Artificial
Organs

Artificial Organs
33(4):318–327, Wiley Periodicals, Inc.

Real-time Monitoring of Force Response Measured in Mechanically Stimulated Tissue-Engineered Cartilage

*Orahn Preiss-Bloom, *Joseph Mizrahi, †Jennifer Elisseeff, and *Dror Seliktar

**Faculty of Biomedical Engineering, Technion—Israel Institute of Technology, Technion City, Haifa, Israel; and †Department of Biomedical Engineering, The Johns Hopkins University, Baltimore, MD, USA*

Abstract: Mechanical stimulation improves tissue-engineered cartilage development both in terms of biochemical composition and structural properties. However, the link between the compositional changes attributed to mechanical stimulation and the changing structural properties of the engineered cartilage is poorly understood. We hypothesize that transient events associated with construct stiffening can be documented and used to understand milestones in construct development. To do this, we designed and built a mechanical stimulation bioreactor that can continuously record the force response of the engineered construct in real time. This study documents the transient changes of the stiffness of tissue-engineered cartilage constructs over the first 14 days of their development under cyclic loading. Compressive strain stimulation (15%, 1 Hz) was applied to poly(ethylene glycol) (PEG) hydrogels seeded with primary articular chondrocytes. The average compressive modulus of strain-stimulated constructs was 12.7 ± 1.45 kPa after 2 weeks, significantly greater ($P < 0.01$) than the average compressive moduli of both unstimulated constructs (10.7 ± 0.94 kPa) and nonviable stimulated constructs (11.2 ± 0.91 kPa). The system was able to document that nearly all of the stiffness increase occurred over the last 2 days of the experiment, where live-cell constructs demonstrated a rapid 20% increase in force response. The system's ability to track significant increases in stiffness over time was also confirmed by Instron testing. These results present a novel view of the early mechanical development of tissue-engineering cartilage constructs and suggest that the real-time monitoring of force response may be used to noninvasively track the development of engineered tissue. **Key Words:** Chondrocytes—Poly(ethylene glycol)—Mechanical stimulation—Tissue engineering—Biomechanics.

Hyaline cartilage is an avascular tissue that lines the articular surface of long bones to provide near frictionless diarthrotic motion under physiological loading conditions (1). The unique biochemical composition and structure of hyaline cartilage, which are essential for its function, are difficult to reproduce ex vivo using tissue-engineering methodologies because the cartilage cells require both biochemical and physical cues to sustain proper tissue composition (2). Several attempts to grow tissue-engineered cartilage using chondrocytes and a biomaterial scaffold have also incorporated some form of physiological mechanical stimulation in order to recapitulate the physical environment of the articulating joint (3–5). A dynamic mechanical environment has been shown by others to be an integral part of cartilage development as well as homeostasis (6–10), and thus may improve the biochemical and mechanical properties of the engineered cartilage constructs (11).

Independent studies have demonstrated that engineered cartilage cultured under dynamic stimulation exhibits very different development patterns when compared with static controls (12–16). Generally, the biochemical and structural differences between stimulated and static constructs can be observed after a couple of weeks, including marked increases in extracellular matrix (ECM) synthesis and superior mechanical properties of the stimulated tissue. Incidentally, some of these studies reported an indirect link between construct stiffening and the accumulation of specific ECM components such as glycosaminoglycans (GAG) (15,17).

doi:10.1111/j.1525-1594.2009.00723.x

Received September 2007; revised March 2008.
Address correspondence and reprint requests to Dr. Dror Seliktar, Faculty of Biomedical Engineering, Technion—Israel Institute of Technology, Technion City, Haifa 32000, Israel. E-mail: dror@bm.technion.ac.il

Attributing the physical maturation of the construct to elevated levels of ECM associated with dynamic culture may underestimate the impact of mechanical stimulation. A more complete perspective requires some consideration for the natural breakdown of the biomaterial scaffold in this transient development process, as well as other factors that may influence the structural development of the construct, including catabolic activity and enzymatic cross-linking (18). Indeed, tissue engineering is premised on the concurrent biochemical maturation of the tissue relative to the biodegradation of the provisional scaffold matrix (19). In this regard, it is important to study the incremental development of tissue-engineered cartilage constructs under dynamic culture to identify transient developmental milestones that can be linked to chondrogenic metabolism, enzymatic activity, scaffold degradation, and other factors that may be influenced by mechanical stimulation (20).

The first step toward achieving the goal of documenting construct development is to track the incremental changes in mechanical properties of the construct during the first days and weeks in culture. This task is particularly challenging when the constructs are subjected to dynamic mechanical stimulation. For this purpose, we modified an existing bioreactor system used in our ongoing studies to determine biochemical differences in mechanically stimulated chondrocyte-seeded and stem cell-seeded hydrogels made from poly(ethylene glycol)-diacrylate (PEG-DA) (21,22). The bioreactor was outfitted with load-sensing capabilities that enable pseudo-real-time monitoring of structural changes to engineered constructs as they mature. The load sensor continuously logs the construct's resistance to physical deformations that are prescribed by the mechanical stimulation. The resistance is converted into a force response, which helps to understand how the stiffness of the construct is incrementally changing over the time course of the experiment.

In the present investigation, the force-sensing bioreactor system was practically applied to monitor the structural changes that occurred in PEG-based tissue-engineered cartilage constructs during a 14-day development period with 15% cyclic strain stimulation. The force-response data were able to identify transient structural changes to the constructs that were verified by comprehensive mechanical testing. The results were helpful in drawing conclusions about the interconnection between mechanical stimulation, the reported changes in chondrocyte-mediated ECM synthesis (21), and the changing mechanical properties of the tissue-engineered cartilage throughout the course of their first 2 weeks of development.

