# Effect of Impact Injury on Engineered Cartilage using a High Throughput Mechanical Screening Device

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#### ABSTRACT

Post-traumatic osteoarthritis (PTOA) occurs when a joint suffers a traumatic injury causing progressive degeneration of the cartilage. Our goal was to develop, in a micro-scale format, methods to mechanically impact native cartilage or cartilage tissue analogs (CTAs) using a high throughput device to initiate the degradative pathways that are associated with PTOA. Our device is designed to measure 48 samples simultaneously, using a customized matrix array of force sensitive resistors (FSRs). These FSRs, when compressed above a threshold, show a repeatable change in resistance with increasing load application. By measuring the voltage drop across each sensor, we determined the relationship between applied load and voltage, and then used this calibration curve to determine the load response of a series of hydrogel samples. We also calculated the peak stress of these materials from the area of the sample measured prior to application of the impact load. For validation of impact protocols using our high throughput device, we evaluated cell viability, biochemical content, and release of cell stress signaling molecules in cell-based engineered constructs that had been grown for at least 16 weeks in the laboratory (so that they could take on cartilage-like properties before impact). Results showed that the response of the CTAs after high throughout impact followed the trends of degeneration observed previously in impact tests of single samples. Following this validation process, the device stands ready to identify molecules important in chondrocyte response to injury, and ultimately provide a tool to characterize small molecules that may aid therapeutically in the cartilage repair response.

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# 1. Introduction

Post-traumatic osteoarthritis (PTOA) occurs when a joint suffers a traumatic injury causing progressive degeneration of the cartilage lining the joint surfaces, leading to osteoarthritis (OA). Brown et al. [4] reported that 79.5% of individuals with ankle OA had suffered one or more previous joint injuries. The risk of PTOA ranges from 20% to more than 50% in individuals with a significant joint injury [9]. The number of patients with PTOA of a lower-extremity approaches 6 million, and accounts for nearly \$11.79 billion in annual expenses [4]. More than 1/3 of people older than 45 years of age report OA symptoms ranging from occasional joint stiffness to constant deep pain [5, 6, 7, 8, 19, 20]. The physical impairment



Fig 1. Factors involved in the development of PTOA after injury [3].

caused by OA is said to be equivalent to that caused by end-stage kidney disease or heart failure [24].

When a joint is injured, the degree of injury depends on the energy of impact sustained by the joint. While low energy impact injuries cause cell and tissue level damage, higher energy impacts, such as motor vehicle accidents, can lead to articular and bone fractures. The resulting joint damage can progress over time, leading to PTOA. Surgical or biological interventions such as total joint replacement or autologous chondrocyte implantation procedures can be utilized in severe cases of PTOA (Figure 1) [3].

Currently, researchers are investigating biological therapeutics to inhibit or reverse the joint degeneration caused by impact injuries. Rundell et al. [23] reported that the surfactant P188, added to joints following impact, can limit chondrocyte necrosis. D'Lima et al. [10] similarly showed that mechanically induced chondrocyte apoptosis could be decreased by inhibition of caspase (enzymes involved in controlled cell death), while Martin et al. [15, 16, 22] demonstrated that anti-oxidants could prevent progressive mechanically induced chondrocyte damage if applied within hours of injury.

Our goal was to create an in vitro model of PTOA using engineered cartilage constructs. This enables us to understand the progression of degeneration caused by impact injuries, as well as serve as a tool with which to discover novel therapeutics to limit or prevent cartilage degradation. In particular, our project used a high throughput mechanical screening (HTMS) device to induce impact injuries to engineered cartilage constructs, and to evaluate the effect of injury using several biological assays and outcome measures.

