TISSUE ENGINEERING
Volume 13, Number 6, 2007

DOI: 10.1089/ten.2006.0339

Mechanical Stimulation Increases Collagen Type I and Collagen Type III Gene Expression of Stem Cell–Collagen Sponge Constructs for Patellar Tendon Repair

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ABSTRACT

Our group has shown that mechanical stimulation increases the stiffness of stem cell–collagen sponge constructs at 14 days in culture and subsequent rabbit patellar tendon repairs at 12 weeks postsurgery. What remains unclear is which genes might be responsible for this increase in stiffness. Therefore, the objective of this study was to determine how a tensile stimulus affects the gene expression of stem cell–collagen sponge constructs used to repair rabbit central patellar tendon defects. Tissue-engineered constructs were created by seeding mesenchymal stem cells (MSCs) from 10 adult rabbits at 0.14×10^6 cells/construct in type I collagen sponges. Half of the constructs were mechanically stimulated once every 5 min for 8 h/d to a peak strain of 2.4% for 2 weeks. The other half remained in an incubator without mechanical stimulation for 2 weeks. After 14 days in culture, half of the stimulated and nonstimulated constructs were prepared to determine the expression of collagen type I, collagen type III, decorin, fibronectin, and glyceraldehyde-3-phosphate dehydrogenase genes using real-time quantitative reverse transcriptase polymerase chain reaction. The remaining constructs were mechanically tested to determine their mechanical properties. Two weeks of *in vitro* mechanical stimulation significantly increased collagen type I and collagen type III gene expression of the stem cell–collagen sponge constructs. Stimulated constructs showed 3 and 4 times greater collagen type I ($p = 0.0001$) and collagen type III gene expression ($p = 0.001$) than nonstimulated controls. Stimulated constructs also had 2.5 times the linear stiffness and 4 times the linear modulus of nonstimulated constructs. However, mechanical stimulation did not significantly increase decorin or fibronectin gene expression ($p = 0.2$) after 14 days in culture. This study shows that mechanical stimulation of cell–sponge constructs produces similar increases in the expression of 2 structural genes, as well as linear stiffness and linear modulus.

INTRODUCTION

TENDON STRUCTURES are frequently injured during sports and other rigorous activities, requiring varied treatment approaches. Significant dysfunction and disability can result from incomplete healing of these load-bearing structures. Partial tendon ruptures are often treated conservatively, although chronic partial ruptures usually require surgery.[1] Repair is recommended in part due to the large and impulsive forces that the tendon's in-series muscle can deliver. Currently, surgeons repair the failed tendon ends directly or fill larger defects with autografts, allografts, xenografts, synthetic polymers, or resorbable biomaterials.[2–7] Although autografts are usually preferred to avoid rejection, donor site morbidity is a common problem.[8,9] Allografts and xenografts offer biological alternatives but remain at risk of

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tissue rejection.[3,10] Donor tissue scarcity can also be a problem with allografts. Synthetic materials are readily available but also pose problems of inflammation and rejection. Given the varied success of these procedures, orthopedic scientists have been exploring the emerging field of tissue engineering, particularly for damaged and diseased tissues with limited healing capacity. Tissue engineering offers certain advantages because it combines principles of engineering and biology to enhance the repair outcome.[11,12]

Our group has been using tissue engineering to create and mechanically stimulate mesenchymal stem cell–collagen gel–collagen sponge constructs in culture to accelerate repair of central-third defects in the rabbit patellar tendon (PT). We initially found that stimulating these constructs increased the resulting repair linear stiffness and linear modulus to as much as 80% and 40% of normal PT values at 12 weeks postsurgery, respectively.[13] Stimulated repairs also match the tangent stiffness of normal PT not only up to peak *in vivo* forces recorded during inclined hopping (100 N),[14] but to 25% beyond this level (125 N). When the scaffold was replaced with a commercial collagen sponge and the gel was removed, the stimulated stem cell–collagen sponge repairs matched the tangent stiffness of normal PT up to 50% beyond peak *in vivo* force levels for inclined hopping (150 N)[14,15] at 12 weeks postsurgery. Maximum force, linear stiffness, maximum stress, and linear modulus for the stimulated (versus nonstimulated) repairs averaged 70% (versus 55%), 85% (versus 55%), 70% (versus 50%), and 50% (versus 40%) of corresponding values for the normal central third of the PT. This stimulus also significantly increased the stiffness of these stem cell–collagen sponge constructs at 14 days in culture and the stiffness of the subsequent rabbit patellar tendon repairs at 12 weeks postsurgery.[15]

Although it is reasonable to speculate that improvements in tensile stiffness of tissue-engineered constructs are related to synthesis and assembly of fibrillar collagens, little is known about which genes are up-regulated to effect such changes. Altman *et al.* showed increases in collagen type I, collagen type III, and tenascin-C gene expression when bovine stem cells were seeded on collagen gel and then exposed to 21 days of mechanical stimulation in culture.[16] However, this study did not report corresponding changes in the mechanical properties of these constructs, nor did it examine how the mechanical signal affected other fibrillar assembly genes important to the creation of load-bearing structures.

