Annals of Biomedical Engineering, Vol. 35, No. 7, July 2007 *(© 2007)* pp. 1188–1195
DOI: 10.1007/s10439-007-9295-9

In Vitro System for Applying Cyclic Loads to Connective Tissues Under Displacement or Force Control

KRISHNA R. ASUNDI, KATHY KURSA, JEFF LOTZ, and DAVID M. REMPEL

Ergonomics Program, Department of Bioengineering, University of California at Berkeley, 1301 South 46th Street, Building 163, Richmond, CA 94804, USA

(Received 13 July 2006; accepted 7 March 2007; published online 24 March 2007)

Abstract—Overuse is thought to be the primary cause of chronic tendon injuries, in which forceful or repetitive loading results in an accumulation of micro-tears leading to a maladaptive repair response. *In vitro* organ culture models provide a useful method for examining how specific loading patterns affect the cellular response to load which may explain the early mechanisms of tissue injury associated with tendinopathies and ligament injuries. We designed a novel tissue loading system which employs closed-loop force feedback, capable of loading six tissue samples independently under force or displacement control. The system was capable of applying loads up to 40 N at rates of 100 N s^{-1} and frequencies of 2 Hz, well above loads and rates measured in rabbit tendons *in vivo*. Loading parameters such as amplitude, rate, and frequency can be controlled while biomechanical factors such as creep, force relaxation, tangent modulus and Young's modulus can be assessed. The system can be used to examine the relationship between each loading parameter and biomechanical factors of connective tissues maintained in culture which may provide useful information regarding the etiology of overuse injuries.

Keywords—Overuse injuries, Tendinopathies, Organ culture, Bioreactor, Mechanical stimulation.

INTRODUCTION

Tendon injuries due to repetitive loading are a significant problem for workers and athletes, accounting for nearly half of all occupational illnesses in the United States[6] and 30–50% of all sports related injuries.[11] Overuse is thought to be the primary cause of such injuries, in which forceful or repetitive loading results in an accumulation of micro-tears leading to a maladaptive repair response.[1,13,17]

Studies using *in vivo* models have been able to cause overuse-like injuries in rabbits[16] and rats[5,19] allowing a better understanding of the degenerative process. These models, however, are limited in their ability to control specific loading parameters such as strain, stress, loading rates, and frequency. Other studies using cell cultures seeded on flexible membranes have provided useful information regarding the effects of mechanical stimulation on connective tissue cells[4,14,25] but are limited because they may exclude interactions between adjacent cells and between cells and the matrix that occur in a tendon or ligament *in vivo*. *In vitro* organ culture models are an appealing alternative, allowing precise control over various loading parameters while maintaining the interactions between cells and the surrounding matrix.

Previous *in vitro* organ culture models have measured the effects of cyclic loading on factors such as morphology, cellularity, and collagen orientation[9] as well as DNA and protein synthesis.[4,21] More recent studies have examined the effects of loading on gene expression.[3,12] While these studies demonstrate the feasibility and value of *in vitro* organ culture models, most applied low loads at low frequencies relative to those experienced *in vivo*, limiting their ability to study loading patterns associated with overuse injuries which include high forces and high repetition rates.[2,17] Most models also employed displacement control making it difficult to account for factors such as force relaxation and creep, factors which may be associated with overuse.[3]

We designed a novel tissue loading system which employs closed-loop force feedback, capable of loading six tendons independently under force or displacement control. By using closed-loop force feedback we can account for factors such as creep and force relaxation. The system can control specific load parameters such as peak strain (e.g., maximum change in length relative to initial length), peak stress (maximum force relative to cross-sectional area), strain rate, stress rate, loading frequency, and force–time integral. Measuring the effects of these loading parameters on

Address correspondence to David M. Rempel, Ergonomics Program, Department of Bioengineering, University of California at Berkeley, 1301 South 46th Street, Building 163, Richmond, CA 94804, USA. Electronic mail: david.rempel@ucsf.edu

connective tissues can provide valuable insight into the mechanical behavior and cellular response to loading which may explain the early mechanisms of tissue injury associated with tendinopathies. In this paper we describe and characterize the system. We also load tendons under force and displacement control to determine the ability of the system to affect and monitor mechanical properties of tendons exposed to cyclic loads.