# 2. Background

The overall goal of this project was to develop, in a micro-scale format, methods to mechanically impact native cartilage or cartilage tissue analogs (CTAs). CTAs are prepared by isolating chondrocytes (the cells in cartilage) from juvenile bovine (1-3 months old) knees

which were then digested overnight. CTAs were fabricated in multi-well plates where cells coalesce and form a single mass. In a previous study by Kim et al. [11] CTAs were analyzed at early (6 month) and aged (4 years) time points. Their histological sections displayed abundant type II collagen, with higher concentrations near the edges, for both time points. They also reported that proteoglycan content was higher in the early CTAs and distributed near the center. The aged CTAs showed more intense calcification near the edge due to long-term culture. [11] Kim et al. reported that the early CTA PG/collagen ratio was 2.8 times greater than native cartilage at 1 week; however, it was balanced to native cartilage by 36 weeks (Figure 2) [11]. This data motivates the use of CTAs that can be produced in a high throughput format, and used in the evaluation of induced pathologic changes in our screening system.



While it is well recognized that impact has adverse effects on joint cartilage, study of this phenomenon has been limited by the throughput of precious systems. In particular, previous impact models (both in animals and in vitro) have only been able to individually test one sample at a time. Researchers, including our group, are now looking to scale up these tests, to not only evaluate mechanical injury in engineered cartilage, but also to screen chemical libraries for drug discovery efforts related to PTOA. In a model by Lujan et al. [14] a device was fabricated in which mechanical assessment of up to six samples was possible, though that device was not designed to measure injurious compression. The device uses electromagnetic actuators to measure the force and various strains. In addition, this particular device could account for the difference in height of various constructs; however, machining would be required [14]. In a device proposed by Salvetti et al. [25] individual sample sensing was possible for up to 12 constructs. This device measured force using individually placed resistance sensors, and could create various strains by using different plunger materials. They could correct for various heights by changing the height of the plunger used [25]. In contrast to these devices, our device is able to measure 48 samples simultaneously using a customized matrix array of force resistant sensors (FRS), enabling higher throughput studies (Figure 3).

	Proposed	Lujan	Salvetti
Number of Samples	48	6	12
Force Measurement	Matrix array of resistance sensors	Electromagnet ic actuators	Resistance Sensors
Offset Height Variation	Force applied in step motion	Machining required	Various plunger heights

Figure 3. Comparison of previous designs alongside our proposed model.

The impact protocols used with this device will be based on previously validated models of impact injury induced in osteochondral explants. Quinn et al. [21] performed tests using strain rates between 3 x  $10^{-5}$  and 0.7 s<sup>-1</sup> and peak stresses between 3.5 and 14MPa. They reported that "at higher strain rates, matrix mechanical failure (tissue cracks) and cell deactivation were most severe near the cartilage superficial zone and were associated with sustained increased release of proteoglycan from explants." Quinn et al. [21] stated that at low strain rates cell activity and proteoglycan synthesis were suppressed throughout the cartilage depth, but were most severe in the center of the explants. Natoli [18] reported similar patterns of behavior, stating that increasing impact caused increased cell death focused in the superficial zone and spreading to deeper zones in the weeks following impact. In a study by Kurz et al. [12] GAG content of tissues decreased slightly after impact, corresponding with an increase in strain rate. On a molecular basis, expression and activation of several catabolic enzymes (molecules that degrade cartilage matrix) are up-regulated after impact. MMP-3 increased 250-fold following injury in a study by Lee et al. [13]. They also noted an increase in ADAMTS-4 by 2-3-fold after impact [13].

Similarly, during validation of impact protocols using our HTMS device, we evaluated cell viability and gene expression and performed biochemical assays to measure extracellular matrix content and release to the medium. Results show that the CTA response after high throughput impact injury followed the trends of degeneration observed previously. Following validation, the developed platform will stand ready to identify molecules important in chondrocyte response to injury, and ultimately provide a tool to characterize small molecules that may aid therapeutically in the cartilage repair response.