METHODS

Cell isolation and culture

Bovine chondrocytes were isolated from the talus of young cows by enzymatic digestion according to published methods (23). The digest was made from 0.2% collagenase solution containing 0.06 g type-II collagenase (Worthington Biochemical Corporation, Lakewood, NJ, USA) and 30 mL of high glucose Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Grand Island, NY, USA), 5% fetal bovine serum (FBS) (Gibco), and 2% penicillin-streptomycin (Biological Industries, Kibbutz Beit Haemek, Israel). The isolated chondrocytes were maintained and passaged according to standard tissue-culture techniques using culture medium containing DMEM supplemented with 20% FBS, 10 μg/mL ascorbic acid (Spectrum, Gardena, CA, USA), 1.25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Sigma-Aldrich, Steinheim, Germany), 1% nonessential amino acids (Biological Industries), 0.4 mM proline (Merck, Darmstadt, Germany), and 2% penicillin-streptomycin (Biological Industries).

Preparation of engineered constructs

The tissue-engineered cartilage constructs were prepared using second passage chondrocytes seeded in PEG-DA hydrogels. The hydrogels were prepared by suspending chondrocytes in a 10% wt/vol PEG-DA solution (10 kDA) containing 150 mM phosphate buffered saline (PBS) and 0.1% wt/vol Irgacure 2959 photoinitiator (Ciba Specialty Chemicals, Tarrytown, NY, USA). The final cell density in the solution was 30×10^6 cells/mL. Small samples of the suspension (50–60 μL) were transferred into sterile 4-mm inner diameter silicon tubes and placed under ultraviolet light (365 nm, 4–5 mW/cm^2) for 5 min (24,25). Control constructs made with nonviable cells were prepared similarly using cells treated for 10 min in a culture medium solution containing 50% dimethyl sulfoxide. PEG-only controls were made similarly without the cell suspension.

Perfusion and compression bioreactor system

A bioreactor was designed to alternate between cyclic compressive strain stimulation and culture medium perfusion of six engineered hydrogel constructs (5 mm diameter and 5.5 mm tall). The bioreactor, which was described in detail by Schmidt et al. (21), was outfitted with a miniature load cell (Fig. 1). Other minor modifications were made to the system for the purpose of this study, including the addition of a second ventilation port and a high-efficiency particulate air filter, which were added to the bioreactor

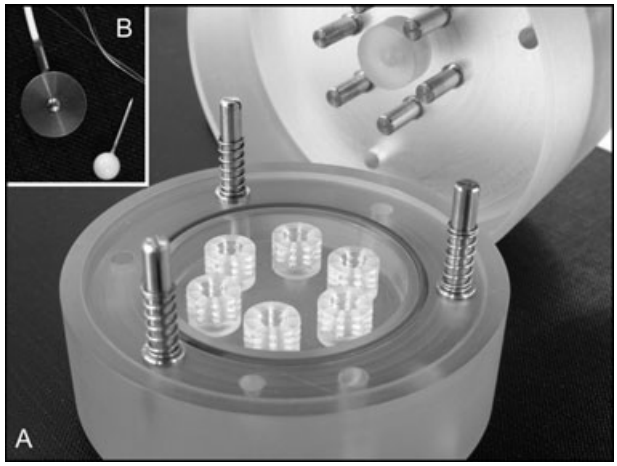

FIG. 1. A bioreactor system described by Schmidt et al. (21) for dynamic mechanical stimulation of engineered cartilage constructs (A), adapted with a miniature load cell (B).

in order to balance the forces acting on the system. A vibration-dampening mat was placed under the bioreactor during operation to reduce internal jarring of the load cell. A digital controller was used to regulate air pressure into a linear position actuator in order to position the loading piston during the loading cycle. The linear actuator was cyclically pressurized for 0.5 s and depressurized for 0.5 s.

Experimental design

The experimental design consisted of cyclic strain stimulation experiments with cellularized constructs and corresponding static control experiments. Each experiment started with first passage chondrocytes that were thawed from liquid nitrogen and dispersed into constructs after a single passage to 90% confluence. A total of twelve chondrocyte-seeded constructs were created for each 2-week experiment: six constructs in the bioreactor and six static controls. All the stimulation experiments were performed using 15% cyclic compressive strain, a duty cycle of 0.5 s on and 0.5 s off (1 Hz frequency), and an actuation pressure of 0.1 MPa. After the initial 24-h period, compressive strain stimulation was intermittently applied for 1.5 h, followed by static perfusion for 1.5 h. The experiment lasted for 14 days, with an initial 24-h static culture followed by a 13-day mechanical stimulation period.

Real-time force-response measuring

In order to facilitate real-time force-response measurements of the hydrogel constructs in the compression bioreactor system, we incorporated a 10 N load cell (Kyowa, Tokyo, Japan) into the bioreactor system as detailed later. Prior to sterilization, a stainless steel rod was attached to the load cell using a high-strength steel epoxy (Devcon, Danvers, MA, USA). A specialized assembly frame was used during epoxy application to maintain the rod in a perfectly vertical position, perpendicular to the load cell surface. In order to maintain sterility, we compressed the load cell onto a Viton O-ring as part of bioreactor assembly by means of a polycarbonate screw (load cell assembly can be viewed as part of Fig. 2). The bioreactor load cell was connected to a nearby computer by way of an A/D signal conditioning box (SC-2345, National Instruments, Austin, TX, USA). The force-response signals sensed by the load cell were recorded in real time on the attached PC using LabView software (National Instruments). In each 1.5-h period of dynamic compressive loading, short segments of force-response measurement (30 s) were recorded at seven separate intervals. The real-time force-response measurements were validated by comparisons to force-response results obtained from an Instron 5544 single-column material testing system when loading constructs to 15% strain at a duty cycle of 0.5 s on and 0.5 s off (1 Hz frequency). The Instron system was fitted with a 2530 series low-profile static load cell (5 N capacity).