METHODS

In Vitro Tendon Loading System

The loading system (Fig. 1) consists of six independently controlled high-precision lead-screw actuators (PM500-1A, Newport Corporation, Irvine, CA). These actuators are connected to a controller (PM500-C, Newport Corporation, Irvine, CA) that interfaces with a personal computer by General Purpose Interface Bus (GPIB). Using Labview software (V 6.2, National Instruments, Austin, TX), commands can be sent to each actuator independently to control the displacement of a rod. Actuators displace the rod with an accuracy of ± 0.75 μm, at movement velocities between 0.2 μm s^{-1} and 25 mm s^{-1} with a total travel of 25 mm. Attached in series to each rod is a compact load cell (ELPS-T2E-10L, Entran Devices Inc., Fairfield, NJ), whose output is amplified (PS-A, Entran Devices Inc., Fairfield, NJ), digitized (NI-DAQ, PCI-6052E, National Instruments, Austin, TX), and inputted to a PC. In series with each load cell is a stainless steel bar at the end of which is a ridged clamp. Maximum inertial effects due to the weight of the steel bar, clamp, and load cell (88 g) and the acceleration of the actuator (350 mm s^{-2}) are less than 0.03 N, much less than the applied loads. Attached to the base of the actuator is a second stationary parallel stainless steel bar with a clamp extending further than the first clamp. Both clamps can be easily detached from the actuator in order to sterilize them.

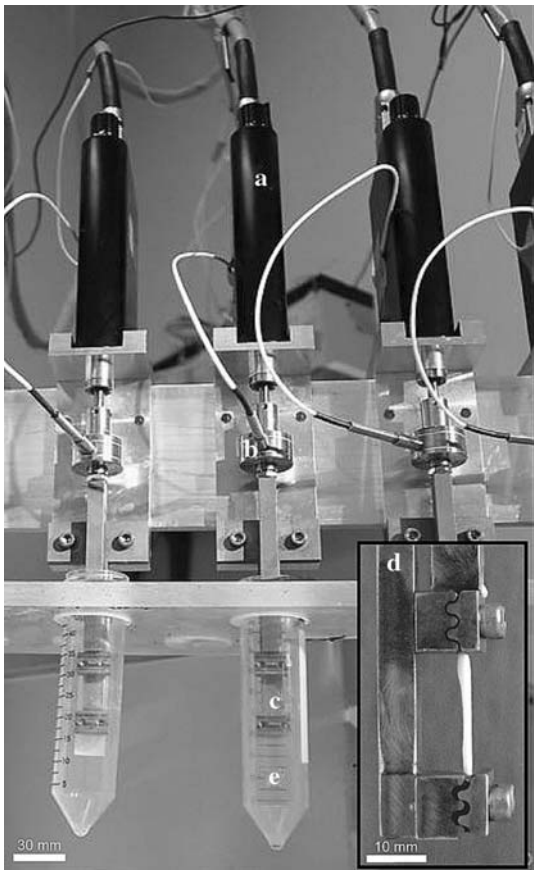

FIGURE 1. Image of three of the six actuators of the loading system. Letters identify system components. (a) Ultra-precision actuator (accuracy $\approx$ 0.75 μm; speed range: 0.02 μm–25 mm s^{-1}); (b) load cell (accuracy $\approx$ 0.1 N; force range: ±50 N); (c) ridged clamps; (d) close up of a tendon secured within the clamps. Fibrocartilage region can be seen protruding from the upper clamp. (e) Tube which can be filled with saline or media. Water bath not shown.

Tendons are aligned visually during clamping with the goal to ensure they are perpendicular and centered within the clamps with enough slack to prevent premature tensional load. Clamps are secured with stainless steel screws tightened to 1 N m. The actuators are fixed to a support allowing the clamps and tendons to be suspended in 50 mL sterile centrifuge tubes which can be filled with media or saline solution. The tubes are submerged in a 20-L water bath and temperature is maintained at 37 °C by an immersion circulator (Isotemp* Immersion Circulators Analog Model 2100, Fisher Scientific).

Five known loads (range 0–40 N) were suspended from each load cell to determine the correlation between load and voltage output. Force measurements over a 14-h period were collected and used to estimate drift.

Control Software

For displacement control loading, Labview software supplied with the controller was used to issue position and velocity commands to each actuator. These position commands allowed displacements in increments as small as 0.1 μm to be applied to tissues secured between the clamps. Under displacement control, a typical force–displacement curve was obtained for a single flexor digitorum profundus tendon harvested from the hind paw of a young (age 2–4 months) New Zealand White Rabbit.

To load tissues under force control, a custom Labview program was written using force and force rate feedback to apply proportional gain, force control

cyclic loading. The software allows the peak and trough force as well as the peak force rate to be controlled individually for each actuator; however, frequency and duty cycle settings are the same for all the actuators. Briefly, a velocity command was issued to each actuator. Next, a position command to increase displacement, thereby increasing force, of the tissue secured in the clamps was issued followed by a position command to decrease displacement of the tissue. This constituted one cycle of load. During the movement, force data was collected at 200 Hz. Data processing identified the maximum and minimum forces as well as the peak rate of force increase. These values were compared to the values set as the target by the user and incremental new values for velocity and position were issued to the actuator. In this way position and velocity were adjusted after each cycle in order to apply the desired forces at the desired force rates.

