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A High Throughput Mechanical Screening Device for Cartilage Tissue Engineering

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Abstract

Articular cartilage enables efficient and near-frictionless load transmission, but suffers from poor inherent healing capacity. As such, cartilage tissue engineering strategies have focused on mimicking both compositional and mechanical properties of native tissue in order to provide effective repair materials for the treatment of damaged or degenerated joint surfaces. However, given the large number design parameters available (e.g. cell sources, scaffold designs, and growth factors), it is difficult to conduct combinatorial experiments of engineered cartilage. This is particularly exacerbated when mechanical properties are a primary outcome given the long time required for testing of individual samples. High throughput screening is utilized widely in the pharmaceutical industry to rapidly and cost-effectively assess the effects of thousands of compounds for therapeutic discovery. Here we adapted this approach to develop a high throughput mechanical screening (HTMS) system capable of measuring the mechanical properties of up to 48 materials simultaneously. The HTMS device was validated by testing various biomaterials and engineered cartilage constructs and by comparing the HTMS results to those derived from conventional single sample compression tests. Further evaluation showed that the HTMS system was capable of distinguishing and identifying ‘hits’, or factors that influence the degree of tissue maturation. Future iterations of this device will focus on reducing data variability, increasing force sensitivity and range, as well as scaling-up to even larger (96-well) formats. This HTMS device provides a novel tool for cartilage tissue engineering, freeing experimental design from the limitations of mechanical testing throughput.

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Keywords

Mechanical Testing; 3D culture; High Throughput Screening

Introduction

Cartilage tissue engineering has made marked progress, with numerous studies arriving at methods for the production of mechanically functional cartilage, based on either native chondrocytes (Kelly, Ng et al. 2006; Novotny, Turka et al. 2006; Lima, Bian et al. 2007; Byers 2008; Bian, Fong et al. 2010; Cheng, Estes et al. 2011; Ng, O'Connor et al. 2011) or mesenchymal stem cells (MSCs) grown as three dimensional (3D) constructs (Mauck, Yuan et al. 2006; Huang, Farrell et al. 2010; Moutos and Guilak 2010; Thorpe, Buckley et al. 2010; Erickson, Kestle et al. 2012). However, the degrees of freedom present in any experimental design can make even the simplest of tissue engineering studies difficult to execute, where an investigator can vary materials (Mouw, Case et al. 2005; Chung, Beecham et al. 2009; Chung and Burdick 2009; Hwang, Varghese et al. 2011), cell number (Mauck, Wang et al. 2003; Weinand, Xu et al. 2009), growth factor doses and combinations (Blunk, Sieminski et al. 2002; Gooch, Blunk et al. 2002; Appel, Baumer et al. 2009; Johnstone, Alini et al. 2013), and the mechanical loading environment (Ng, Mauck et al. 2009; Thorpe, Buckley et al. 2010). Moreover, complexity in experimental design leads to difficulties in capturing outcome parameters in a cost- and time-efficient manner. The need for increased throughput in assessing outcomes is not unique to tissue engineering. Indeed, high throughput screening (HTS) methods emerged very early in the pharmaceutical industry (Drews 2000), where such methods were essential for screening large chemical libraries for biologic activity relevant to disease.

The underlying premise of HTS is that if a suitable assay can be developed that is 1) sufficiently sensitive to measure a relevant cellular response, 2) of a low cost per sample, 3) easy to automate, and 4) reproducible, then one can expedite drug discovery. While most HTS assays are performed in monolayer culture, recent studies have begun to implement assays in 3D constructs as well. For example, 3D multi-cellular spheroids have been used to screen for tumor suppressive agents (Kunz-Schughart 2004). A few studies have applied HTS principles towards applications in bone and cartilage biology and regeneration. For instance, HTS-based assays focused on MSC osteogenesis in monolayer (Brey, Motlekar et al. 2011) and chondrogenesis in micro-scaled pellet cultures (Huang, Motlekar et al. 2008) have been used to screen small molecule libraries in a 384-well format. The potential of such HTS approaches is perhaps best illustrated by a recent study, employing an image-based HTS method that identified molecules that promoted the formation of chondrogenic MSC nodules, and protected cartilage from degeneration in a small animal model of joint instability (Johnson, Zhu et al. 2012).