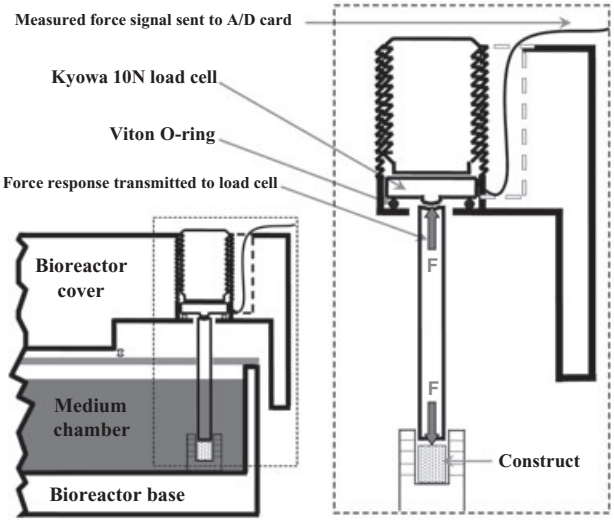

FIG. 2. Schematic illustration of the modified bioreactor with a miniature load cell. A cross-sectional view of the bioreactor shows the loading mechanism relative to the cartilage construct. The components of the mechanism of force transmission are highlighted in the insert (right), which highlights the relative position of the stainless steel loading piston, the load cell, and the construct. A Viton O-ring seals the bioreactor chamber at the load cell interface and maintains sterility of the cartilage constructs. The displacement of the bioreactor cover relative to the stationary base provides a uniform strain stimulation of 15% strain at 1 Hz frequency (21).

Force-response data processing

A process chart depicting the experimental data-collection procedure is included as Fig. 3. Force data acquisition was taken directly during the loading portion of the cyclic stimulation at a sampling frequency of 1.6 kHz. Each loading cycle was characterized by a loaded and unloaded plateau force response (see Fig. 4A). A single force-response measurement was obtained by averaging 28 individual force readings in a 30-s segment of data acquisition. The force reading in each cycle was measured in relation to the unloaded plateau value of the corresponding cycle. A force-response data point was comprised of seven force-response measurements taken at equal intervals in each 1.5-h loading period.

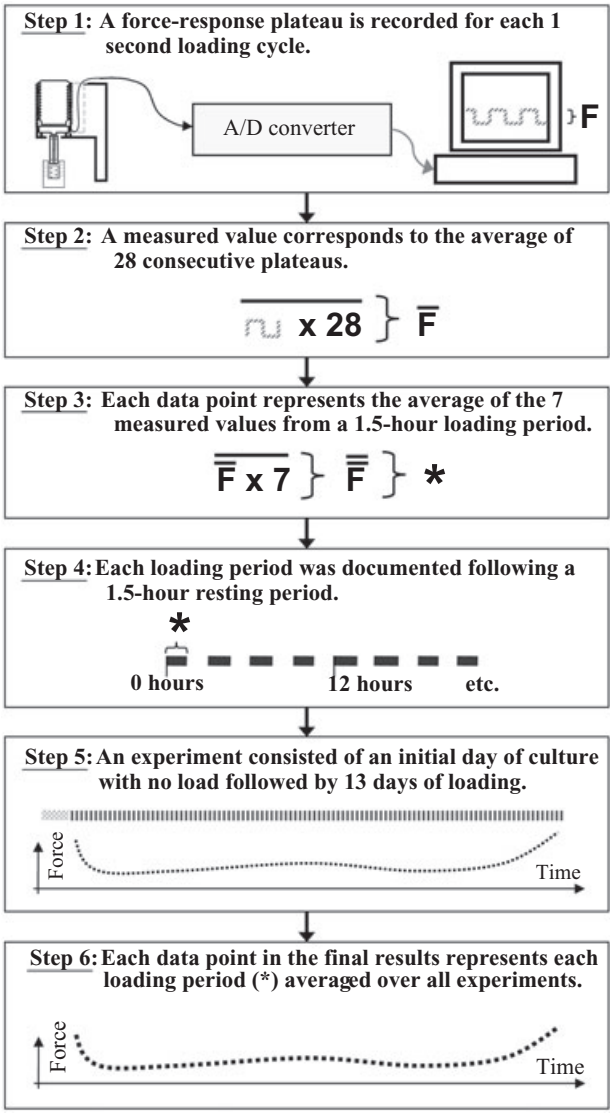

FIG. 3. Flowchart of the real-time force-response signal processing. The load cell's raw data go through a six-step process to convert it into an accurate representation of the average force of the measured construct.

Unconfined compressive testing

After each experimental period, the compressive stress–strain characteristics of the stimulated and control constructs were characterized using an Instron 5544 single-column material testing system with Merlin software (Instron Industrial Products, Grove City, PA, USA). Each construct was compressed uniaxially in physiologically buffered saline (25°C) at a rate of 2.5×10^{-3}/s to an ultimate strain of 15% while the force and displacement were digitally recorded. The raw data were converted into a stress–strain relationship, and the elastic modulus was determined directly from the linear portion of the stress–stain curve. Three groups of engineered constructs were tested at the 2-week time point, including 10% PEG-DA constructs with live cells ($n = 21$), with nonviable cells ($n = 18$), and acellular controls ($n = 8$). Treatments lasting less than 2 weeks were also characterized as follows: 10% PEG-DA constructs with live cells ($n = 6$) and constructs with nonviable cells ($n = 6$) cultured dynamically or statically for 2, 4, 6, 8, 10, and 12 days.

Statistical analysis

Quantitative data for each experiment were represented as the mean ± SD. Significant differences in the unconfined compressive modulus or force response between treatment groups were determined by two-tailed Student's *t*-test. In all cases, a P value less than 0.05 was considered statistically significant.

RESULTS

Bioreactor system validation

The bioreactor system was validated by comparing the force-response data from the load cell with the displacement of the loading piston (Fig. 4A). Each of the 1 Hz frequency loading cycles was characterized by an initial 100 ms loading spike followed by 400 ms of plateau force readings. The sample unloading consisted of an initial unloading response lasting 100 ms followed by a plateau-unloaded force reading of 400 ms in duration. The loading and unloading force data were slightly out of phase with the independent compressive strain measurements gathered using a linear variable differential transformer attached to the bioreactor lid assembly. The bioreactor force readings were compared with Instron data using identical loading conditions (Fig. 4B).