Thus, the purpose of this study was to determine how a controlled mechanical stimulus applied to a stem cell–collagen sponge construct in culture influences the expression of two fibrillar genes (collagen types I and III) and two fibrillar assembly genes (decorin and fibronectin), as well as linear stiffness and linear modulus. We hypothesized that 14 days of mechanical stimulation would increase collagen type I, collagen type III, decorin, and fibronectin gene expression, as well as linear stiffness and linear modulus.

EXPERIMENTAL DESIGN

Ten skeletally mature, female New Zealand white (NZW) rabbits (Myrtles Rabbitry, Thompson Station, TN) were purchased (weighing 4.6 ± 0.3 kg; mean $\pm$ standard error of the mean) for the current *in vitro* study. Bone marrow biopsies were performed from which autologous mesenchymal stem cells (MSCs) were isolated and expanded.[17] Sample size was calculated to achieve a power of 85% to detect differences if they existed. All constructs ($n = 4$ for each animal) were created by seeding these MSCs (0.14×10^6 cells/construct) in a type I collagen sponge (see details below). Half of the constructs ($n = 2$ for each animal) were then mechanically stimulated, and the other half remained in an incubator without stimulation for 2 weeks. After 14 days in culture, half of the stimulated (S) and nonstimulated (NS) constructs were prepared to evaluate changes in the expression of collagen type I, collagen type III, decorin, fibronectin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes using real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR). The remaining constructs were mechanically tested to determine their structural and material properties.[15]

MATERIALS

Mesenchymal stem cells

MSCs were isolated from the iliac crest and processed as previously described.[18,19] Briefly, cells were plated at 22×10^6 cells per 100-mm dish, placed in an incubator, and fed with growth medium formulated from 93% advanced Dulbecco's modified Eagle medium (ADV-DMEM, Gibco BRL/Life Technologies Inc., Gaithersburg, MD), 1% antibiotic/antimycotic (Gibco), 1% glutamax (Gibco), and 5% fetal bovine serum of selected lots (Atlanta Biologics, Norcross, GA) for 12 to 14 days to optimize the cell growth rate.[18,19] MSCs proliferated to form colonies between 6 and 8 days in primary culture. After cells reached confluency, they were retrieved, counted, and subcultured again to passage 2.

Scaffold and construct preparation

Kensey Nash Corporation (Exton, PA) provided collagen type I sponges (P1076 collagen pads). The sponges were sterilized using a minimum of 25 kGy of gamma irradiation. Each sponge (94% pore volume; 62 μm mean pore diameter) was then cut to fit in the base of each of 4 wells in a silicone dish containing 2 restraining posts protruding from the base (Fig. 1).[15,19] Two 4-mm-diameter holes were also created, permitting each sponge to be positioned in the base of each well.[15] The length, width, and thickness of the scaffolds averaged 23 ± 0.8 mm, 9 ± 0.8 mm, and 3 ± 0.1 mm (mean $\pm$ standard deviation), respectively.

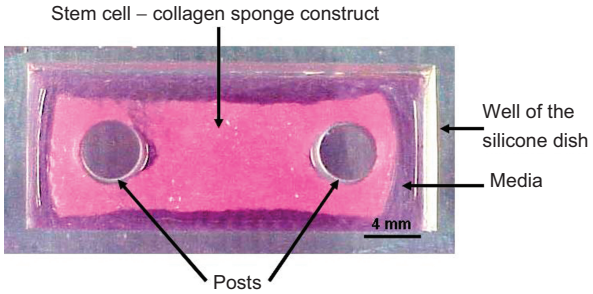

FIG. 1. Stem cell–collagen sponge construct in one of the wells of the silicone dish after 14 days in culture.[15] Color images available online at www.liebertpub.com/ten.