While most HTS assays focus on molecular events, functional outcomes are equally important for musculoskeletal tissues (Vandenburg 2010). This is particularly relevant for cartilage as the properties of the engineered tissue will dictate function in the load-bearing joint environment (Ateshian and Hung 2005). Thus, it would be ideal if HTS approaches

could be modified to include mechanical measures. However, traditional one-at-a-time assessments of mechanical properties can be prohibitively time consuming, where a typical stress relaxation test can take several hours per sample (Mauck, Soltz et al. 2000; Soltz and Ateshian 2000). In even relatively simple experimental designs (Erickson, Kestle et al. 2012), involving just two different seeding densities, four different material formulations, one growth factor at a single dose (and a growth factor-free control), and five samples per group, over eighty hours of testing is required at each time point. Given the continued development of novel materials and new factors influencing cartilage growth, and the requirement that each of these inputs be carefully evaluated in a combinatorial context, throughput in mechanical analysis has become a significant barrier to further advances. As such, development of a high throughput mechanical screening (HTMS) system would represent a valuable tool to advance cartilage tissue engineering.

Towards this end, several mechanical testing systems have been introduced that enable multi-sample evaluation. For instance, the Myoforce Analysis Device was developed to monitor bioartificial muscles to identify compounds that alter contractile strength (Vandenburg 2010). The MATE system incorporated real-time measures of load during dynamic stimulation of engineered cartilage, using a six-sample actuating system (Lujan, Wirtz et al. 2011). Still more recently, a 12-sample tissue stimulator was developed that recorded load from each sample via individual force sensitive resistors (Salveti, Pino et al. 2012). These devices illustrate how real-time and multi-sample mechanical analysis can be incorporated into tissue systems. While promising, throughput in these devices is restricted to a relatively small sample capacity, and expansion to higher throughput formats might be limited by sensor technology (Lujan, Wirtz et al. 2011; Salvetti, Pino et al. 2012). Additional development is needed to make such devices compatible with HTS of chemical libraries.

We developed a novel high throughput mechanical screening (HTMS) device that can assess mechanical properties of biomaterials and engineered cartilage in a 48-well format. Our system utilizes a custom force sensitive resistor (FSR) array to measure instantaneous and time-dependent mechanical response of up to 48 samples simultaneously. The increased capacity of this device provides a platform to evaluate properties in complex, combinatorial studies for the screening and optimization of engineered cartilage. The objective of this study was to design, optimize, and validate this system, and to screen mechanical properties of multiple materials and engineered cartilage in several experimental configurations.

Materials and Methods

HTMS Device: Components

The HTMS device was designed to interface with mechanical testing systems utilized in most orthopaedic and bioengineering laboratories. A schematic is shown in Figure 1. The device housing consists of an aluminum base plate and two parallel side plates onto which linear bearings (Maintenance-Free Ball Bearing Carriages and Guide Rails, McMaster-Carr, GA) are affixed to align and maintain smooth vertical displacement of the platen. The sensor platen was integrated via two plates: an upper plate to which it is directly attached, and a bottom plate fixed to the upper plate. A custom force sensitive resistor (FSR) array was mounted via adhesive backing to this plate (Custom 48 Matrix FSR Sensor Array,

Sensitronics, WA). To control vertical displacement of the sensor platen, an Instron (Model 5848, Instron, MA) was connected via an adaptor to the sensor platen (Figure 1A,D).