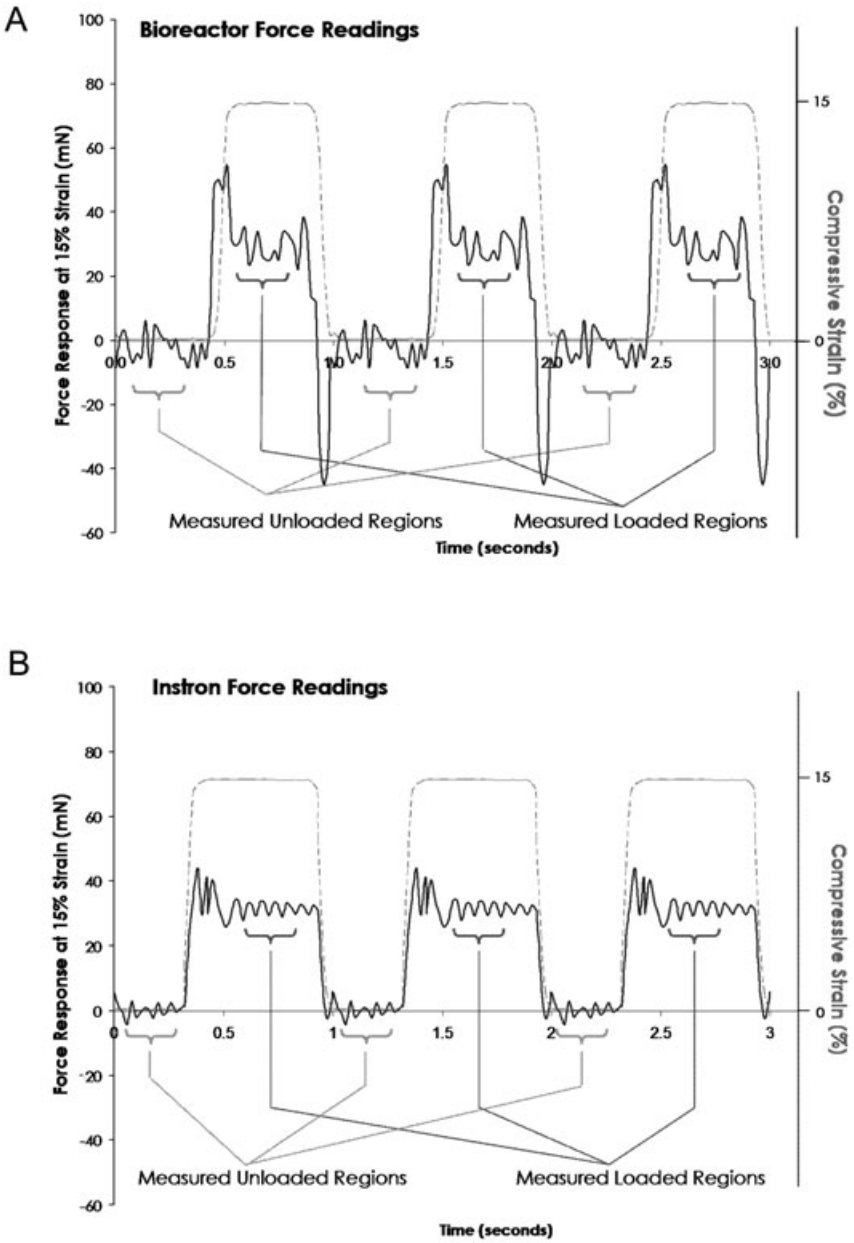

FIG. 4. Bioreactor load cell force response and strain calibration. (A) The characteristic output of the force signal from the load cell is plotted together with the compressive strain measurements of a linear variable differential transformer. The characteristic force-response signal during the dynamic loading of a tissue-engineered cartilage construct shows similarity to the Instron validation data using an identical strain loading pattern (B). The loading and unloading force-response measurement is taken directly from the region indicated on the graph to minimize the bioreactor inertial effects.

Real-time force-response measurements

The calibrated bioreactor system was used to track the force response of engineered constructs during 2-week stimulation experiments. Figure 5 shows the normalized averages of six bioreactor experiments (2 weeks) using live-cell constructs ($n = 4$) and nonviable cell constructs ($n = 2$). Both the live-cell and nonviable cell constructs exhibited an initial decrease in force response during the first 3 days of the experiment. The subsequent 9 days of the experiment exhibited relatively constant force response in both treatments. The last 2 days of the experiment demonstrated a rapid 20% increase in the force response of live-cell constructs and an unchanged force response in nonviable cell constructs.

Mechanical properties testing

In order to corroborate the overall force-response increase in the live-cell constructs, we performed a comprehensive mechanical testing on the constructs after the 2-week experimental period. The live-cell stimulated constructs demonstrated an average compressive modulus of 12.7 ± 1.45 kPa, significantly greater ($P < 0.01$) than the average compressive modulus of unstimulated control constructs (10.7 ± 0.94 kPa) (Fig. 6A). The average force response of

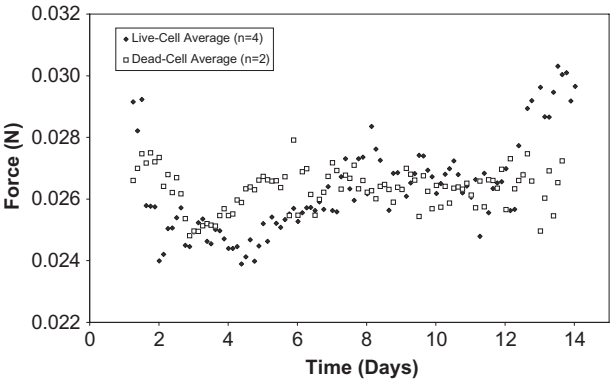

FIG. 5. The real-time force responses for live-cell constructs ($n = 4$) and dead-cell constructs ($n = 2$) during the 2-week dynamic culture experiments. The constructs were cultured in the mechanical compression bioreactor environment, and the real-time force data were measured continuously and averaged so that each point on the graph represents a 1.5-h segment of the experiment. The live-cell constructs demonstrated an increase in force response during the final 2 days of culture when compared with dead-cell controls.