Mid-substance Strain

Previous studies have concluded that clamped tendons exhibit uneven strain distribution along the length of the tendon.[29] To examine the relationship between clamp-to-clamp strain and mid-substance strain in our system, strain distribution along tendon length was determined optically and compared to the applied clamp-to-clamp strain.

Eight flexor digitorum profundus tendon from the hind paws of eight adult New Zealand White rabbits were harvested and stored at −20 °C until tested. Individually, tendons were thawed and positioned in the clamps of a single loading station, immersed in saline solution, and preconditioned to 5% strain with 1000 cycles at 0.75 Hz. The gauge length was defined as the clamp-to-clamp length of the tendon under a 0.5 N (approximately 0.38 MPa) load. Small dots were applied at equal 2-mm intervals along the longitudinal axis of the tendon using an insect pin and tattoo ink. A DC-330 camera attached to a dissecting microscope was used to acquire images of each region between two consecutive dots at applied clamp-to-clamp strains of 1, 3, and 5% (resolution of 0.0023 mm). The distance between the centroids of two marks on each image was determined with SCION Image (SCION, Frederick, MD). Local tendon strain values were calculated for two tissue segments located within the central portion of the loaded tendon. The proximal region was located 4 mm away from the stationary bottom clamp and consisted of a 4-mm long segment. The distal region consisted of the 4 mm of tendon adjacent to the proximal region and contained a small fibrocartilaginous zone. The proximal region experiences predominantly tensile loads, while the distal region containing fibrocartilage, experiences tensile and compressive forces *in vivo*. The effect of tendon region (proximal vs. distal) and magnitude of clamp-to-clamp strain (1, 3, 5%) on the ratio of tendon to clamp strain was examined with a two-factor repeated analysis of variance. A p-value below 0.05 was considered significant.

Tendon Loading

Two loading tests were conducted to determine the ability of the system to affect and monitor mechanical properties of tendons exposed to cyclic loads. The first experiment loads tendons under displacement control, while the second loads tendons under force control.

In the first test, tendons were cyclically loaded to 1 or 5% strain under displacement control. Young's modulus and force relaxation were measured to determine how these properties were affected by peak strain. Sixteen flexor profundus tendons, approximately 45 mm in length, were harvested from the hind paws of eight young (2–4 months) New Zealand White rabbits. The tendons were secured in the clamps and gauge length was defined as the length of the tendon under a 0.5 N load. Under this load, width and thickness of the tendons were measured using a light microscope and the cross-sectional area (CSA) was calculated by assuming it to be elliptical. Tendons were preconditioned by loading to 3% clamp-to-clamp strain for 1000 cycles. After preconditioning, gauge length was reassessed and tendons were then cyclically loaded to a 1 or 5% peak clamp-to-clamp strain for 24 h (86,400 cycles). Preconditioning and loading was applied using a saw-tooth wave form at 1 Hz with a 50% duty cycle and 20% per second strain rate. All loading was done with tendons submerged in Dulbecco's Modified Eagle Medium and maintained at 37 °C. Before and after loading, tendons were slowly ramped to 10 MPa to obtain a stress–strain curve in order to measure Young's modulus. Stress at peak and trough strain was measured for every cycle. Force relaxation was defined as the ratio of peak force of the first cycle and last cycle of loading. Differences in Young's modulus and force relaxation were compared using paired t-test. A p-value below 0.05 was considered significant.

For the second test, tendons were cyclically loaded between 4 and 6 MPa or 1 and 9 MPa under force control. These loads were selected to compare tendons loaded to different peak stresses with equal force–time integrals. Tangent modulus and residual strain were monitored to determine how these parameters were affected by peak stress. Eighteen flexor profundus tendons, each approximately 45 mm in length, were harvested from the hind paws of three young (2–4 months) New Zealand White rabbits. Cross-sectional

area was measured using a load applied micrometer[7] (Electronic Digital Thickness Gauge, Suncoast Precision Tools, Largo, FL) applying a 0.05 MPa load for 30 s (resolution = 0.013 mm^2). Without preconditioning, tendons were cyclically loaded between 4 and 6 MPa ($n = 9$) or 1 and 9 MPa ($n = 9$) for 4 h at 20 MPa s^{-1} at 1 Hz with a 50% duty cycle. All loading was done with tendons submerged in PBS and maintained at 37 °C. At time 0 and every 15 min thereafter, cyclic loading was paused and gauge length and tangent modulus were measured. Gauge length was defined as the length of the tendon with a 0.5 N load applied. Tangent modulus was determined by applying a 3.5% strain to each tendon and measuring the resulting stress. Residual strain was defined as the percent change in gauge length from time 0. Differences in tangent modulus and residual strain were compared at each time point using a paired *t*-test. A *p*-value below 0.05 was considered significant.