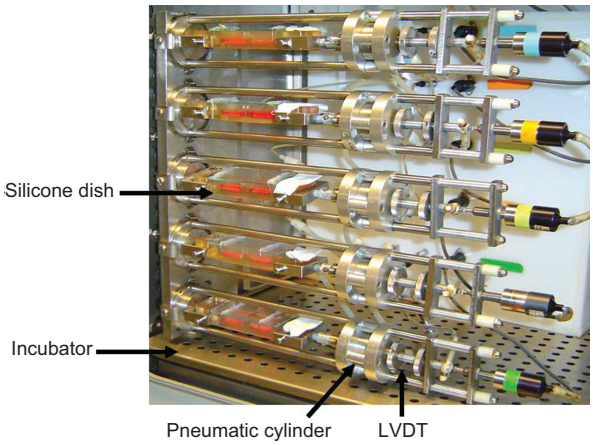

FIG. 2. Mechanical stimulation system showing 5 stations in the incubator. Each station contains a silicone dish, a pneumatic cylinder to stretch the dish, and a linear variable differential transducer to monitor the end-to-end displacement of the dish.[15] Color images available online at www.liebertpub.com/ten.

Each construct was formed by suspending 0.14×10^6 cells in 0.4 mL of ADV-DMEM. The mixture was placed on top of the collagen type I sponge in the bottom of each well in the dish (Fig. 1). Inspection under a fluorescence microscope (Axiovert 25, Carl Zeiss Inc., Göttingen, Germany) revealed that the cells penetrated uniformly into the interstices of the sponge.[15] All S and NS constructs were placed in an incubator (Steri-Cult Model 3033, Forma Scientific, Mariette, OH; 37°C, 5% carbon dioxide, 95% relative humidity) for 2 weeks and fed 3 times weekly with ADV-DMEM, 5% ascorbic acid, 1% antibiotic/antimycotic, 1% glutamax, and 5% fetal bovine serum.

METHODS

Mechanical stimulation

After 2 days of incubation, the silicone dishes containing the constructs to be stimulated were placed into a pneumatic mechanical stimulation system (Fig. 2). The computer-controlled system consists of 5 stations mounted within an incubator (Steri-Cult Model 3033, Forma Scientific). The system allows for control of strain and time parameters (amplitude, frequency, rest period between cycles) and collects displacement data from linear variable differential transducers (LVDTs). Each station contains a silicone dish, a pneumatic cylinder to stretch the dish, and a LVDT to monitor the end-to-end displacement of the dish. Post-to-post displacement was previously calibrated with respect to end-to-end displacement of the dish.[20] The constructs were stretched once every 5 min for 8 h/d to a peak amplitude of 2.4% strain. These loading conditions were used because it has been previously shown that they increase the construct stiffness at 14 days in culture and the repair biomechanics at 12 weeks postsurgery.[15] After 12 days, the dishes were removed from mechanical stimulation, half of the constructs were prepared for evaluation of gene expression using real-time quantitative RT-PCR, and the other half were failed in tension.

Biomechanical evaluation of the constructs

The constructs for biomechanical evaluation were placed in cryovials and stored at –80°C for no more than 3 days. On the day of testing, constructs were thawed, and each end of the construct containing the post hole was covered with gauze. The gauze sections were placed into custom grips attached to an electromechanical testing system (100R6, TestResources Inc., Shakopee, MN), and photographs were taken to determine the length, width, and thickness of the construct (from which area was computed). Each construct was placed in a chamber of phosphate buffered saline (pH 7.4, 25°C) and failed in tension at a constant strain rate of 10%/s while continuously recording force and grip-to-grip elongation. The force-elongation and stress-strain curves were plotted to determine maximum force, linear stiffness, maximum stress, and linear modulus.

Ribonucleic acid extraction and conventional gene expression analysis

The constructs for gene expression analysis were treated with RNAlater (QIAGEN Inc., Valencia, CA) for 6 h, quick frozen with liquid nitrogen, and taken to –80°C to prevent Ribonucleic acid (RNA) degradation. RNA from each construct was extracted using a 0.45-μm centrifuge filter (Millipore, Billerica, MA) and an RNeasy mini kit (QIAGEN Inc.). First-strand complementary deoxyribonucleic acid (cDNA) was generated using a reverse transcriptase (RT) reaction (MuLV reverse transcriptase, Applied Biosystems, Foster City, CA). The conventional RT reaction was performed according to the following protocol: 25°C for 10 min, 42°C for 15 min, 99°C for 5 min, 5°C for 5 min. Rabbit-specific primers were used for collagen type I,

collagen type III, decorin, fibronectin, and GAPDH. The detailed forward and reverse primer sequences and the resultant products are summarized in Table 1.[21,22] Before use in the experiment, all primers were extensively tested under conventional and real-time quantitative RT-PCR conditions. The conventional PCR of the reverse-transcribed RNA was performed according to the following protocol: denaturation at 94°C for 20 s; annealing at 60°C for 30 s; and extension at 72°C for 30 s, 35 cycles. The amplified products were verified using 2% agarose gel electrophoresis in Tris-acetate-ethylenediaminetetraacetic acid and SYBR safe DNA gel stain (Invitrogen Molecular Probes, Eugene, OR).