live-cell stimulated constructs, measured using Instron testing, was 35 ± 3 mN, which was significantly greater ($P < 0.01$) than the average force response of 29 ± 3 mN of unstimulated control constructs (Fig. 6B). No significant differences were noted in either the nonviable cell or PEG-only control constructs when comparing stimulated and unstimulated treatments. The average modulus of the PEG-only controls on day 1 was 50.1 ± 0.55 kPa, and it was not significantly different than the modulus of the PEG-only constructs after 2 weeks in culture ($P < 0.05$). The average live-cell stimulated construct modulus and force response were also significantly greater ($P < 0.01$) than the average modulus and force response of nonviable cell stimulated constructs
(11.2 ± 0.91 kPa and 31 ± 2 mN, respectively). Conversely, the average modulus and force response of the live-cell unstimulated constructs were significantly greater ($P < 0.01$) than the average modulus and force response of the nonviable cell controls (11.6 ± 0.74 kPa and 31 ± 2 mN, respectively). The average moduli and force responses of both the stimulated (16.6 ± 0.59 kPa, 45 ± 2 mN) and unstimulated (17.2 ± 0.91 kPa, 47 ± 2 mN) PEG-only constructs were significantly greater than those of both the viable and nonviable cell-seeded constructs. Further Instron testing was performed intermittently during the course of the 2-week stimulation experiments in order to validate the real-time force-response data and confirm the timing of the increase in force response. Instron provided modulus data to supplement the force-response data obtained from the bioreactor experiments. Other than at day 14, there were no statistically significant differences in compressive modulus between each of the treatment groups at any of the other time points.

DISCUSSION

Mechanical stimulation is an essential part of the chondrocyte environment and may be necessary for successful cartilage tissue engineering. This study utilizes a novel bioreactor that enables real-time force measurements of tissue-engineered cartilage constructs in a sterile culture environment as they are subjected to dynamic mechanical stimulation in vitro. The objective of this study was to assess if such a system could document transient development of the constructs as they mature over the course of a 2-week culture period. The results underscore the practical benefits of dynamic mechanical stimulation on tissue-engineered cartilage construct development and provide detailed clues about the timing of structural and mechanical changes that occur during this process. The use of traditional mechanical testing devices to measure mechanical properties of engineered cartilage constructs can only be carried out invasively and therefore provide only end-point measurements. The novel system described herein was able to provide a continuous, noninvasive monitoring of the developmental effects of mechanical stimulation on the tissue-engineered cartilage constructs. Indeed, one of the immediate conclusions that can be drawn from this study is that real-time force data can be used to detect the beneficial effect of dynamic mechanical compression on the development of tissue-engineered cartilage after 14 days of intermittent stimulation, with the most significant increase in the force response occurring during the final 24–48 h of culture. Although the extent of such benefit in cartilage tissue engineering greatly depends on the scaffold type, the cell type, and the experimental duration, our system was able to detect even modest changes to the construct development at the earlier time points of development.

In the real-time force-feedback bioreactor system, it was not possible to perform the force-response measurements without applying mechanical stimulation to the constructs. Even the negative control group situated in the bioreactor system would be subjected to dynamic stimulation by default when obtaining the real-time force response of that group. Therefore, the negative control group used for the real-time force-response experiments was chosen in such a way that the dynamic stimulation of those

