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DESIGN PARAMETERS FOR TISSUE ENGINEERED IMPLANTS FOR RABBIT PATELLAR TENDON AND ACHILLES TENDON REPAIRS

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DESIGN PARAMETERS FOR TISSUE ENGINEERED IMPLANTS FOR RABBIT PATELLAR TENDON AND ACHILLES TENDON REPAIRS

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ABSTRACT

Previous work performed in Noyes-Giannestras Laboratory has shown that mechanical alignment of undifferentiated mesenchymal stem cells about a suture causes alignment of cells and contraction of constructs in culture in a form that is suitable for implantation for tendon repair. With preliminary proof of concept, it is now our goal to fine-tune this procedure to determine the various factors that will lead to the highest quality tissue from a biomechanical standpoint and the fastest cell proliferation rates in culture. The basis for this step assumes that natural *in-vivo* conditions are optimal for *in-vitro* culture and that if we can simulate *in-vivo* forces or strains for a variety of activities we can precondition the implant and cells to the signals they will receive after surgery. However, these *in-vivo* force patterns are generally not known for a tissue for different activities and likely vary from tendon to tendon. Knowing tendon and ligament forces during normal activities is important in order to understand the levels of *in-vivo* forces the constructs will be expected to bear once implanted. While researchers have to date failed to develop tissue-engineered replacements that match the ultimate mechanical properties of normal tissues, it is conceivable that less stringent design requirements based on normal activity forces (rather than ultimate or failure properties) may be sufficient for functional efficacy.

The purpose of this research study was to determine the *in-vivo* force-time patterns acting on the rabbit patellar tendon and Achilles tendon models for two speeds of activity and for two inclinations of activity. In addition, we sought to determine the failure properties of these tissues so as to compute safety factors (i.e. ratios of failure tissue force to *in-vivo* operating force). This data will provide design parameters for preparing tissue engineered implants containing

mesenchymal stem cells (MSCs) that will more effectively repair surgical defects in these same tissues.

Eight rabbit patellar tendons (PT) and eight rabbit Achilles tendons (AT) were instrumented using implantable force transducers (IFTs), and each rabbit was subjected to an experimental design involving five activity levels. Peak tensile forces and rates of rise and fall in tendon force increased significantly with increasing activity ($p<0.001$). These data will be employed to mechanically stimulate tissue engineered implants in culture.

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Chapter 1: Introduction

1.1 Background

Tendon injuries are among the most common orthopaedic injuries experienced by patients. The most common tendon disorders are observed in the rotator cuff in the shoulder, Achilles tendon in the ankle, and patellar tendon in the knee. For example, at least 100,000 Achilles tendon (AT) injuries are diagnosed and treated annually in the US alone [Praemer et al. 1992]. Tendon inflammation (tendinitis) and degeneration (tendinosis) are particularly common. It is also estimated that 75,000 to 125,000 anterior cruciate ligament (ACL) injuries occur each year [Butler et al., 1989] and an estimated 300,000 ACL tears are reported worldwide. All of these conditions can produce significant disability for the patients. In fact, between 1985 and 1988, soft connective tissue injuries accounted for 33% to 47% of all musculoskeletally related hospitalizations, work and school loss days reported [Praemer et al., 1992].

Treatment of tendon disorders has traditionally involved reduction of inflammation, restoration of flexibility and surgical repair [McCaroll et al. 1995, Kuwada 1995, Winter et al. 1998, Ahmad et al. 2000, Cofield et al. 2001]. More recently new approaches to repair have been evaluated. Functional Tissue Engineering is one of these new approaches. Functional Tissue Engineering ideally seeks to regenerate and substitute damaged tissue, and replicate the mechanical performance of normal tissue. Applying the concept of Functional Tissue Engineering [Butler et al., 2000] new generations of reparative tissue constructs to regenerate and functionally replace damaged tissue can be developed.