Real-time quantitative RT-PCR

To quantify messenger RNA levels of the genes, real-time quantitative RT-PCR was performed with a continuous fluorescence detector (DNA Engine Opticon 2 System, MJ Research Inc., Waltham, MA) by monitoring SYBR Green fluorescent dye (SYBR Green PCR master mix, Applied Biosystems, Foster City, CA) bound to double-strand DNA. The 50-μL reaction included 25 μL of X SYBR Green PCR master mix, 6 μL of primer mix (5 μM), 14 μL of RNAase-free water, and 5 μL of cDNA (40 ng/μL) template. Real-time quantitative RT-PCR was run according to the following protocol: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. All samples were run in duplicate because differences in CT (cycle number) values between samples were less than 0.3 cycles. Each PCR product was verified according to fragment size on agarose gel. Standard curves were created for each target gene to quantify the gene expression for each cell–sponge construct. To obtain standard curves for each gene, PCR products were cloned into a plasmid (pCR®2.1-TOPO®, TOPO TA cloning® kits, Invitrogen), and dilutions of each gene cDNA in a range from 1 to 10^{-4} ng were subjected to real-time quantitative RT-PCR analysis. The absolute amount of the corresponding gene messenger RNA contained in each sample was obtained by deduction from the corresponding gene standard curve. The gene expression was normalized by calculating the ratio between each target gene and GAPDH for each sample.

Statistical analysis

The duplicate gene expression measures for each rabbit were averaged before statistical analysis. Gene expression and mechanical properties of the S versus NS stem cell–collagen sponge constructs were compared using a paired Student *t*-test. All conclusions regarding the significance of mechanical stimulation on gene expression and mechanical properties were made at the $\alpha = 0.05$ experiment-wise level.

RESULTS

Two weeks of *in vitro* mechanical stimulation significantly increased collagen type I and collagen type III gene expression of stem cell–collagen sponge constructs. S constructs had 3 times greater collagen type I gene expression ($p < 0.001$) than NS controls and 4 times greater collagen type III gene expression ($p = 0.001$) (Fig. 3A). Collagen type I gene expression for the S and NS construct groups averaged 11.6 ± 3.8 and 3.8 ± 1.3 (mean $\pm$ SD), respectively, and collagen type III gene expression averaged 17.3 ± 7.1 and 4.3 ± 1.8 (mean $\pm$ SD), respectively. However, mechanical stimulation did not significantly increase decorin, fibronectin, and GAPDH gene expression ($p = 0.2$) after 14 days in culture. Decorin gene expression for the S and NS construct groups averaged 3.5 ± 3.9 and 3.5 ± 5.8 (mean $\pm$ SD), respectively, and fibronectin gene expression averaged 0.2 ± 0.2 and 0.1 ± 0.1 (mean $\pm$ SD), respectively (Fig. 4). GAPDH gene expression for the S and NS construct groups averaged 0.0003 ± 0.0001 and 0.0003 ± 0.00007 (mean $\pm$ SD), respectively.

Although mechanical stimulation did not significantly alter construct dimensions after 14 days in culture (Table 2, $p > 0.05$), the treatment increased 4 mechanical parameters. Two weeks of *in vitro* mechanical stimulation significantly increased construct maximum force, linear stiffness, maximum stress, and linear modulus (Tables 2, $p = 0.002$). The S constructs had 2.5 times the linear stiffness (Figure 3B) and 4 times the linear modulus (Figure 3C) of the NS constructs ($p = 0.002$). Linear stiffness and linear modulus were chosen as outcome measures because we had previously showed

Table 1. Sequence of Primers Used for Gene Expression Analysis and Product Size in Base Pairs