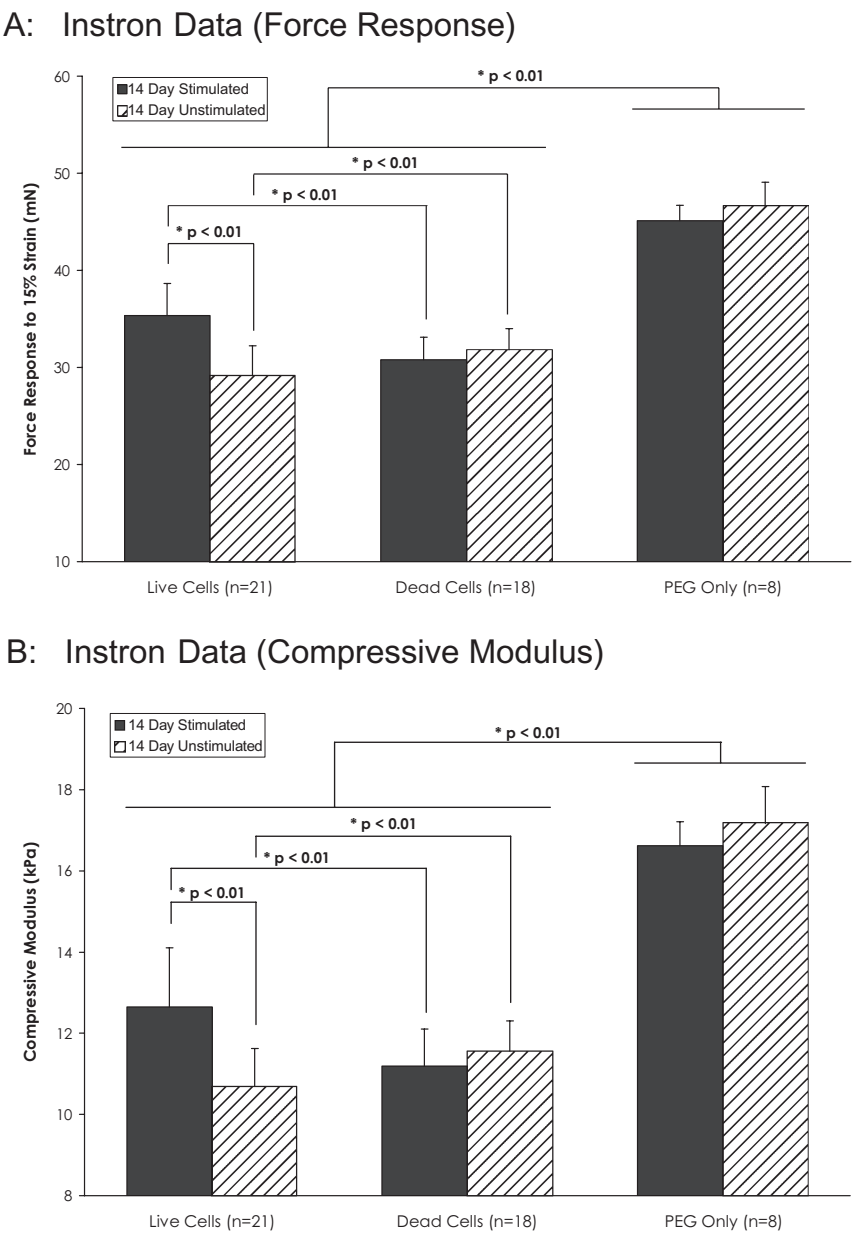

FIG. 6. Instron data of 2-week stimulated and unstimulated constructs. The force response (A) and compressive modulus (B) of the cultured constructs were determined by Instron testing to verify the real-time experimental results at the 2-week time point. The live-cell stimulated constructs demonstrated a significant increase in both their force response and compressive modulus values when compared with static and dead-cell controls. Poly(ethylene glycol) (PEG)-only stimulated constructs (acellular) did not demonstrate a significant difference in force response or compressive modulus when compared with static control.

constructs would not alter the inherent biosynthetic activity. One possible control was the PEG-only group; however, the mechanical differences between PEG-only constructs and chondrocyte-seeded constructs were significant enough to necessitate an alternate nonviable negative control for the real-time force-response experiments (26). The dead-cell constructs were used as a negative control in force-feedback experiments based on the rationale that these controls were mechanically similar to the live-cell constructs, yet their biosynthetic activity would not be altered by the cyclic mechanical stimulation inherent to the experimental system. Static controls were also a necessary part of the overall experimental design and were included in the Instron analysis for comparison with the stimulated live-cell constructs and the dead-cell controls. The unconfined uniaxial compressive testing of the specimen did demonstrate a significant difference in the modulus and force response (at 15% strain) of the stimulated live-cell constructs after 14 days relative to stimulated dead-cell constructs and to static controls. Interestingly, the dead-cell stimulated constructs and PEG-only (acellular) stimulated constructs actually had a lower modulus on average than their respective static control constructs, although these differences

were not statistically significant ($P > 0.05, n \geq 8$). This could suggest that physical perturbations to the scaffold resulting from the mechanical stimulation accelerate the deterioration of the polymer network over time (27).

An increase in the unconfined compressive modulus of the stimulated live-cell constructs must be associated with cellular remodeling, mainly with the production of new ECM that reinforces the deteriorating polymer network of the scaffold. In a previous publication, our group demonstrated that mechanical stimulation was responsible for a 37% significant increase in sulfated GAG (sGAG) content in constructs seeded with chondrocytes, relative to static controls (21). Incidentally, there was no statistically significant difference in total deoxyribonucleic acid, type-II collagen levels, or cell orientation in the mechanically stimulated constructs as compared with static controls. Other groups published similar results of increased biosynthesis by chondrocytes in response to mechanical stimulation of tissue-engineered cartilage (17,28–32). Table 1 summarizes some of these results in relation to scaffold type and mechanical loading parameters. It is therefore possible that the sGAG accumulation or other biosynthetic activity of the cells in the constructs is the primary contributor to the increased compressive modulus of the stimulated specimen.

Other studies have reported similar trends in both sGAG production and mechanical properties of dynamically cultured cartilage constructs. Mauck et al. discovered that chondrocyte-seeded constructs cultured under cyclic mechanical compression for 14 days demonstrated a significant rise in sGAG content alongside a rise in average peak stress response and aggregate modulus (12). Although their mechanics data did not prove to be statistically significant in comparison to static controls, the stress response and modulus increases of the stimulated constructs were on the same order as that reported in the present study. The similar trends reported in the various studies reinforce the hypothesis that mechanics and sGAG production are linked in some way to dynamic stimulation (15,33). It is reasonable to assume that the increased sGAG production resulting from dynamic stimulation has some effect on the stiffness of the construct, although this has yet to be proven experimentally in our system. It is also important to highlight that the other studies employed different construct materials, cell densities, and loading parameters, yet the results were very similar.

Despite some consensus in the literature regarding the effects of mechanical stimulation on engineered

TABLE 1. *Mechanical stimulation effects biosynthesis in tissue-engineered cartilage*

Scaffold	Density	Loading	Cycle	Duration	Results	Reference
Poly(ethylene glycol) hydrogel	30×10^6 cells/mL	15% strain, 1 Hz	1.5 h on 1.5 h off Continuous	2 weeks	37% increase in sGAG	Schmidt O. et al. (21)
Porous calcium polyphosphate	2×10^6 cells per construct	5% strain, 1 Hz	400 cycles every second day	4 weeks	30% increase in sGAG; 40% increase in collagen	Waldman S.D. et al. (28)
Agarose hydrogel	10×10^6 cells/mL	15% strain, 1 Hz	Continuous	48 h	40% increase in GAG RNA	Lee D.A. et al. (29)
Nonwoven polyglycolic acid	125×10^6 cells/mL	5% strain, 0.1 Hz	Continuous	24 h	Increase in GAG RNA	Davisson T. et al. (30)
Agarose hydrogel	20×10^6 cells/mL	10% strain, 1 Hz	1 h on 1 h off Three cycles per day	4 weeks	~150% increase in biosynthesis	Mauck R.L. et al. (33)
Chitosan hydrogel	40×10^6 cells/mL	5–15% strain, 0.1 Hz	0.75 h on 5.25 h off Continuous	3 weeks	fivefold increase in proteoglycan synthesis	Fehrenbacher A. et al. (31)
Oligopeptide and agarose hydrogel	15×10^6 cells/mL	2.5% strain, 1 Hz	0.75 h on 5.25 h off Every other day	22 days static and 11 days	twofold increase in proteoglycan synthesis	Kisiday J.D. et al. (32)

sGAG, sulfated glycosaminoglycans; RNA, ribonucleic acid.