# 3. Methods

# **3.1. Engineered Cartilage Preparation**

# 3.1.1. CTAs

CTAs were prepared using a method adapted from Kim et al. [11]. We began by isolating chondrocytes (the cells in cartilage) from juvenile bovine (1-3 months old) knees. Femoral head cartilage was cut into small pieces before being removed from the joint and placed in



Figure 4. Dimensions of engineered cartilage analog (CTA).

phosphate-buffered saline (PBS) with 2% penicillin streptomycin fungizone (PSF). Any pieces appearing to contain bone marrow were removed before the cartilage was minced. All of the PBS with 2% PSF solution was removed and basal media containing collagenase-2 (an enzyme that breaks down the cartilage matrix around cells) at 1mg/ml was added. It was then left to digest overnight in a petri dish at 37°C. The digested sample was passed through a 70µm nylon mesh and placed into a 50 ml tube. Then PBS with 2x PSF was added. The tubes were centrifuged at 1750 rpm for 15 minutes at 10°C, washed 3 times, and then the cells were counted. The CTAs were fabricated in 96 -well plates. The cells coalesced and formed a single mass which grew quickly and maintained the chondrocyte phenotype for long periods in culture [17]. The cells were cultured for 4 to 6 months before use in experiments. The CTAs were cultured in DMEM (Mediatech, Inc., Manassas, VA) supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100  $\mu$ g/ml), amphotericin B (2.5  $\mu$ g/ml), 1% MEM vitamin (Mediatech, Inc.), ascorbic acid (50  $\mu$ g/ml) (Gibco-Invitrogen, Carlsbad, CA), and HEPES buffer (25 mM) (Gibco-Invitrogen). [11]

# 3.1.2. Acellular Agarose Constructs

Type VII agarose was dissolved in sterile PBS to create 10 w/v% stock agarose solutions which were microwaved until molten. Agarose solutions were then cast using an electrophoresis casting system in which the solution was pipetted between two glass plates spaced 2.25mm apart to create a gel with a uniform 2.25mm height. A sterile 4mm biopsy punch was then used to punch out acellular agarose constructs which were kept in PBS to maintain the constructs in a hydrated state.

# 3.2. High Throughput Mechanical Screening

### 3.2.1. Set-Up

To induce impact injuries in CTAs our lab has designed a custom device to apply simultaneous impact injuries to CTAs in a high throughput fashion. The device consists of aluminum housing with linear bearings that guide the sensor-pressing plate. The plate then impacts the PTFE indenters that contact the engineered cartilage samples on the opposing surface (Figure 5). The sensor simultaneously measures the pressure applied during the compression of 48 engineered cartilage analy



Figure 5. High throughput mechanical "screening (HTMS) device. (A) Assembled 48-well HTMS device, (B) close in view of the Well Plate Assembly with the Hole Plate positioned above a 48 well plate, and (C) Close in view of Indenter with pin to allow free movement in only one direction

compression of 48 engineered cartilage analogs.

When the force sensitive resistors (FSRs) are contacted, a change in resistance occurs with load. The device measures the voltage difference which corresponds to a load or pressure. The sensor is connected to a DAQ board, which collects inputs and supplies 5V input. The DAQ board also reads in the displacement of the Instron from an analog output channel. Once a resistor is loaded there is a change in output voltage. By individually testing each sensor, we determined the relationship between load and voltage, and then used the voltage

read outs to determine the unknown load values for each sample. The peak stress was then calculated using the area calculated prior to impact.

### 3.2.2 CTA Experimental Impact Protocols

For validation, standard acellular agarose gels produced by our lab were used. We looked for consistency of the results by comparing them to the impact parameters of single tests. Due to the results being similar we continued with the device. The next step was to develop a method to analyze the data. We were able to compare the stress relaxation, peak stress levels, strain rates, and other values for the various protocols, using the HTMS device or a single Instron test.