RESULTS

In Vitro Loading System

The linear calibration fit for the six load cells had an average correlation value of 0.995. Drift in output did not exceeded more than 0.25 N over a 14-h period. The maximum variation in force readings was 0.1 N.

Control Software

Figure 2 is a plot of the force–displacement curve for a single flexor tendon. Typical toe region followed by the linear region are identified.

During cyclic loading the system was capable of converging on the desired force and force rates within 10 cycles. Dynamic loading tests demonstrate that the system is capable of cyclically loading tendons independently at a range of loads between 1 and 40 N, using loading rates up to 100 N s^{-1} (Figs. 3a–3d). The 40 N limit is set to protect the load cells. The system can also load tendons at frequencies up to 2 Hz. Over 900 cycles, with a target peak load of 9.98 N and loading rate of 66.56 N s^{-1}, actual mean (SD) peak load and loading rate were 9.99(0.08) N and 66.57(1.08) N s^{-1}, respectively.

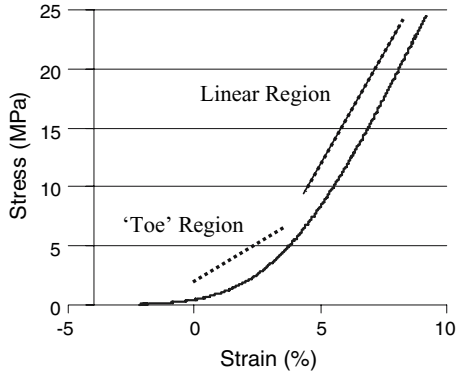

FIGURE 2. Typical stress–strain curve for a rabbit flexor digitorum profundus tendon. Gauge length (e.g., 0% strain) was defined as the length of the tendon under a 0.5 N load. (Negative strain indicates loads below 0.5 N, not compression.) Sample cross-sectional area was 1.5 mm^2. 'Toe' and linear regions are identified on the curve.

Strain

Strain at the mid-substance increased when clamp-to-clamp strain was increased (Fig. 4a). However the mean tendon strain ratio between these two strains, 32(12)%, did not differ based on region or on the amount of clamp-to-clamp strain applied ($p > 0.05$) (Fig. 4b).

Tendon Loading

Tendons loaded to failure initially failed at or near the distal clamp, indicating failure was due to stress concentrations at the clamp site. Consequently, a new method for clamping tendons was developed. The fibrocartilage zone of the tendon (Region B/C according to Okuda *et al.* 1987)[18] was secured in the proximal clamp while the distal clamp was padded with sterile gauze before securing the distal end of the tendon. In a preliminary study, tendons which were secured in the clamps with this method failed at the mid-substance rather than at the clamps when loaded to failure.

Tendons loaded under displacement control did not fail after 24 h of cyclic load. Maximum stress for tendons loaded to 1% peak strain dropped from 1.85(5.7) to 0.70(0.31) MPa and those loaded to 5% peak strain dropped from 8.96(2.03) to 2.48(1.04) MPa. Stress relaxation was significantly greater for tendons loaded to 5% peak strain compared to 1% peak strain (Fig. 5). Cyclic loading to either 1 or 5% peak strain significantly increased the Young's modulus of the tendons, with 5% strain producing a significantly greater increase (Fig. 5).

Cyclic loading under force control led to failure at the mid-substance of tendons in both loading groups. While loading to a peak stress of 9 MPa led to a faster increase in residual strain (Fig. 6) and failed earlier (5343(904) cycles), compared to loading to a peak stress of 6 MPa (12520(1487) cycles), final residual strain before failure was not significantly different between loading groups. Cyclic loading led to an initial increase followed by a decrease in tangent modulus until failure in both loading groups (Fig. 7). This occurred faster in tendons loaded to a peak stress of 9 MPa.