Opposing the sensor surface, a well plate assembly was designed to accommodate standard 48-well plates (BD Falcon, Multiwell Cell Culture Plate, #75875, NJ) and an indenter array and hole plate to align with each sensor on the FSR array (Figure 1B,D). On top of the 48-well plate, a hole plate was used to guide vertical and restrict horizontal movement of each indenter during loading. Indenters were composed of PTFE (McMaster-Carr, GA) rods with a flat bottom surface that interacted with the sample during loading, and a beveled top for centered activation of the sensor (Figure 1C,D). Each indenter had a diameter of 9.5mm to fit within the 10mm diameter well of the 48-well plate to allow fluid displacement during unconfined compression testing. A small cotter pin (Dowel Pins, McMaster-Carr, GA) inserted at the top of the indenter allowed for simultaneous removal of all indenters after testing, without interfering with the motion of adjacent indenters in the array.

HTMS Device: Sensor, Electronics, and Software

The custom force sensor includes four layers: a force sensitive resistor (FSR) shorting layer, a spacer layer (0.0005"), a trace layer, and an adhesive layer (3M) (Figure 2B). The spacer layer between the FSR layer and trace layer creates a gap between the two conductive layers. Upon compression, the conductive layers come into contact, resulting in a decrease in resistance in the circuit. The sensor contains 48 "sensels" (force-sensitive locations) that match the well-plate layout and individually capture load during compression (Figure 2B). The sensor was connected via a voltage supply and resistor (10k Ω) to create a voltage divider to measure change in resistance. Each sensor on the trace layer has an individual conducting pin out, and all sensors have a common power supply pin (connected to a 5V source in the NI DAQ board, NI USB-6225 M Series DAQ, NI, TX). A ribbon (Nicomatic) connected the sensor to a custom wiring box (Figure 1D) with the circuit illustrated in Figure 2A. Each sensel voltage was captured as analog input to DAQ board and recorded using a custom LabVIEW program (LabVIEW 8.6, NI, TX), with data post-processing in MATLAB (MATLAB R2010a, Mathworks, MA).

HTMS Device: Sensor Calibration

The sensor was calibrated by inverting the sensor platen and applying displacement controlled compression to each sensel. Sensors were compressed to ~5N at a rate of 0.0004 mm/s via an indenter connected to the Instron. Force and voltage was recorded in LabView, and the resulting force-voltage data fit to an exponential curve (Figure 2C); this calibration was used for all subsequent testing. An example 3D representation of force output for silicone compression is shown in Figure 2D. The most sensitive (greatest force resolution) and nearly linear range of the sensor was 0–1V, corresponding to a load of ~1N. This sensitivity is determined by the thickness of the spacer layer; increasing the thickness of the spacer layer decreases low-load sensitivity, but would allow for higher forces to be measured.

HTMS Device Testing: Protocols and Data Analysis

To test the HTMS device, we first evaluated the properties of several common elastic (e.g., silicone rubber) and viscoelastic (e.g., agarose) materials. To accommodate differences in sample height, we devised a step-wise stress relaxation testing profile consisting of multiple ramps of 10% compressive strain (relative to original average sample height) applied at 0.05% strain/sec with a 1000 sec hold after each step. At each step, the relaxation phase was followed by a dynamic deformation phase (1% strain applied at 0.1 Hz), followed by a further 60 second hold (Figure 2E). Before starting each test, the device displacement was zeroed to the height of the tallest sample. The number of steps was determined by measuring the height of all samples, calculating average height and displacement per step, and determining how many steps would be required to ensure that multiple compression steps were applied to all samples. The sensor successfully captured elastic and viscoelastic (relaxation behavior) of these hydrogels (Figure 2D,F).

To calculate compressive equilibrium modulus for each sample, voltage data was imported into a custom MATLAB program to extract strain and equilibrium load for each step. All sample and test information, including sample height, width, and step displacement magnitude was likewise imported into the program. The starting actuation point for each sensor was determined using a pre-determined voltage threshold, which was then used to calculate applied strain for the sample for each step. A “first step” was selected for calculation of compressive modulus based on an 8% strain threshold, i.e. for an individual sample the step was required to reach at least 8% strain for it to count as a first step. Since samples differed in height, this ‘first step’ was in some cases the first actuation of the sensor (i.e., for samples making good contact initially), and sometimes the second actuation of the sensor (i.e., for shorter samples that were not in contact, or not compressed by at least 8%, during the first actuation). Following capture of the transient and equilibrium response during this ‘first step’, all data (including step number, step strain, total strain, and equilibrium modulus) were exported to Excel for further analysis.