cartilage, few have reported tracking the development of a construct in dynamic culture in real time. As such, most published data reflect end-point measurements that leave much uncertainty about the transient development of the constructs. In our view, it is important to understand the precise process of development in order to account for nonuniform kinetics of scaffold degradation or ECM synthesis by dynamically stimulated chondrocytes. The real-time force measurements represent a certain advance in this regard in that they provide highly detailed tracking of the mechanical changes that occur in response to dynamical stimulation, even if those structural changes are modest. It is evident from the transient force-response data that the mechanical properties of the constructs do not increase linearly, but rather, the constructs weaken over the first 3–4 days of the experiment. The initial weakening of the constructs may be attributed to the fatigue of the polymer because of the physical perturbations prescribed by the bioreactor in the aqueous environment. Although the PEG-DA hydrogels are susceptible to degradation by hydrolysis, the amount of degradation occurring in 2 weeks of culture should not significantly reduce the modulus of a 10% PEG-DA hydrogel. After this initial decline in force response, both the live- and dead-cell constructs appear to stabilize and maintain their structural stability through day 12 of the experiment. After day 12, the force measurements depict a rapid increase in the stimulated live-cell constructs to a final force value that was up to 13% higher than that of the dead-cell constructs. This was consistent with the Instron results that show stimulated live-cell constructs having an average force response that was 14.8% higher than that of the stimulated dead-cell constructs after 14 days. The rapid increase is noteworthy as it represents new insight into how mechanical stiffening of stimulated tissue-engineered cartilage constructs transpires in time.

The real-time force data presented in Fig. 5 exhibit some fluctuations that may be attributed to variability between samples and experimental runs, as well as errors introduced by the dynamic stimulation system. For example, the bioreactor lid moves at a very high rate of speed during each cycle (15% strain in 0.03 s or ~3 cm/s), with an actuation pressure driving this motion being above 100 kPa. The accelerations and decelerations of the system relative to the sensitivity of the force transducer are believed to be the main cause of the high fluctuations of the raw data. Nevertheless, using an algorithm designed to deal with these conditions (Fig. 2), we were able to provide a real-time indicator for the actual force response of each construct with a ±1 mN resolution. This resolution is sufficient to draw conclusions about the progressive enhancement of mechanical properties associated with the stimulation of the engineered cartilage. Although the bioreactor represents a suboptimal milieu for precise mechanical properties testing of engineered constructs, and the 10 N force sensor used was reaching its lower sensitivity limits, there was only a 6% discrepancy between the bioreactor force-response results and the Instron force results. This level of accuracy was also confirmed by simulations using the Instron to dynamically load the constructs as part of the validation of the system (Fig. 4). The overall trend of bioreactor and Instron force measurements during a loading cycle was similar. The bioreactor force reading contained several spikes that were associated with inertial effects of the pneumatic system, mainly the initial loading and final unloading of the piston manifold as it reaches its stationary positions. The measured loading and unloading force-response data collected did not include these spiked regions on the graph. Moreover, the absolute value of force response of the bioreactor and the Instron (at 15% strain) was very similar.

CONCLUSIONS

A dynamic stimulation bioreactor was developed with the capabilities of measuring real-time force response of mechanically stimulated cartilage constructs. Constructs made from primary chondrocytes, embedded in a PEG-based scaffold, were physically deformed dynamically for 14 days, and their development was tracked through the resistance to deformation of the construct. The force response to deformation initially declined but ultimately increased during the final 2 days of the experimental period, confirming that the system was able to document even modest structural changes to the construct. The transient changes in force response were confirmed by compressive modulus measurement and attributed to the degradation of the scaffold as well as the production of sGAG by the stimulated chondrocytes. The acellular and dead-cell control groups did not demonstrate a similar increase in force response.

Acknowledgments: The authors gratefully acknowledge the financial support of the United States–Israel Binational Science Foundation (grant number 2003184), the joint Johns Hopkins University–Technion Program for the Biomedical Sciences and Biomedical Engineering, and the support of the Israel Science Foundation (grant number 1140/04).