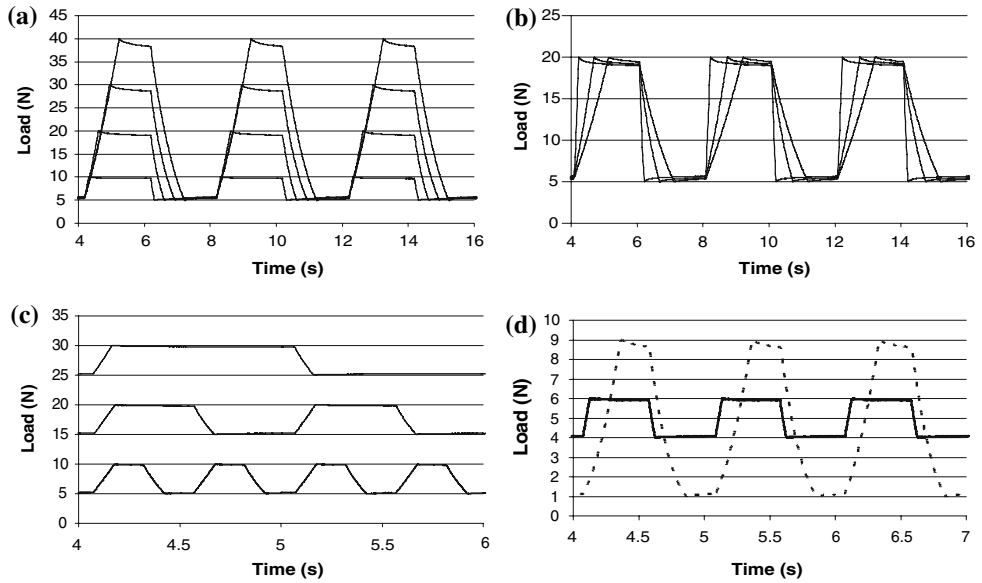

FIGURE 3. Various loading patterns applied to tendons under force control: (a) Four patterns with different peak forces: 5–10, 5–20, 5–30, and 5–40 N at 40 N s^{-1}, 0.25 Hz with a 50% duty cycle; (b) three loading patterns with different loading rates (15, 25, and 100 N s^{-1}) but equal peak loads (5–20 N), frequency (0.25 Hz) and duty cycle (50%); (c) three loading patterns with loading frequencies of 0.5, 1, and 2 Hz but equal peak loads (5–10 N, curves have been displaced along the *Y*-axis for clarity) at 50 N s^{-1} with a 50% duty cycle; (d) two loading patterns with different peak and trough loads (1–9, 4–6 N) but equal mean load (5 N), loading rate (20 N s^{-1}) and force–time integral (5 N s).

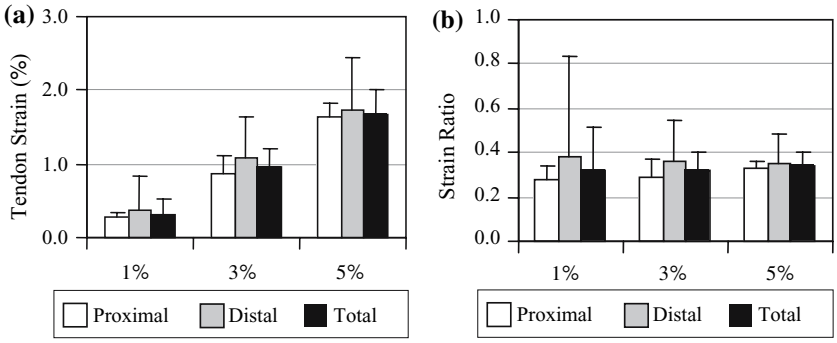

FIGURE 4. (a) Mean mid-substance tendon strains and strain ratios with clamp-to-clamp strains of 1, 3, and 5%; (b) the mid-substance to clamp-to-clamp strain ratio is similar (0.32 ± 0.12) across peak clamp-to-clamp strains. Values are presented for the proximal and distal zones, as well as those for the total 8 mm region. Error bars are 1 SD (*n* = 8).

DISCUSSION

This paper describes an *in vitro* tissue loading system capable of simultaneously applying different, specific loading patterns to six tissues under force or displacement control. Loading parameters such as amplitude, rate, and frequency can be controlled while biomechanical factors such as creep, force relaxation, and modulus can be monitored. The system can be used to examine the relationship between each loading parameter, biomechanical factors, and the cellular response which may provide useful information regarding the etiology of overuse injuries.

Loading patterns were applied to rabbit flexor tendons with a maximum load of 40 N at 100 N s^{-1} and frequencies up to 2 Hz. For the samples examined in our lab, where the average tendon cross-sectional area was 1.32 mm^2, these loads translate to a peak stress of 30.3 MPa at a rate of 75.8 MPa s^{-1}. *In vivo* tendon force measurement studies report mean stresses of 6.7–12.8 MPa and rates of 16.7–26.0 MPa s^{-1} in rabbit flexor,[15] Achilles,[27] and patellar tendons.[10] By selecting tendons with cross-sectional areas below 3 mm^2 (40/3 = 13.3 MPa) such as the rabbit flexor tendons, the system can be used to apply loads within physiologic range and above in order to simulate exposures associated with overuse injuries.