First we evaluated impact protocols using single Instron tests to determine an approximate threshold for inducing mechanical injury in CTAs (as seen in Figure 7 below). Protocols were set to test to either 50% or 75% strain, at a rate of 0.1 or 0.5 s<sup>-1</sup>. They were impacted for a total time of 10 seconds as can be seen in Figure 6. Samples were collected at multiple time points and biochemical content, histology, and gene expression



Figure 6. Impact protocols relating stress to time.

were analyzed. Based upon these results, we set up in a preliminary study to induce high throughput mechanical injury using the HTMS device. CTAs were compressed to 60% strain at 50%/s. In this study media was collected from all wells at 24 hrs (n=8 for impact, n=6 for control) with constructs being harvested for  $\frac{1}{2}$  of the samples (n=4 for impact, n=3 for control). The remaining constructs and media were harvested at 120 hrs post-impact, with a media change at 60 hrs.

	Final Strain	Strain Rate	Hold Period	Harvest Time Points
Group A	50%	0.5 s <sup>-1</sup> (1s impact)	9 seconds	12, 24, 120 hrs
Group B	50%	0.1 s <sup>-1</sup> (5s impact)	5 seconds	12, 24, 120 hrs
Group C	75%	$0.5  \text{s}^{-1}  (1.5 \text{s impact})$	8.5 seconds	12, 24, 120 hrs
Group D	75%	0.1 s <sup>-1</sup> (7.5s impact)	2.5 seconds	12, 24, 120 hrs
Group E	No impact			12, 24, 120 hrs

Figure 7. Individual impact protocols and harvest time points.

### 4. Analysis

# 4.1. Biochemical Assays

### 4.1.1. DMMB Assay: Glycosaminoglycan Content

Glycosaminoglycans (GAGs) are a negatively charged part of the extracellular matrix. GAG concentrations can be determined by adding a DMB Dye Solution (as described below) to the sample. The dye will bind to the negatively charged GAGs and absorb light at 540-600nm wavelengths. The dye for this assay was made by dissolving 1 g of sodium formate in 490ml of deionized water before adding 1 ml of formic acid. The pH was checked to verify it was around 3.5. A mixture of 8 mg of 1,9-dimethelene blue (DMB) and 2.5 ml ethanol was added to the previous solution. The total volume was brought to 500 ml by adding an additional 7.5 ml ddH2O and then the solution was stored at 4°C in the dark until use. The standards used were dilutions of chondroitin-6-sulfate at concentrations of 5, 10, 15, 20, 25, 30, 35, 40, 45, and  $50\mu$ g/ml. The samples were serially diluted [ex. 1:10, 1:20, 1:40, 1:80] in each well 40µl of the sample was combined with 250µl of the DBM dye. The plate was then read immediately. In these tests the amount of GAG was normalized to DNA to account for variation in sample size.

# 4.1.2. PicoGreen

This assay measures the amount of double-stranded DNA. PicoGreen is a fluorescent nucleic acid stain which reaches its maximum emission at 530nm when bound specifically to double-stranded DNA. Standards were created by creating a 2-fold dilution series of Lambda DNA stock ( $100\mu g/ml$ ) to create concentrations of 20, 10, 5, 2.5, 1.25, .625, .313, and  $0\mu g/ml$ . The standards were placed in triplicate in the first 3 columns of a 96-well plate. Samples were then run in triplicate in the subsequent columns. A working solution of PicoGreen Reagent, 20x TE buffer and ddH2O, was added to all wells. The plate was then incubated for 5 minutes and read at 480nm excitation and 520nm emission.

#### 4.2. Gene Expression

CTAs were flash frozen in liquid nitrogen, and then crushed in Trizol and stored at -80°C until RNA extraction. A Qiagen RNEasy Mini kit was used during RNA extraction. RNA quality and concentration was determined using a NanoDrop spectrometer. cDNA was then synthesized using the BioRad Reverse Transcriptase Supermix kit. Real Time PCR was used to quantify gene expression levels of extracellular matrix proteins, proteases, and cell stress molecules following impact of CTAs and in control samples. In particular the following

genes were investigated: Matrix Metalloproteinase 13 (MMP13), Aggrecan (AGG), Collagen II (COLII), and inducible Nitric Oxide synthase (iNOS)