HTMS Validation: Multi-Sample Versus Sample-By-Sample Testing

To validate the HTMS device, we measured several materials with a range of compressive properties and compared the results to those derived from single sample-by-sample tests. Two hydrogels, agarose (Type VII, Sigma) and polyacrylamide (National Diagnostics) were cast between glass plates to create gels of uniform thickness and punched into cylindrical samples (H: 2.25mm, Ø 4mm). Agarose gels were cast at 4 or 10% (w/v) and polyacrylamide was cast at 15% (w/v). Construct dimensions were measured, and all samples were arranged in a 48-well plate for HTMS testing (n=10–11/material). HTMS testing consisted of a five-step stress relaxation, and for comparison, single sample-by-sample testing used a two-step stress relaxation test. Since HTMS results provide a modulus for compression ranging between 10 and 20% applied strain (depending on when the sensor actuated for a given sample), the equilibrium modulus at both strain levels were reported for the sample-by-sample testing. Materials screening experiments were conducted at least three times, with one representative set of results shown here.

Fabrication and Screening of Engineered Cartilage

To evaluate engineered constructs using the HTMS device, mesenchymal stem cells (MSCs) were isolated from bone marrow from juvenile bovine femurs, and expanded (passage 2 or 3) as previously described (Huang 2010). Methacrylated hyaluronic acid (MeHA) was prepared as previously described (Burdick, Chung et al. 2005; Erickson, Huang et al. 2009). To generate cell seeded constructs, MSCs were trypsinized, centrifuged (300 rcf for 5 minutes), counted, and encapsulated in 1% (w/v) MeHA at 20 or 60 million cells/mL (Erickson, Kestle et al. 2012). The MeHA solution was cast between two glass plates and exposed to UV light for 10 minutes; gels were punched to form cylindrical constructs (H: 2.25 mm, Ø 4mm). Constructs were cultured in a chemically defined chondrogenic medium (CM+) as previously described (Mauck, Yuan et al. 2006).

Two HTMS evaluations were performed with MSC-seeded MeHA constructs. In the first, we evaluated the concentration-dependent effect of a pro-inflammatory cytokine on the mechanical integrity of constructs. For this, constructs (20 million cells/mL; 1% MeHA) were cultured in CM+ for 12 weeks, at which point, constructs were cultured for a further 6 days in CM- medium (lacking TGF- β 3), and treated with increasing concentrations of TNF- α (0, 1, 5, and 10ng/mL). Media was changed on day 3 and TNF- α replenished; all media was collected for further analysis of GAG loss using the DMMB assay (Farndale, Buttle et al. 1986) and nitric oxide production via the Griess Assay (Promega). Mechanical properties were evaluated 6 days after exposure to TNF using the HTMS device (n=10–12/group). To validate HTMS results, single sample unconfined compression (10% step only) was performed on a parallel set of samples (n=4/group) (Mauck, Yuan et al. 2006). Construct GAG (DMMB assay) and DNA (PicoGreen, Invitrogen) content was also evaluated following mechanical testing.

In the second test, we evaluated the ability of the HTMS device to distinguish differences in properties arising from constructs seeded with MSCs at two densities (20 vs. 60 million cells/mL) in 1% w/v MeHA hydrogels. Mechanical properties of constructs were evaluated after 10 weeks of culture using the HTMS device (n=8–9/group) and compared to single sample-by-sample testing using the two-step protocol. Biochemical content of gels was evaluated as described above.