Mid-substance strain was approximately 30% of clamp-to-clamp strain, consistent with previously reported values of 28 and 40%.[28] In a recent study,

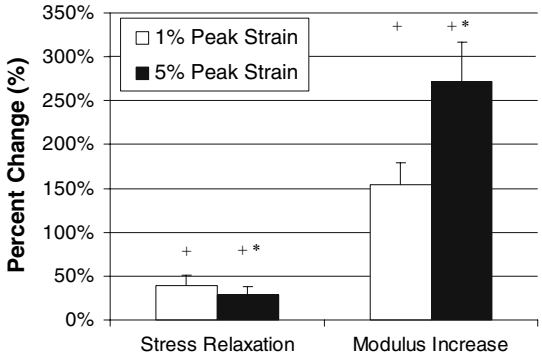

FIGURE 5. Stress relaxation and modulus change after 24 h of cyclic loading under strain control. Peak stresses decreased to 39 and 28% of their initial value in tendons cyclically loaded to a peak strain of 1 and 5%, respectively. The same loading patterns led to increases of 154 and 272% in the Young's modulus. Error bars = 1 SD, + indicates significant difference compared to initial values, * indicates significant difference compared to 1% peak strain, *n* = 8.

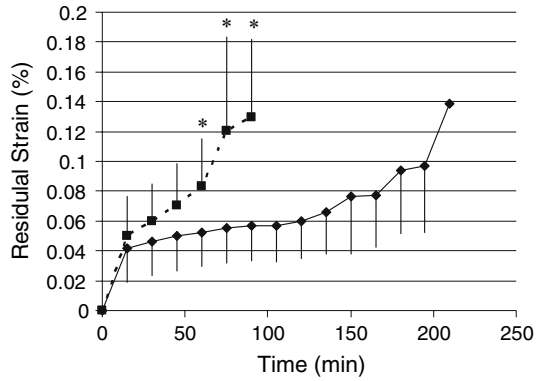

FIGURE 6. Residual strain due to cyclic loading between 4 and 6 MPa (solid line) and between 1 and 9 MPa (dashed line). Error bars are 1 SD, * indicates significant difference in residual strain between loading groups (*n* = 9, *n* = 8 at 90 min time point for 1–9 MPa loading group).

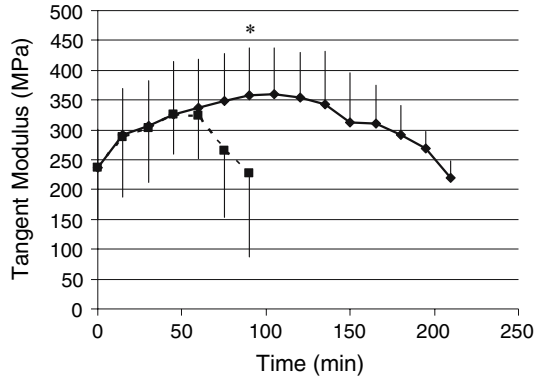

FIGURE 7. Tangent modulus throughout cyclic loading between 4 and 6 MPa (solid line) and between 1 and 9 MPa (dashed line). Error bars are 1 SD, * indicates significant difference in tangent modulus between loading groups (*n* = 9, *n* = 7 at 90 min time point for 1–9 MPa loading group).

Devkota and Weinhold[8] found that the relationship between mid-substance strain and clamp-to-clamp strain changed with cyclical loading which suggests that the cause of uneven strain distribution may be transient, possibly due to settling of the tendon in the clamps, fiber recruitment or fiber alignment. While the uneven strain distribution found in our system is consistent with other tissue loading devices, it does limit the ability of the system to assess tendons loaded under displacement control, as the system cannot routinely measure mid-substance strain during cyclic loading.

Cyclic loading to 1 or 5% peak strain did not result in tendon failure; however a significant amount of force relaxation occurred after 24 h. The increase in tangent modulus indicates that the decrease in stress is not due to a decrease in tendon strength, but rather an accumulation of residual strain. This increase in residual strain could be caused by fiber straightening, sliding, or ruptures,[20] changes which are likely to alter the mechanical environment of cells within the tendon. The mechanical response of tendons has been well characterized at various strains.[20,24] Crimped fibers are straightened under a 2–4% strain, while micro-damage may occur with 4–8% strain. Strains of 12–14% can result in complete rupture. Loading tendons under strain control with this *in vitro* system will allow us to examine the cellular response to such strains.

Cyclic loading under force control initially resulted in an increase in tangent modulus followed by a decrease and ultimately failure for tendons in either loading group. The initial increase in tangent modulus with load has been previously documented in tendons[23] and ligaments[22] while studies that loaded tendons to failure found a similar decrease in modulus with aggressive loading.[26,28] A limitation of this experiment was that cyclic loading was paused every 15 min to assess tangent modulus. While the assessments may have affected the tissue response, changes in tangent modulus were different depending on loading pattern. This indicates how tendons were loaded played a significant role on how tangent modulus changed. The changes in tangent modulus demonstrate that the system can be used to produce and measure different levels of damage in tendons through cyclic load. Examining the cellular response to such damage may help identify exposure thresholds and biomarkers of injury.