# 4.3 Nitric Oxide

Nitric Oxide (NO) is a molecular messenger in many cell response pathways, but in particular has been related to cell stress and inflammation mediated pathways which regulate cell death and matrix degradation [1]. One way to measure NO is by measuring the amount to nitrite in a solution, which is a breakdown product of NO. The Griess Assay tells us how much NO is in media by measuring the amount of nitrite. A standard was prepared by diluting 1µl of 0.1M Nitrite Standard with 999µl of complete media. The standard was serially diluted twofold to create 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 µM standards. 50µl of the standards were placed in the first 3 columns of a 96-well plate. Experimental samples were placed in triplicate in the remaining columns of the plate. Using a multichannel pipette 50µl of Sulfanilamide Solution was added to all wells. The plate was incubated at room temperature, protected from light, for 5-10 minutes before 50µl of NED solution was added. The plate was again incubated at room temperature for 5-10 minutes before being read at 540 nm.

#### 4.4 AnaSpec Assay of MMPs

Matrix metalloproteinases (MMPs) function to degrade extracellular matrix proteins and also play a large role in apoptosis, or controlled death, of a cell. The assay works by using MMPs to cleave 5-FAM/QXL<sup>TM</sup>520 into 5-FAM and QXL<sup>TM</sup>520 [2]. This Fluorescent Resonance Energy Transfer (FRET)



probe acts such that when located close to one another the QXL<sup>TM</sup>520 prevents the 5-FAM from fluorescing. MMPs separate the two fragments and 5-FAM fluoresces, as show in Figure 8 from AnaSpec [2]. This fluorescence can be measured using excitation/emission wavelengths of 490nm and 520nm.

## 4.5. Histology – Live/Dead

Assessing the live and dead fractions of a cell population is one way to determine cell viability and health locally. Calcein AM is a molecule that can easily cross the cell membrane of live cells. Once in the cell, esterases cause the calcein AM to lose an acetomethyoxy group. Without this acetomethyoxy group the calcein cannot leave the cell

and fluoresces green, indicating a living cell. Ethidium homodimer-1 cannot penetrate live cells; however, when a cell is dead it can pass through the nuclear membrane and bind directly to the nucleic acid in a cell, and fluoresces red.

## 4.6 Statistics

Statistical analysis for various assays was completed using a one-way ANOVA with Tukey's post hoc test with p<0.05.

### 5. Results

### **5.1 Acellular Agarose Gels**

The sensor used in these preliminary tests had 5 active sensors. As shown in Figure 9 each sensor responds individually to a specific sample deformation generating a load response in the sensor. While all sensors responded slightly differently, they were consistent in their relaxation patterns. All showed an immediate spike, followed by a rapid drop in voltage, consistent with the sample stress relaxation. All sensors then relaxed slightly before returning to their original values. As can be seen in Figure 9, the sensors can be activated at various points. This is due to variation in indenter and sample heights. Sensors were also loaded cyclically and no change in signal was noted after 3 cycles to 20N at a rate of 50%/s. (Figure 10)



Figure 9. (Left) Individual response of sensors. Figure 10. (Right) Cyclic loading of a sensor to 20N at 50%/s

When impacting acellular agarose gels a voltage is recorded for each sensor; this voltage is then converted into a load. The voltage at each protocol was recorded before being converted to a load and averaged as shown in Figure 11. While the values vary for the



Figure 11. Peak and average voltage and load values for acellular agarose gels.

peak load between each sensor and protocol, the average values indicate a trend. For example, at 25% strain at 50%/s there is a much smaller peak stress than at 75% strain at 50%/s (Figure 11). The variations seen in each protocol with a different sensor is likely due to variations in sample heights as previously stated, or perhaps improper alignment of the sensor panel. These tests were run with a sample sensor; a custom sensor may be more accurate and allow for more samples to be tested at the same time. In addition, the custom sensor will have a lower load threshold so it may be more sensitive to changes in load.

# **5.2 Single Instron Impact**

To evaluate the biologic response of CTAs to our impact protocols, individual samples were impacted one at a time using the Instron. Biochemical and metabolic outcomes were assessed at regular intervals after impact loading.