Statistical Analysis

Mechanical properties of biomaterial and engineered cartilage constructs evaluated using the HTMS device were compared to single sample testing, and effect of TNF- α on construct maturation was determined using one-way ANOVA with Tukey's post-hoc test ($p < 0.05$). To perform quality control (QC) and to assess validity of 'hit' criteria with mechanical screening, the strictly standardized mean difference (SSMD) was utilized (see below), with positive and negative controls from the log-transformed (for normalization) HTMS data set (Zhang 2007; Zhang 2011).

Results

HTMS of Materials and Engineered Cartilage

To test the HTMS platform, three acellular hydrogel formulations, 4% agarose, 10% agarose, and 15% polyacrylamide were simultaneously evaluated for compressive properties using the HTMS device, with results compared to sample-by-sample testing (Figure 3A). HTMS-derived equilibrium moduli for all materials closely approximated the results from single sample first and second step equilibrium moduli. Indeed, the means from HTMS testing were not different from sample-by-sample testing (HTMS vs. single samples: 4% agarose: 31.1 ± 24.0 vs. 49.6 ± 18.7 , 10% agarose: 173.6 ± 74.8 vs. 194.2 ± 46.8 , and 15% polyacrylamide: 140.9 ± 61.6 vs. 109.9 ± 32.2 ; $p > 0.05$). Engineered cartilage formed at 20 or 60 million cells/mL (20M or 60M) were also evaluated for mechanical properties. As shown in Figure 3B, the mean values from HTMS testing were not different from sample-by-sample testing, with 20M constructs having an equilibrium modulus of 105.6 ± 99.1 vs. 128 ± 65.9 and 60M constructs of 187.3 ± 65.7 vs. 179.2 ± 62.1 ($p > 0.05$). These results show that the HTMS device can effectively determine and distinguish mechanical properties ranging from ~25 to ~300 kPa in engineered constructs.

HTMS of Engineered Cartilage Treated with Inflammatory Cytokines

Treatment of engineered constructs with TNF- α resulted in a dramatic loss of properties in a concentration-dependent manner. HTMS measurement of properties captured differences between treatment groups in a manner similar to that of the sample-by-sample testing (Figure 4A). Both testing methods showed that exposure to higher concentrations (5 and 10 ng/mL) of TNF- α resulted in declining properties compared to control or 1 ng/mL groups ($p < 0.05$). Measurement of GAG in the media confirmed matrix breakdown, where sustained GAG release was observed during six days of treatment, with the higher TNF concentrations resulting in ~3–5 times more GAG release compared to control and 1 ng/mL groups (Figure 4B). These results illustrate that the HTMS device can capture loss of properties in engineered cartilage as a consequence of graded exposure to TNF- α .

Quality Control and ‘Hit’ Criteria for HTMS Screening

The value of a high throughput screening system lies not just in its ability to measure multiple samples at once, but specifically in its ability to distinguish between groups to identify ‘hits’, or factors that produce a response that is different from a given control. To further evaluate our HTMS system in terms of quality control (QC) and hit selection criteria, we used a statistical parameter developed by Zhang termed the strictly standardized mean difference (SSMD) (Zhang 2007; Zhang, Ferrer et al. 2007; Zhang 2011). SSMD measures the magnitude of difference between two populations, and can better account for non-normality, variability, skewness, and outliers within a population, compared to other statistical methods (such as the ‘z-factor’) (Zhang, Chung et al. 1999). Non-normality and skewness are common features of HTS data, where ‘hits’ are few, and most conditions produce negative results (i.e., remain at the baseline level). To perform a quality control (QC) analysis, one first selects a condition that provides an acceptable baseline (negative control) level, and another condition that constitutes a ‘hit’ (or positive control). For our biomaterial screen, we chose the 4% and 10% agarose groups as negative and positive