Tendons loaded under force control failed within 4 h while tendons loaded under displacement control did not fail. This is due to the nature of such loading. Tendons have a typical failure strain around 12–20%.[24] Since the strain in tendons loaded under displacement control will never exceed the set strain (in our case 5%) tendons will not fail, however force will

drop due to force relaxation. In force control, if the loads are high enough, the strain in tendons will constantly increase due to creep until failure occurs. In this way, force control is a more physiologically relevant loading method, as muscles will act in a similar fashion, increasing displacement such that the desired force at the limb can be achieved.

Under force control, tendons were subjected to loads within physiologic range resulting in failure in less than 4 h, much earlier than would be expected *in vivo*. A possible factor which may have led to such early failure is the high repetition rate. While the applied loads were within those experienced by rabbits *in vivo*,[15] it is unlikely that rabbits would experience such loads at 1 Hz continuously for a period longer than a few minutes. Another factor which may have played a role is the young age of the tendons examined. Younger tendons may be weaker than skeletally mature tendons. Further investigation is required to better understand the mechanisms of failure seen in this study.

A unique feature of our system is its ability to load tendons under force or displacement control while precisely controlling and measuring several parameters. Since the system uses force feedback it is able to adjust the applied position and velocity to achieve the desired stress and stress rates. This allows us to examine the effects of creep, changes in modulus, and force relaxation. Another advantage of our system is that it can independently load six tendons simultaneously which allows for repeated measures experiments to be run, increasing the power of our experiments while reducing the number of animals required.

A limitation to the system is the inability to routinely measure mid-substance strain during cyclic loading. While markers could be placed in the center portion of the tendon, as was done to examine the relationship between clamp-to-clamp strain and mid-substance strain, the tendons need to be removed from the media in order to make these optical measurements. A possible alternative is using an LVDT that inserts directly into the mid-substance region of the tendon. A second limitation is that the system is unable to load tendons at different frequencies simultaneously. Finally, while this study has demonstrated the systems ability to affect and monitor biomechanical properties of tissues, its effects on tissue viability and cellular behavior were not assessed. The system needs to be further validated by examining how clamping and loading tissues affects their viability and normal protein expression before it can be used to examine the effects of detrimental loading. So far, in preliminary studies, tendon viability has been maintained for at least 24 h.

CONCLUSION

We have designed a tissue loading system capable of applying independently controlled loads under force or displacement control simultaneously to six tissue samples maintained in culture. Ultimately, the system may lead to an improved understanding of how different loading parameters such as peak stress, stress rate, or duty cycle affects the initial cellular response to repetitive load. This knowledge may lead to the development of more effective prevention and treatment methods.

ACKNOWLEDGMENTS

The authors would like to acknowledge Xing Chen and Alan Barr for their contributions to this study. Funding for this study was provided by the Centers for Disease and Prevention, National Institute for Occupational Safety and Health Training Grant (5T42OH008429).

REFERENCES

[1]Archambault, J. M., J. P. Wiley, and R. C. Bray. Exercise loading of tendons and the development of overuse injuries. A review of current literature. *Sports Med.* 20(2):77–89, 1995.

[2]Armstrong, T. J., L. J. Fine, S. A. Goldstein, Y. R. Lifshitz, and B. A. Silverstein. Ergonomics considerations in hand and wrist tendinitis. *J. Hand Surg. [Am.]* 12:830–837, 1987.

[3]Arnoczky, S., T. Tian, M. Lavagnino, and K. Gardner. *Ex vivo* static tensile loading inhibits MMP-1 expression in rat tail tendon cells through a cytoskeletally based mechanotransduction mechanism. *J. Orthop. Res.* 22:328–333, 2004.

[4]Banes, A. J., P. Weinhold, X. Yang, M. Tsuzaki, D. Bynum, M. Bottlang, and T. Brown. Gap junctions regulate response of tendon cells *ex vivo* to mechanical loading. *Clin. Orthop. Relat. Res.* 367:s356–s370, 1999.

[5]Barbe, M. F., A. E. Barr, I. Gorzelany, M. Amin, J. P. Gaughan, and F. F. Safadi. Chronic repetitive reaching and grasping result in decreased motor performance and widespread tissue responses in a rat model of MSD. *J. Orthop. Res.* 21:167–176, 2003.

[6]Bureau of Labor Statistics, U.S. Department of Labor. www.bls.gov/iif/home.htm, May 2002.

[7]Butler, D. L., E. S. Grood, F. R. Noyes, and R. F. Zernicke. Biomechanics of ligaments and tendons. *Exerc. Sport Sci. Rev.* 6:125–181, 1978.

[8]Devkota, A., and P. S. Weinhold. A tissue explant system for assessing tendon overuse injury. *Med. Eng. Phys.* 27:803–808, 2005.

[9]Hannafin, J. A., S. P. Arnoczky, A. Hoonjan, and P. A. Torzilli. Effect of stress deprivation and cyclic tensile loading on material and morphological properties of canine flexor digitorum profundus tendon: an *in vitro* study. *J. Orthop. Res.* 13:907–914, 1995.