Gene	*Primer sequence*	*Product size (bp)*	*Primer source*
Collagen I	GAT GCG TTC CAG TTC GAG TA GGT CTT CCG GTG GTC TTG TA	312	Boykiew *et al* [21]
Collagen III	TTA TAA ACC AAC CTC TTC CT TAT TAT AGC ACC ATT GAG AC	255	Boykiew *et al* [21]
Decorin	TGT GGA CAA TGG TTC TCT GG CCA CAT TGC AGT TAG GTT CC	419	Boykiew *et al* [21]
Fibronectin	TCG GGA GGA AGA AGA CAG ATG AGC ACC ACT GCC AAA GCC TAA GCA C	767	Guehring *et al* [22]
GAPDH	TCA CCA TCT TCC AGG AGC GA CAC AAT GCC GAA GTG GTC GT	293	Boykiew *et al* [22]

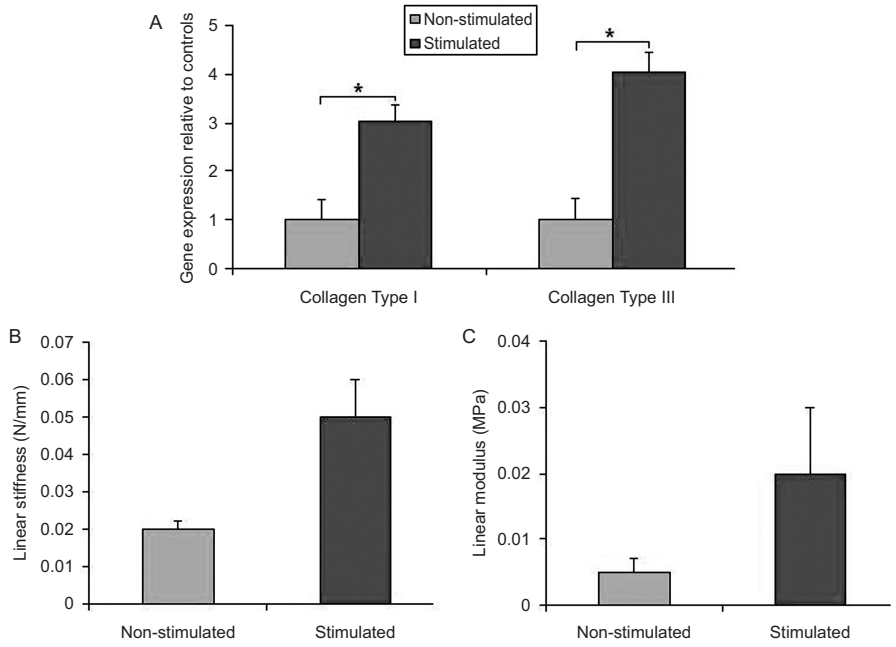

FIG. 3. Stimulated constructs showed significantly higher collagen type I ($p < 0.001$) and collagen type III ($p = 0.001$) gene expression relative to controls than did nonstimulated constructs after 14 days in culture ($n = 10$; mean ± standard deviation (SD)). * Significantly different from nonstimulated construct values (**A**). Stimulated constructs showed significantly higher linear stiffness (**B**) and linear modulus (**C**) than nonstimulated constructs after 14 days in culture ($n = 10$; mean ± SD; $p = 0.002$).

that both measures increased significantly with mechanical stimulation at 14 days in culture and correlated with repair stiffness and modulus at 12 weeks postsurgery.[15] Constructs from different cell donors showed similar gene expression and mechanical properties in each group.

DISCUSSION

Although numerous surgical approaches are available to treat tendon and ligament injuries, surgeons routinely encounter problems with the quality of direct repairs, availability of autograft tissues, potential disease transmission from allografts, and inflammation and long-term biocompatibility problems with synthetic implants. Such challenges make tissue engineering of cell–scaffold constructs an attractive alternative for improving tendon and ligament repair. Ideally, such constructs should possess sufficient initial mechanical properties to support rapid restoration of joint mobility and loading to prevent formation of tissue adhesions

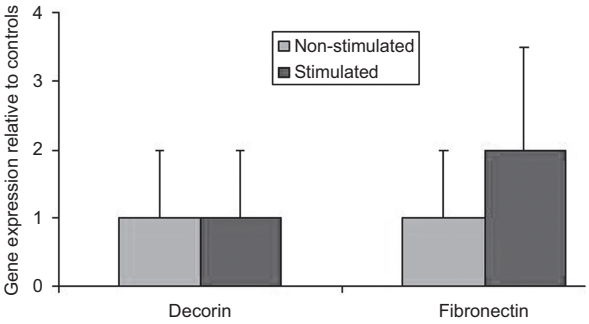

FIG. 4. Stimulated constructs showed similar decorin and fibronectin gene expression relative to controls as nonstimulated constructs after 14 days in culture ($n = 10$; mean ± SD; $p = 0.2$).