After surgeons repair AT ruptures, patients frequently report weakness in the repair site and often must undergo long periods of rehabilitation. Many complications with AT repair go unreported [Kuwada et al. 1995] and re-injury is not uncommon [Kvist et al. 1994], especially

during the early rehabilitation period when the repair sites are still weak. When injuries are localized and the tendon ends can be sutured in apposition, scar tissue forms and slowly remodels, but never regenerates to match the original structure and biomechanics [Leadbetter et al. 1992]. Functional tissue-engineered constructs containing mesenchymal stem cells (MSCs) can be designed to fill these defects in the AT and PT, and accelerate repair in the wound site.

The researchers in the Noyes-Giannestras laboratory seek to accelerate tendon repair using rabbit mesenchymal stem cells (MSCs) to fabricate the implants. Some studies have improved tendon repair by simply placing cells and collagen gel in the wound site [Awad et al, 1999] and by aligning the cells along sutures before implantation [Young et al, 1998; Awad et al, 2000]. However, these studies did not design the MSC constructs while also considering the biomechanical function of the tissue being replaced. Since the biomechanical properties of a repairing tendon are likely critical to its proper function *in-vivo*, the thresholds of force, stress, and strain that normal tissues transmit or encounter must be determined as the tissue is subjected to expected *in-vivo* stresses and strains. Moreover, it is important to scale these *in-vivo* results to the failure properties of the tissues in order to determine the "safety factor" for the tissue engineered implants being designed [Butler et al., 2000]. Knowing the mechanical threshold and safety factor for normal tissues for different *in-vivo* activities will be essential if the tissue repairs are to meet functional demands after surgery. While these measurements can be difficult to make, they establish the patterns of activity and the bounds of expected usage.

1.2 Research Objectives

The purpose of this study was to establish design parameters for tissue engineered implants containing mesenchymal stem cells (MSCs) that are to be used to repair injured tendons

like the rabbit patellar and Achilles tendons. These parameters need to be determined within the working ranges of *in-vivo* forces for each tissue type. In order to accomplish these objectives, patterns of *in-vivo* force were measured in normal rabbit patellar tendon and Achilles tendon during various activities. Corresponding safety factors were also established for threshold values of these forces. The following three hypotheses were also tested as part of this study, including: 1) peak *in-vivo* forces and the rates of rise and fall in these forces increased significantly with increasing levels of activity; 2) the safety factor for the patellar tendon remained above 2.5 for all activities tested. 3) Finally, the rabbits were applying the same force in both limbs. Knowing these patterns and thresholds will permit MSC-based tissue engineered implants to be designed with greater likelihood of success after surgery.

In order to test the first and second hypotheses, the following specific aims were accomplished:

a. Measure voltages *in-vivo* in the rabbit patellar and Achilles tendon for different levels of activity under controlled conditions (treadmill to control the speed of the activity and to vary the level of the forces).

b. Record on videotape the rabbit activity on the treadmill.

c. Calibrate the IFTs *in-vitro* by applying force to each tissue specimen until the same *in-vivo* voltage levels are reached.

d. Determine the failure forces for the rabbit PT and AT in contralateral normal tendons.

e. Calculate the safety factor as the ratio of failure force to *in-vivo* force for each level of activity.

Finally to test the third hypothesis, a TEKscan Pressure Measurement System was employed to determine in a qualitative way if the rabbits were applying equal force on both limbs.

1.3 Organization of Text

Chapter 2 gives a historical background of previous work done on determine patterns of *in-vivo* forces for different connective tissues, including work done on ligament and tendon. In these studies different sensors were employed to measure *in-vivo* forces: buckle E-type gages, implantable force transducers (IFTs), and modified pressure transducers (MPTs).

Chapter 3 presents the study in which *in-vivo* forces in the normal patellar tendon (PT) were quantified for different activities. The study determines different measures of the dynamic tensile forces in the PT using an implantable force transducer (IFT). The results of this study show that measures of *in-vivo* force in the PT are highly activity-dependent for the level of inclination of the activity.

Chapter 4 describes the study in which we tested that *in-vivo* forces in the normal Achilles tendon (AT) increased with increasing levels of inclination of the activity and that the safety factor for the Achilles tendon remained above 2.5 for all activities tested.