control groups, respectively. For the TNF- α treatment data set, we chose free swelling controls as the baseline, and 10ng/mL as the positive control. Using log-transformed data, we then calculated the estimated SSMD from the ratio of median and median absolute deviation of both populations (Zhang 2011). From this analysis, SSMD was computed to be -2.16 for the biomaterial screen and -1.43 for TNF- α treatment. Comparing these values against published SSMD QC criteria (Zhang 2011), and assuming that our controls were of 'moderate strength', these SSMD values indicate that the HTMS screen is a "good to excellent" tool for identifying 'hits'. Indeed, by randomly selecting three values (a reasonable number of replicates for HTS) from each group in the TNF- α study, and computing SSMD value for each, the value for 1ng/mL was -4.8, indicating a strong effect, while the values for 5ng/mL and 10ng/mL treatments were -23.5 and -8.4, respectively, indicating that these two concentrations had extremely strong effects ($|\text{SSMD}| > 5$) according to RNAi hit criteria (Zhang 2011). Candidate molecules with such strong effects would be identified as 'hits' based on empirically determined thresholds in a high throughput screen, and the molecules or conditions they represented would be further investigated in follow up screens.

Discussion

While mechanical properties are an essential outcome in any study of engineered cartilage, current sample-by-sample testing methods represent a significant bottleneck, constraining experimental designs. To address this limitation, we developed a novel HTMS device to enable evaluation of up to 48 samples at one time. Our data show that this device can successfully determine the properties of various biomaterials and engineered constructs, in a rapid manner, while producing data that closely matches that of sample-by-sample testing. The current device was designed to measure mechanical properties of soft biomaterials and engineered cartilage (in the range of 25–300 kPa). However, given the flexibility of the sensor technology, stiffer materials or tissues (e.g. bone) could be evaluated by modifying the removable sensor to shift the load-sensitivity range for the desired application. With minor modifications (inclusion of an independent displacement control system), the device could likewise be adapted to apply long-term controlled mechanical stimulation to engineered tissues in culture, while measuring real-time evolution of properties. More importantly, this device reduces the burden of time spent in evaluation, where one can sequentially test several 48-well plates, thereby enabling a single user to derive properties from ~400 samples in a single 8-hour day. In contrast, single-sample testing would require at least 200 hours. The step protocol implemented here also takes into account differences in sample height, and allows the user to select a "first step" after completing the test, which is then used to calculate the equilibrium modulus. In contrast to previous HTS devices that used individual actuators (Lujan, Wirtz et al. 2011) or varying height plungers (Salveti, Pino et al. 2012) to account for differences in sample heights, this protocol requires no additional parts and is easily tuned to apply a range strain magnitudes. Additional advantages of this system include individual force sensing capability, a semi-automated MATLAB program for data analysis, and the ability to fabricate and assemble the device at a reasonable cost (~\$6000 circa 2013).

While our data suggest that the HTMS device is a useful tool for the rapid evaluation of multiple materials and factors for cartilage tissue engineering, it is not without its limitations and opportunities for refinement. Namely, while the mean values from HTMS testing matched sample-by-sample testing, the standard deviations were larger. In developing screening tools, a balance must be achieved between accuracy, throughput, and cost. To address this, a number of statistical tests exist to evaluate whether a screening tool can ably distinguish ‘hits’ from conditions that fail to generate a response. One such test, the SSMD parameter, has been validated for high throughput RNAi assays, and its use is suggested for other HTS small-molecule screening (Zhang 2007). Using this method and data from HTMS testing, we validated the HTMS device as a “good-excellent” screening tool. One caveat to this statistical method, however, is the consideration of the strength of the controls, as this factor influences QC cutoff criteria. For some RNAi screens, hits by definition fall within range between an extremely strong positive control and negative reference defining the dynamic range (Zhang, Chung et al. 1999). In contrast, when screening properties in engineered materials, moderate or strong controls may be sufficient, as the effect size is unknown for assayed molecules, and their action might improve or reduce properties beyond controls. If we had considered our QC groups to be ‘extremely strong controls’, meaning that they represented a maximum possible compressive modulus, then the assay would only fall into the “inferior-good” range. This highlights the importance of understanding the expected differences in effect size in order to correctly set QC criteria as well as hit thresholds. Despite this limitation, using “moderate” controls, SSMD analysis identified the two higher doses of TNF- α as having extremely strong effects compared to controls, validating the HTMS device as a screening tool. Moving forward, it will be necessary to empirically determine SSMD hit thresholds based on the desired number of molecules or materials to be evaluated in more rigorous secondary (*in vitro*) and tertiary screens (*in vivo*) (Johnson, Zhu et al. 2012).