TABLE 2. STIMULATED AND NONSTIMULATED CONSTRUCT DIMENSIONS AND MECHANICAL PROPERTIES AFTER 14 DAYS IN CULTURE (MEAN ± STANDARD DEVIATION)

	Stimulated construct n = 10	*Non-stimulated construct n = 10*
Dimensions		
Thickness (mm)	2.1 ± 0.1	2.1 ± 0.3
Width (mm)	5.0 ± 0.5	5.1 ± 0.7
Length (mm)	21.2 ± 0.7	21.2 ± 0.5
Structural and mechanical properties		
Maximum Force (N)	0.11 ± 0.03*	0.08 ± 0.02
Linear Stiffness (N/mm)	0.05 ± 0.02*	0.02 ± 0.01
Maximum Stress (MPa)	0.005 ± 0.002*	0.003 ± 0.001
Linear Modulus (MPa)	0.02 ± 0.01*	0.005 ± 0.002

*Significantly different from non-stimulated construct values, $p = 0.002$.

and limit atrophy of surrounding muscle and bone. Two strategies that are currently under investigation are immediate implantation of cell–scaffold constructs with subsequent remodeling under *in vivo* loads[23] and *in vitro* mechanical preconditioning of cell–scaffold constructs before implantation.[15] Regardless of approach, there remains a fundamental need to understand how external loads applied to these constructs alter gene expression and protein production to ultimately improve construct and repair mechanical properties.

This study was primarily designed to determine how mechanical stimulation affects gene expression of stem cell–collagen sponge constructs used to repair central defects in rabbit patellar tendon. The mechanically induced increases we measured in the expression of 2 fibrillar genes (collagen type I and collagen type III) after 14 days in culture (Fig. 3A) emphasize the benefit of mechanical stimulation of cell-based constructs before construct implantation.

A second objective of this study was to determine how changes in gene expression of collagen types I and III relate to changes in the construct linear stiffness and linear modulus after mechanical stimulation. We chose to study only the relationships between gene expression and these subfailure parameters even though we had previously found that both measures increased significantly with mechanical stimulation and correlated with repair stiffness and modulus postsurgery.[15] Two weeks of mechanical stimulation significantly increased the construct linear stiffness (Fig. 3B) and linear modulus (Fig. 3C) by approximately the same amount (2.5–4 times) as the increases we observed in collagen type I and collagen type III gene expression (3–4 times). No statistically significant correlation was observed between collagen type I gene expression and linear stiffness. However, it is important to recognize that these observed changes in gene expression must first alter protein expression and then result in assembly before effecting any improvements in the biomechanical integrity of the structure. The exact temporal relationships between gene expression, protein production, and biomechanical improvements must still be elucidated.

The results of this study are consistent with the findings of 3 previous studies on the up-regulation of collagen type I and collagen type III gene expression. Unfortunately, these studies are difficult to compare because of different cell types, cell source, bioreactors, and mechanical stimulation signals. In one study, bovine stem cells seeded on collagen gel showed an increase in collagen type I and collagen type III gene expression when exposed to mechanical stimulation for 21 days in culture.[16] In that study, translational strain (10%, 2 mm) and rotational strain (25%, 90°) were applied concurrently at a frequency of 0.0167 Hz to collagen gels seeded with stem cells. However, the mechanical properties of the constructs were not reported in that study. Our study also demonstrated increases in collagen type I and collagen type III gene expression after 14 days in culture, but we also showed corresponding increases in the construct linear stiffness and linear modulus due to mechanical stimulation. Two other studies also found increases in collagen type I and collagen type III gene expression, but these investigators stimulated human anterior cruciate ligament fibroblasts in monolayer culture using sinusoidal rather than trapezoidal profiles and for only 24 h in culture.[24,25] Furthermore, these studies also performed these cyclic oscillations to 5% strain at 1 Hz[24] and 10% peak strain at 0.167 Hz,[25] making direct comparisons of results difficult.

Alterations in cellular activity due to mechanical stimulation provide an adaptive capability for a tendon or ligament to withstand physiological loads for different activities. Collagen type I is the primary protein responsible for resisting these loads, whereas collagen type III is generally thought to be involved in the early stages of tendon and ligament healing. In the early stages of tendon healing, the ratio of collagen type III to collagen type I increases and later in the healing process decreases.[26,27] However, proteoglycans are also believed to be critical to maintaining function in these tensile structures. Decorin, for example, is the predominant proteoglycan in the tensile region of tendons. Decorin mediates the process of fibrillogenesis, transmits forces between fibrils, and regulates the physiological remodeling of collagen.[28] By contrast, fibronectin is known to serve as the mediator of cellular adhesions and post-translational collagen fibril modifications and assembly.[29] The absence of mechanically induced changes in decorin and fibronectin gene expression might mean that these two proteins are later-term events in culture or do not occur until after surgical implantation or that our mechanical stimulus did not favor such up-regulation. Further studies are needed to identify which of these explanations might be valid or whether other mechanisms might explain this lack of effect.