The final chapter, Chapter 5 discusses the overall implications of the research presented in this thesis, the limitations in performing *in-vivo* forces measurements and the future research directions.

An important aspect of this thesis is that Chapter 3 and 4 are manuscripts that will be submitted for publication. The papers in these chapters will be submitted to the Journal of Biomechanics. This approach emphasized the need for the dissertation to be widely distributed through peer-reviewed publications.

Chapter 2: Literature Review

Numerous investigators have determined *in-vivo* forces and strains in ligaments and tendons. *In-vivo* forces have been recorded using buckle E-type gages [Biewener et al. 1988, Lewis et al. 1982, Komi et al. 1990, Komi et al. 1992], implantable force transducers [Cummings et al. 1991, Glos et al. 1993, Herzog et al. 1992, Holden et al 1994, Korvick et al 1996, Malaviya et al 1998, Ronsky et al 1995, Xu et al 1992], and modified pressure transducers [Holden et al 1994, Korvick et al 1996, Ronsky et al 1995, Xu et al 1992, Korvick et al 1992].

Studying the Achilles tendons (AT) in humans, Komi and coworkers [Komi 1990; Komi et al, 1992] showed that forces increased with increasing levels of activity, rising to as high as 9000 N. The results of these experiments imply that Achilles tendon forces are unexpectedly high in certain activities (e.g., hopping) and that the rates of loading rather than the absolute magnitudes of the recorded forces may be more relevant for clinical purposes as well as for the construction of artificial tendon materials. They further argued that these high forces give rise to quite high rates of *in-vivo* force generation and dissipation, making both parameters as clinically important as the peak levels achieved.

Herzog et al. have assessed *in-vivo* forces of the gastrocnemius tendon in the cat model [Herzog et al. 1992]. The purpose of their study was to measure isometric force-length properties of cat soleus, gastrocnemius and plantaris muscle-tendon units, and to relate these properties to the functional demands of these muscles during everyday locomotor activities. Knee mechanics were obtained by measuring patellar tendon forces, gastrocnemius forces, and hind limb kinematics. Gastrocnemius forces were determined using an E-shaped transducer placed on the isolated gastrocnemius tendon. Patellar tendon (PT) forces were recorded using a custom-made, implantable force transducer (IFT) inserted into the mid-third of the PT. Maximum PT force was

approximately 120 N and maximum gastrocnemius force was 25 N. Their results suggest that isometric force-length properties of cat soleus, gastrocnemius and plantaris muscles, as well as the region of the force-length relation that is used during everyday locomotor tasks, match the functional demands.

In-vivo forces in the goat patellar tendon have been measured by Korvick et al. [Korvick et al 1996]. Patellar tendon (PT) force was assessed during three different levels of activity with an implantable force transducer (IFT). The goat's speed was determined using two photoelectric sensors 2.5 m apart on the walkway. PT force, vertical ground reaction force (VGRF) and the animal's speed were recorded for standing, walking and trotting. Standing PT force averaged 207 N. During gait activity, they found that the PT was loaded during the stance phase and relatively unloaded during the swing phase of gait. They found that the force measured in the PT during the swing phase is most likely related to recoil in the stretched elastic elements of the muscle and tendon and falls off rapidly with toe-off. Maximum PT force was approximately 800 N for walking and 1000 N for trotting and occurred at mid-stance. For each activity, the PT force increased with increases in VGRF and speed. Maximum *in-vivo* PT stress occurred during trotting and measured 29 MPa. They also found that PT forces were non-zero throughout gait and that these forces approach 32% of failure force. This study demonstrates the IFT's usefulness in assessing tendon force directly.