# 5.2.1 GAG/DNA

When looking at GAG/DNA at 12 hrs, 24 hrs, and 120 hrs post-impact various things were noticed. In all protocols the amount of GAG/DNA had dropped at the 12 hr time point (Figure 12). While this was not always significant, it shows that following an injurious impact the



Figure 12. Individual impact GAG values normalized to DNA. P<0.05: \* vs 50% and 75% strain impact groups; & vs 24 and 120 hrs; # vs control and 50% strain

CTAs lose GAG, which makes them more susceptible to compression injuries. Another interesting note is that for 75% strain the 24 hr time point seemed to recover some GAG/DNA that had originally been lost. At the 24 hr and 120 hr time points the drop in GAG/DNA for 50% and 75% strain was statistically significant compared to the control at those time points.

#### 5.2.2 NO Assay

There were large changes in NO levels in the media for various time points for all impact protocols. The control levels for 12 and 24 hrs were statistically different from the 50% and 75% strains. There was also a large difference between the 24 and 120 hr time points when compared to the 75% strain. There was also a spike in NO concentration at 24 hrs for the 75% strain that was statistically different from the 12 and 120 hr time points. These results show that NO is a good early indicator of CTA response to injury.

#### 5.2.3 Gene Expression

Shown in Figure 14 are the expression levels of various genes at 12, 24, and 120 hrs post-impact at 2 different impact protocols. In this matrix study metalloproteinase 13 (MMP13), which plays an important role in the breakdown of the extra cellular matrix, was dramatically increased at 120 post-impact for CTAs impacted at 75% strain at 50%/s. In all other cases the MMP13 levels remained similar to the control values. When looking at Aggrecan (AGG), which functions to provide cartilage with structural support, the expression levels after impact at 50% strain at 50%/s increased over time. There is also in increase in Aggrecan with an impact of 75% strain at 50%/s from 12 to 120 hrs.



Figure 13. NO concentrations at two different protocols. P <0.05: \* vs 50% and 75% strain for the same time point; \$: vs 75% strain; @ vs other time points.



Figure 14. Relative gene expression of (A) Aggrecan and (B) MMP13. P<0.05: \* vs 12hr; \$: vs 24hr; # vs 75% strain; @ vs control for the same time point.

# 5.2.4 Peak stress

Single Impact tests were conducted for CTAs using the protocols previously described. A peak load was recorded for each test, and the peak stress was calculated. CTAs impacted to 75% recorded higher peak stresses and loads than those impacted to 50%. It was also noticed that higher strain rates corresponded to higher peak stresses.



Figure 15. (Left) Impact protocol schematic for single impacts. Figure 16. (Right) Peak Stresses for single impact protocols.

#### 5.2.5 Histology – Live/Dead

In these images CTAs were impacted individually according to the protocols mentioned, and the green stain represents live cells, whereas the red stain represents dead cells. The CTAs impacted at 50% strain show little to no dead cells at 24 hrs post-impact. At 75% strain at both 50%/s and 10%/s CTAs show large cracks and fissures. There are also large areas of dead cells located throughout these CTAs.



Figure 17. Histology images from individual impacts at (A) 50% strain at 50%/s, (B) 75% strain at 50%/s, (C) 50% strain at 10%/s and (D) 75% strain at 10%/s.

### 5.2.6 Mechanics

The dynamic modulus and equilibrium modulus were calculated for CTAs at various points as they matured before single impact testing. As the constructs matured they became stiffer; thus these values increased as shown in Figure 18 below.



Figure 18. Equilibrium modulus of CTAs cultured for up to 16 weeks (lightest color: 4 weeks to darkest color: 16 weeks) correlated with (A) GAG and (B) DNA content for multiple donors. Mechanics are strongly correlated with GAG, but less so with DNA.

### 5.3 High Throughput Impact Analysis: Preliminary Results

In a preliminary study, we used the high throughput impact device to apply injurious compression to multiple samples at the same time, and evaluated their response to determine if it was similar to the response of the sample-by-sample impact results described above.