Further studies are also needed to overcome certain limitations in our study. 1) All constructs were evaluated at only one time point in culture (14 days), with one loading condition (2.4% peak strain). Future studies need to evaluate gene expression of these constructs at earlier and later time points and with different loading conditions. 2) The peak applied strain (2.4%) was lower than the amplitude of the largest estimated *in vivo* strain signal (4% strain) calculated from one of our previous studies.[14] Although the rather simple waveform we imposed produced increases in collagen type I and collagen type III gene expression, other signals might further optimize gene expression. 3) Protein production was not determined in this study. Future work needs to focus on finding temporal relationships between gene expression, protein production, and final mechanical properties. 4) Because the aspect ratio for the *in vitro* constructs was 2–1 (less than the ideal 3–1 length-to-width ratio[30]), end effects could have played a role in the *in vitro* mechanical properties. However, low aspect ratio has been shown to have greater effects on failure properties than subfailure properties like linear stiffness and modulus.[30]

Constructs with higher aspect ratios are currently being investigated, and the results of this study will be reported in a separate manuscript.[31]

In conclusion, this study demonstrated that a single mechanical stimulus applied to stem cell–collagen sponge constructs increased collagen type I and collagen type III gene expression. Based on these findings and those of our previous study,[15] we conclude that increases in collagen types I and III gene expression relate to similar increases in the linear stiffness and linear modulus of cell–sponge constructs due to mechanical stimulation (Fig. 3). Collagen type I and collagen type III gene expression might therefore be used as markers or *in vitro* predictors to evaluate tendon repair outcome. We are currently optimizing components of the mechanical signal (peak strain, signal shape, frequency, number of pulses per day) to further improve construct stiffness. Future studies will focus on identifying conditions that trigger increases in decorin and fibronectin gene expression and on understanding how gene expression relates to protein production and subsequent tendon repair mechanical properties.

ACKNOWLEDGMENTS

The authors wish to thank Kensey Nash Corporation for the donation of the collagen sponges. This research was partially supported by a grant from the National Institutes of Health (AR 46574) given to the University of Cincinnati.

