA analysis.

Experiment 8 - Trial 2 - GAG
Anova: Two-Factor With Replication

SUMMARY	Control	DC	Total
Regular			
Count	6	6	12
Sum	1087.307	912.7801	2000.087
Average	181.2179	152.13	166.674
Variance	11039.01	601.6666	5521.972
IA			
Count	6	6	12
Sum	777.552	741.76	1519.312
Average	129.592	123.6267	126.6093
Variance	331.9484	359.0681	323.8035
Total			
Count	12	12	
Sum	1864.859	1654.54	
Average	155.4049	137.8783	
Variance	5895.499	658.2724	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	9631.04	1	9631.04	3.123996	0.092403	4.35125
Columns	1843.09	1	1843.09	0.597838	0.448447	4.35125
Interaction	801.9773	1	801.9773	0.260135	0.61561	4.35125
Within	61658.46	20	3082.923			
Total	73934.57	23				