### 5.3.1 GAG/DNA

For this study we analyzed the amount of GAG/DNA at 24 and 120 post-impact. As shown in Figure 19 the amount of GAG appeared to decrease in the samples 24 hrs after impact; however, this drop was not statistically significant. In addition the amount of GAG/DNA at 120 hrs post-impact had increased from the amount present at 24 hrs.



Figure 19. High throughput impact GAG values normalized to DNA.

### 5.3.2 NO Assay

In this assay there were large differences in NO concentrations that were statistically significant. This means the CTAs impacted with the high throughput device were responding to impact by releasing NO into the media and sending messages to the cell. The largest difference was noticed between the 24 hr control and impact, with the impact value being nearly 6 times the control amount. This amount of NO in the media shows a large release shortly after impact, and is consistent with the sample-by-sample single impact testing described above.



#### 5.3.3 Peak Stress

For the preliminary high throughput mechanical testing, constructs were impacted at 60% strain at a rate of 50%/s. The peak stresses were calculated for 3 different samples, as shown

in Figure 22. These values ranged from 0.8 MPa to 1.1 MPa. These stresses are similar to those recorded for 50% strain in the single impact tests, and lower than those recorded at single impact for protocols involving 75% strain.



Figure 21. (Left) Signal reported from sensors during high throughput impact. Figure 22. (Right) Peak stress values from sensors during high throughput impact

# 5.3.4 Histology – Live/Dead

In these images CTAs were impacted at 60% strain at 50%/s. These images show us that there are micro-fractures caused by impact and that cell death is present in the adjacent areas. There is also a fair amount of consistency among the CTAs, leading us to the conclusion that they were all impacted to the same extent.



Figure 23. Histology images from 4 high throughput impact samples at 60% strain at 50%/s.

#### 5.3.5 Mechanics

For high throughput tests the dynamic and equilibrium modulus was recorded as with single impact tests. The mechanics of these CTAs follows similar trends to those used for single impact tests. These results show that the maturation of these constructs follows a temporal timeline in which GAG gradually increases, leading to increased mechanical properties.



Figure 24. Equilibrium modulus of CTAs cultured for up to 16 weeks (lightest color: 4 weeks to darkest color: 16 weeks) correlated with (A) GAG and (B) DNA content for multiple donors.

#### 6. DISCUSSION

By using high throughput mechanical screening we can drastically reduce the amount of time required to run large amounts of samples. At this point in our testing we are able to make several conclusions. Between 50% and 75% strain there is a threshold for inducing significant damage to CTAs. Without reaching this threshold there is a little change in GAG as well as in MMP13 gene expression and NO production. At the highest strain, impact also causes the matrix to visibly fracture, resulting in an increase in exposed surface area which may be critical for the loss of GAG as well as other cell signaling molecules and extracellular matrix components. In addition we have determined the sensor to be reliable in that when normalizing each sensor to its calibration curve the data remains true to its individual value. Finally, we have shown that the high throughput impact mirrors several key features of both individual CTA response to injury, as well as native tissue, validating it as a tool for studying cartilage response to injury in a high throughput fashion.

### 7. FUTURE DIRECTIONS:

Within the next few months we will have a sensor that is custom designed for our high throughput mechanical screening device. This sensor will have a lower load threshold and therefore be able to detect smaller changes in load during impact as well as mechanical testing. Once the device is validated for 48 samples we hope to scale up to 96 well plates. At this point a single sensor mat will be used instead of individual resistors. A program will be created to isolate individual sample signals so data may be recorded for each individual sample. Once this device is scaled to this level, it will be ready for



Figure 25. Custom high throughput layout.

screening of small molecule libraries of chemicals that can potentially limit degeneration after impact injury. Such a screening tool will be very useful for identifying new therapeutics that can improve patient outcomes after traumatic injury to their cartilage potentially stopping the progression of PTOA and improving patient care.

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