REFERENCES

1. Mankin HJ, Mow VC, Buckwalter JA, Iannotti JP, Ratcliffe A. Form and function of articular cartilage. In: Simon SR, ed. *Orthopaedic Basic Science*. Roseville, IL: American Academy of Orthopaedic Surgeons, 1994;1–44.
2. Silver FH, Siperko LM. Mechanosensing and mechanochemical transduction: how is mechanical energy sensed and converted into chemical energy in an extracellular matrix? *Crit Rev Biomed Eng* 2003;31:255–331.
3. Kuo CK, Li WJ, Mauck RL, Tuan RS. Cartilage tissue engineering: its potential and uses. *Curr Opin Rheumatol* 2006; 18:64–73.
4. Hunter CJ, Imler SM, Malaviya P, Nerem RM, Levenston ME. Mechanical compression alters gene expression and extracellular matrix synthesis by chondrocytes cultured in collagen I gels. *Biomaterials* 2002;23:1249–59.
5. Hunter CJ, Levenston ME. Maturation and integration of tissue-engineered cartilages within an in vitro defect repair model. *Tissue Eng* 2004;10:736–46.
6. Grodzinsky AJ, Levenston ME, Jin M, Frank EH. Cartilage tissue remodeling in response to mechanical forces. *Annu Rev Biomed Eng* 2000;2:691–713.
7. Quinn TM, Grodzinsky AJ, Buschmann MD, Kim YJ, Hunziker EB. Mechanical compression alters proteoglycan deposition and matrix deformation around individual cells in cartilage explants. *J Cell Sci* 1998;111(Pt 5):573–83.
8. Sah RL, Doong JY, Grodzinsky AJ, Plaas AH, Sandy JD. Effects of compression on the loss of newly synthesized proteoglycans and proteins from cartilage explants. *Arch Biochem Biophys* 1991;286:20–9.
9. Sah RL, Grodzinsky AJ, Plaas AH, Sandy JD. Effects of tissue compression on the hyaluronate-binding properties of newly synthesized proteoglycans in cartilage explants. *Biochem J* 1990;267:803–8.
10. Sah RL, Kim YJ, Doong JY, Grodzinsky AJ, Plaas AH, Sandy JD. Biosynthetic response of cartilage explants to dynamic compression. *J Orthop Res* 1989;7:619–36.
11. Freed LE, Guilak F, Guo XE, et al. Advanced tools for tissue engineering: scaffolds, bioreactors, and signaling. *Tissue Eng* 2006;12:3285–305.
12. Mauck RL, Soltz MA, Wang CC, et al. Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. *J Biomech Eng* 2000;122: 252–60.
13. Demarteau O, Jakob M, Schafer D, Heberer M, Martin I. Development and validation of a bioreactor for physical stimulation of engineered cartilage. *Biorheology* 2003;40: 331–6.
14. Seidel JO, Pei M, Gray ML, Langer R, Freed LE, Vunjak-Novakovic G. Long-term culture of tissue engineered cartilage in a perfused chamber with mechanical stimulation. *Biorheology* 2004;41:445–58.
15. Kelly TA, Wang CC, Mauck RL, Ateshian GA, Hung CT. Role of cell-associated matrix in the development of free-swelling and dynamically loaded chondrocyte-seeded agarose gels. *Biorheology* 2004;41:223–37.
16. Hunter CJ, Levenston ME. The influence of repair tissue maturation on the response to oscillatory compression in a cartilage defect repair model. *Biorheology* 2002;39:79–88.
17. Mauck RL, Seyhan SL, Ateshian GA, Hung CT. Influence of seeding density and dynamic deformational loading on the developing structure/function relationships of chondrocyte-seeded agarose hydrogels. *Ann Biomed Eng* 2002;30:1046–56.
18. De Croos JN, Dhaliwal SS, Grynpas MD, Pilliar RM, Kandel RA. Cyclic compressive mechanical stimulation induces sequential catabolic and anabolic gene changes in chondrocytes resulting in increased extracellular matrix accumulation. *Matrix Biol* 2006;25:323–31.
19. Lu L, Zhu X, Valenzuela RG, Currier BL, Yaszemski MJ. Biodegradable polymer scaffolds for cartilage tissue engineering. *Clin Orthop Relat Res* 2001;October(Suppl 391):S251–70.
20. Nesic D, Whiteside R, Brittberg M, Wendt D, Martin I, Mainil-Varlet P. Cartilage tissue engineering for degenerative joint disease. *Adv Drug Deliv Rev* 2006;58:300–22.
21. Schmidt O, Mizrahi J, Elisseeff J, Seliktar D. Immobilized fibrinogen in PEG hydrogels does not improve chondrocyte-mediated matrix deposition in response to mechanical stimulation. *Biotechnol Bioeng* 2006;95:1061–9.
22. Terraciano V, Hwang N, Moroni L, et al. Differential response of adult and embryonic mesenchymal progenitor cells to mechanical compression in hydrogels. *Stem Cells* 2007;25: 2730–8.
23. Elisseeff J, Anseth K, Sims D, et al. Transdermal photopolymerization of poly(ethylene oxide)-based injectable hydrogels for tissue-engineered cartilage. *Plast Reconstr Surg* 1999;104: 1014–22.
24. Lum LY, Elisseeff J. Injectable hydrogels for cartilage tissue engineering. In: Ashammakhi N, Ferretti P, eds. *Topics in Tissue Engineering*. Oulu University Hospital, Oulu, Finland, 2003; Ch. 4, 1–25. e-book.
25. Williams CG, Malik AN, Kim TK, Manson PN, Elisseeff JH. Variable cytocompatibility of six cell lines with photoinitiators used for polymerizing hydrogels and cell encapsulation. *Biomaterials* 2005;26:1211–8.
26. Bryant SJ, Anseth KS. Hydrogel properties influence ECM production by chondrocytes photoencapsulated in poly(ethylene glycol) hydrogels. *J Biomed Mater Res* 2002;59:63–72.
27. Bryant SJ, Anseth KS, Lee DA, Bader DL. Crosslinking density influences the morphology of chondrocytes photoencapsulated in PEG hydrogels during the application of compressive strain. *J Orthop Res* 2004;22:1143–9.
28. Waldman SD, Spiteri CG, Grynpas MD, Pilliar RM, Kandel RA. Long-term intermittent compressive stimulation improves the composition and mechanical properties of tissue-engineered cartilage. *Tissue Eng* 2004;10:1323–31.
29. Lee DA, Bader DL. Compressive strains at physiological frequencies influence the metabolism of chondrocytes seeded in agarose. *J Orthop Res* 1997;15:181–8.
30. Davisson T, Kunig S, Chen A, Sah R, Ratcliffe A. Static and dynamic compression modulate matrix metabolism in tissue engineered cartilage. *J Orthop Res* 2002;20:842–8.
31. Fehrenbacher A, Steck E, Roth W, Pahmeier A, Richter W. Long-term mechanical loading of chondrocyte-chitosan biocomposites in vitro enhanced their proteoglycan and collagen content. *Biorheology* 2006;43:709–20.
32. Kisiday JD, Jin M, DiMicco MA, Kurz B, Grodzinsky AJ. Effects of dynamic compressive loading on chondrocyte biosynthesis in self-assembling peptide scaffolds. *J Biomech* 2004;37:595–604.
33. Mauck RL, Wang CC, Oswald ES, Ateshian GA, Hung CT. The role of cell seeding density and nutrient supply for articular cartilage tissue engineering with deformational loading. *Osteoarthritis Cartilage* 2003;11:879–90.