[10]Juncosa, N., J. R. West, M. T. Galloway, G. P. Boivin, and D. L. Butler. *In vivo* forces used to develop design parameters for tissue engineered implants for rabbit patellar tendon repair. *J. Biomech.* 36:483–488, 2003.

[11]Kannus, P. Tendons—a source of major concern in competitive and recreational athletes. *Scand. J. Med. Sci. Sports* 7:53–54, 1997.

[12]Lavignino, M., S. P. Arnoczky, T. Tian, and Z. Vaupel. Effect of amplitude and frequency of cyclic tensile stress on the inhibition of MMP-1 mRNA expression in tendon cells: an *in vitro* study. *Connect Tissue Res.* 44:181–187, 2003.

[13]Leadbetter, W. Cell-matrix response in tendon injury. *Clin. Sports Med.* 11:533–578, 1992.

[14]Li, Z., G. Yang, M. Khan, D. Stone, S. L. Woo, and J. H. Wang. Inflammatory response of human tendon fibroblasts to cyclic mechanical stretching. *Am. J. Sports Med.* 32:435–440, 2004.

[15]Malaviya, P., D. L. Butler, D. L. Korvick, and F. S. Proch. *In vivo* tendon forces correlate with the activity level and remain bounded: evidence in a rabbit flexor tendon model. *J. Biomech.* 31:1043–1049, 1998.

[16]Nakama, L., K. King, S. Abrahamsson, and D. Rempel. Evidence of tendon microtears due to cyclical loading in an *in vivo* tendinopathy model. *J. Orthop. Res.* 23(5):1199–1205, 2005.

[17]National Research Council and the Institute of Medicine. Musculoskeletal Disorders and the Workplace: Low Back and Upper Extremities. Panel on Musculoskeletal Disorders and the Workplace. Commission on Behavioral and Social Sciences and Education. Washington, DC: National Academy Press, 2001, pp. 196–199.

[18]Okuda, Y., J. P. Gorski, K. N. An, and P. C. Amadio. Biochemical, histological and biomechanical analyses of canine tendon. *J. Orthop. Res.* 5:60–68, 1987.

[19]Perry, S., S. McIlhenny, M. Hoffman, and L. Soslowsky. Inflammatory and angiogenic mRNA levels are altered in a supraspinatus tendon overuse animal model. *J. Shoulder Elbow Surg.* 14(1 Suppl S):79S–83S, 2005.

[20]Screen, H., D. Lee, D. Bader, and J. Shelton. An investigation into the effects of the hierarchical structure of tendon fascicles on micromechanical properties. *Proc. Inst. Mech. Eng. [H]* 218:109–119, 2004.

[21]Slack, C., M. H. Flint, and B. M. Thompson. The effect of tensional load on isolated embryonic chick tendons in organ culture. *Connect Tissue Res.* 12:229–247, 1984.

[22]Thornton, G. M., N. G. Shrive, and C. B. Frank. Ligament creep recruits fibres at low stresses and can lead to modulus-reducing fibre damage at higher creep stresses: a study in rabbit medial collateral ligament model. *J. Orthop. Res.* 20:967–974, 2002.

[23]Torp, S., R. G. Arridge, C. D. Armeniades, and E. Baer. Structure-property relationship in tendon as a function of age. In: Structure of Fibrous Biopolymers, edited by E. D. Atkins and A. Keller. Butterworths, London: The Colston Research Society, 1975, pp. 197–221.

[24]Wang, J. H. Mechanobiology of tendons. *J. Biomech.* 39:1563–1582, 2006.

[25]Wang Jia, J. H. F., G. Yang, S. Yang, B. Campbell, D. Stone, and S. L. Woo. Cyclic mechanical stretching of human tendon fibroblasts increases the production of prostaglandin E_2 and levels of cyclooxygenase expression: a novel *in vitro* model study. *Connect Tissue Res.* 44:128–133, 2003.

[26]Wang, X. T., R. F. Ker, and R. M. Alexander. Fatigue rupture of wallaby tail tendons. *J. Exp. Biol.* 198:847–852, 1995.

[27]West, J. R., N. Juncosa, M. T. Galloway, G. P. Boivin, and D. L. Butler. Characterization of *in vivo* Achilles tendon forces in rabbits during treadmill locomotion at varying speeds and inclinations. *J. Biomech.* 37:1647–1653, 2004.

[28]Wren, T. A., S. A. Yerby, G. S. Beaupre, and D. R. Carter. Mechanical properties of the human Achilles tendon. *Clin. Biomech.* 16:245–251, 2001.

[29]Zernicke, R. F., D. L. Butler, E. S. Grood, and M. S. Hefzy. Strain topography of human tendon and fascia. *J. Biomech. Eng.* 106:177–180, 1984.