While the current HTMS device is sufficient in its capacity to screen differences in material properties, it will be important in future iterations to not only increase throughput of the system, but also to improve the sensitivity and range of force detection and reduce measurement variability. To increase throughput capacity, we are currently scaling up to a 96-well format. This modification will double throughput, making screening of large chemical libraries more practical, enabling identification of compounds that promote cartilage growth and repair. To further adjust the range of load sensitivity, the thickness of the spacer layer in the FSR could be customized for a desired force range. Moreover, other sensor technologies could be incorporated into the design that may provide more stable calibrations and force readings, for example via the inclusion of an integrated sensor with force interpolation. Additionally, other applications for the HTMS system include controlled mechanical stimulation via the addition of an independent displacement control system (LVDT, stepper motor, and feedback control) to replace the Instron displacement control. This possibility opens a new platform whereby testing multiple treatments can be carried out in a setting of real-time mechanical loading. This HTMS device has the potential to dramatically alter the landscape of what is possible in the experimental design of studies directed towards cartilage tissue engineering, injury, and repair. Most sample-by-sample studies are predicated on a specific hypothesis, and as such use a defined set of experimental

conditions to test that hypothesis. Such an approach inherently limits the design space, and biases studies towards evaluation of a low number of conditions and interrogation of already well known mechanisms. In contrast, high throughput screening methods such as this allow discovery to drive hypothesis formation. A multitude of compounds can be tested rapidly, in a cost-efficient manner, and molecules previously unknown to have any relevance to the tissue or growth pattern of interest may unexpectedly produce a desired response. Using this HTMS device, our goal is to identify novel molecules and pathways that not only improve the functional properties of engineered cartilage, but also intervene to enhance cartilage repair in clinically relevant joint pathology.

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Figure 1. Schematic of HTMS device

(A) The system includes an aluminum housing frame, sensor platen controlled via Instron displacement, and (B–C) well plate assembly designed for a standard 48-well culture dish with indenter platens. (D) Fully assembled HTMS device on Instron platform and complete 48 sample array of indenters in well plate assembly.

Figure 2. Sensor calibration and testing protocol

(A) Sensor circuit which measures changes in voltage due to compression and contact of sensor layers within (B) the custom matrix array sensor. (C) Example force-voltage calibration curve for one position in the matrix array sensor. (D) 3D rendering of force signal from one step of HTMS testing. (E) Schematic illustration of multi-step stress relaxation and dynamic testing protocol. (F) Example time dependent response with three applied compression steps to agarose hydrogel (in blue) and silicone rubber (in black) samples in the HTMS device. Note that the sensor can capture the viscoelastic stress relaxation behavior of hydrogel sample.

Figure 3. HTMS evaluation of biomaterials and engineered constructs

(A) Compressive modulus of various biomaterials (single sample, N=4; HTMS, N=10–11) and (B) 1% MeHA hydrogels seeded with MSCs at two densities (20 million or 60 million cells/mL, single sample and HTMS, N=7–9). Comparison between single sample testing and HTMS-derived moduli show no significant differences in mean values ($p>0.05$).

Figure 4. HTMS evaluation of cytokine-mediated degradation of engineered constructs
 (A) Treatment of MSC-seeded constructs with TNF- α results in a significant loss of mechanical properties in a concentration dependent manner that is readily captured using the HTMS device (single sample, N=4; HTMS, N=10–12). (B) Release of GAG to the media (SolGAG) correlates with the loss of mechanical integrity. $p < 0.05$: * vs Control; # vs 1ng/mL.