REFERENCES

1. Kvist M. Achilles tendon injuries in athletes. Sports Med **18,** 173, 1994.
2. Ahmad C.S., Stein B.E., Matuz D., Henry J.H. Immediate surgical repair of the medial patellar stabilizers for acute patellar dislocation. A review of eight cases. Am J Sports Med **28,** 804, 2000.
3. Badylak S.F., Tullius R., Kokini K., *et al.* The use of xenogeneic small intestinal submucosa as a biomaterial for Achilles tendon repair in a dog model. J Biomed Mater Res **29,** 977, 1995.
4. Cofield R.H., Parvizi J., Hoffmeyer P.J., Lanzer W.L., Ilstrup D.M., Rowland C.M. Surgical repair of chronic rotator cuff tears. A prospective long-term study. J Bone Joint Surg Am **83-A,** 71, 2001.
5. Kato Y.P., Dunn M.G., Zawadsky J.P., Tria A.J., Silver F.H. Regeneration of Achilles tendon with a collagen tendon prosthesis. Results of a one-year implantation study. J Bone Joint Surg Am **73,** 561, 1991.
6. Louie L.K., Yanna I.V., Hsu H.P., Spector M. Healing of tendon defects implanted with a porous collagen-GAG matrix: histological evaluation. Tissue Eng **3,** 187, 1997.
7. Winter E., Weise K., Weller S., Ambacher T. Surgical repair of Achilles tendon rupture. Comparison of surgical with conservative treatment. Arch Orthop Trauma Surg **117,** 364, 1998.
8. Natri A., Jarvinen M., Kannus P. Primary repair plus intra-articular iliotibial band augmentation in the treatment of an acute anterior cruciate ligament rupture. A follow-up study of 70 patients. Arch Orthop Trauma Surg **115,** 22, 1996.
9. Reid J.S., Hanks G.A., Kalenak A., Kottmeier S., Aronoff V. The Ellison iliotibial-band transfer for a torn anterior cruciate ligament of the knee. Long-term follow-up. J Bone Joint Surg Am **74,** 1392, 1992.
10. Milthorpe B.K.. Xenografts for tendon and ligament repair. Biomaterials **15,** 745, 1994.
11. Butler D.L., Goldstein S.A., Guilak F. Functional tissue engineering. The role of biomechanics. J Biomech Eng **122,** 570, 2000.
12. Guilak F., Butler D.L., Goldstein S.A., Mooney D. Functional Tissue Engineering. New York: Springer-Verlag, 2003.
13. Shearn J., Juncosa-Melvin N., Boivin G., Galloway M., Goodwin W., Gooch C., Dunn M., Butler D. Mechanical stimulation of tendon tissue engineered constructs: effects on construct stiffness, repair biomechanics and their correlation. J Biomech Eng, in press.
14. Juncosa N., West J.R., Galloway M.T., Boivin G.P., Butler D.L. *In vivo* forces used to develop design parameters for tissue engineered implants for rabbit patellar tendon repair. J Biomech **36,** 483, 2003.
15. Juncosa-Melvin N., Shearn J.T., Boivin G.P., *et al.* Effects of mechanical stimulation on the biomechanics and histology of stem cell–collagen sponge constructs for rabbit patellar tendon repair. Tissue Eng **12,** 2291, 2006.
16. Altman G.H., Horan R.L., Martin I., *et al.* Cell differentiation by mechanical stress. Faseb J **16,** 270, 2002.
17. Young R.G., Butler D.L., Weber W., Caplan A.I., Gordon S.L., Fink D.J. Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. J Orthop Res **16,** 406, 1998.
18. Awad H.A., Boivin G.P., Dressler M.R., Smith F.N., Young R.G., Butler D.L. Repair of patellar tendon injuries using a cell-collagen composite. J Orthop Res **21,** 420, 2003.
19. Juncosa-Melvin N., Boivin G.P., Galloway M.T., *et al.* Effects of cell to collagen ratio in mesenchymal stem cell-seeded implants on tendon repair biomechanics and histology. Tissue Eng **11,** 448, 2005.
20. Schantz E.J. A New Dynamic Culture System Designed to Provide Controlled Strain to Cell Seeded Collagen Constructs for Tendon Repair. Cincinnati: University of Cincinnati, 2001.
21. Boykiw R., Sciore P., Reno C., Marchuk L., Frank C.B., Hart D.A. Altered levels of extracellular matrix molecule mRNA in healing rabbit ligaments. Matrix Biol **17,** 371, 1998.
22. Guehring T., Omlor G.W., Lorenz H., *et al.* Stimulation of gene expression and loss of anular architecture caused by experimental disc degeneration – an *in vivo* animal study. Spine **30,** 2510, 2005.
23. Ouyang H.W., Goh J.C., Thambyah A., Teoh S.H., Lee E.H. Knitted poly-lactide-co-glycolide scaffold loaded with bone marrow stromal cells in repair and regeneration of rabbit Achilles tendon. Tissue Eng **9,** 431, 2003.
24. Hsieh A.H., Tsai C.M., Ma Q.J., *et al.* Time-dependent increases in type-III collagen gene expression in medical collateral ligament fibroblasts under cyclic strains. J Orthop Res **18,** 220, 2000.
25. Kim S.G., Akaike T., Sasagaw T., Atomi Y., Kurosawa H. Gene expression of type I and type III collagen by mechanical

stretch in anterior cruciate ligament cells. Cell Struct Funct **27,** 139, 2002.
26. Frank C., Woo S.L., Amiel D., Harwood F., Gomez M., Akeson W. Medial collateral ligament healing. A multidisciplinary assessment in rabbits. Am J Sports Med **11,** 379, 1983.
27. Frank C., Schachar N., Dittrich D. Natural history of healing in the repaired medial collateral ligament. J Orthop Res **1,** 179, 1983.
28. Reed C.C., Iozzo R.V. The role of decorin in collagen fibrillogenesis and skin homeostasis. Glycoconj J **19,** 249, 2002.
29. Banes A.J., Link G.W., Bevin A.G., *et al.* Tendon synovial cells secrete fibronectin *in vivo* and *in vitro*. J Orthop Res **6,** 73, 1988.
30. Cook R.D., Young W.C. Advanced mechanics of materials. Upper Saddle River, NJ: Prentice Hall, 1999.
31. Nirmalanandhan V., Rao M., Sacks M., Haridas B., Butler D. Effects of length of the engineered tendon constructs on its structure-function relationship in culture. J Biomech, in press.

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