Malaviya et al. [Malaviya et al 1998] have assessed *in-vivo* forces in the rabbit flexor digitorum profundus (FDP) using implantable force transducers (IFTs) for three different levels of activity: quiet standing (QS), level hopping (LH) and inclined hopping (IH). The authors tested three hypothesis: 1) Peak forces and the rates of rise and fall in *in-vivo* force increase significantly with activity. 2) A minimum force is maintained in the FDP model. 3) The FDP

tendon generates forces and stresses which approach one-third of their failure force during activity. Forces during quiet standing remained steady at 13 N. By contrast, the peak forces for level and inclined hopping were approximately 32 and 72 N, respectively. They found that peak *in-vivo* forces and stresses in this tendon were on average within 30% of the tendon's ultimate load and ultimate stress capacities.

In-vivo forces for goat anterior cruciate ligament (ACL) during quiet standing and during gait (walking or trotting) have been recorded by Holden and coworkers [Holden et al 1994]. The objectives of their study were to determine the *in-vivo* forces levels for the ACL in a quadruped, and to examine how joint position and speed of activity may be correlated with ACL loading. The authors employed photocells to assess the speed of each activity. A modified pressure transducer (MPT) was implanted within the anteromedial band of the ligament to make direct measurements of ACL force. One or two days following implantation, assessments were made of ACL force, knee joint flexion angle, ground reaction forces, and speed of locomotion. The ACL was loaded during quiet standing (30-61 N) and during the stance phase of gait. Peak ACL forces were achieved within the first 40% of stance, with magnitudes ranging from 63 to 124 N during walking and from 102 to 150 N during trotting. The average ACL forces during the stance phase ranged from 34 to 68 N while walking and from 46 to 69 N while trotting. The force in the ACL dropped to zero during the swing phase in all trials.

The implantable force transducer (IFT) is a metallic, curved beam with strain gages affixed on the concave and convex sides and is implanted into the mid-substance of a ligament or tendon. The IFT has been tested *in-vitro* using the goat patellar tendon to determine the transducer's performance [Glos et al 1993]. The conditions tested were chosen to simulate possible extremes of anticipated *in-vivo* loading conditions. IFT output had a strong correlation

with induced tissue load over repeated loading trials. The installation of this transducer did not significantly alter the force/elongation relationship for the tissue. Additionally, the relationship between IFT output and tissue load was unaffected by either stress relaxation or by increases in strain rate from 1 to 10% s^{-1}. Implantable force transducers showed uniform and reliable performance in all the studies cited before. Based in the results of these studies, implantable force transducers were used to measure *in-vivo* forces in the rabbit patellar and Achilles tendon in our project.

Investigators have shown that while peak forces can vary both within and between activities, these forces increase with the speed of activity [Holden et al 1994, Korvick et al 1996]. Tendons typically develop forces earlier than joint ligaments, primarily because muscles must first transmit their developed force to an in-series tendon to effect large enough motions before ligaments can develop loads [Korvick et al 1992, Korvick et al 1992 (new)]. When forces are expressed as percentages of their failure capacity, tendons typically develop much larger forces than ligaments, reaching 30 to 40 percent of ultimate strength, whereas ligaments produce forces that rarely exceed 10 to 12 percent of failure force [Cummings et al 1991, Korvick et al 1996, Malaviya et al 1998]. These differences in percentage of failure force suggest that ligaments maintain a safety factor of 8 to 10 whereas tendons have a factor of only 2.5 to 3. In situ loads measured in cadaveric knees demonstrate the large safety factor in the anterior cruciate ligament [Livesay et al 1997, Sakane et al 1997]. These forces can also vary within ligaments, as evidenced by the large variations in both in situ forces and *in-vivo* strains in the anterior cruciate ligament for different levels of activity. In our study a larger sample size for each tissue type, rabbit patellar tendon and Achilles tendon, was employed to ensure the consistency in the level

of *in-vivo* forces within the eight subjects. A treadmill was utilized to control the activity levels of the rabbits.

Butler, Grood et al. have pioneered efforts in directly recording tendon and ligament forces in animal models using miniature implantable force transducers (IFT) [Glos et al 1993, Holden et al 1994, Korvick et al 1996, Xu et al 1992, Malaviya et al 1998]. They have assessed goat patellar tendon (PT) and rabbit flexor digitorium profundus (FDP) tendon forces using miniature implantable force transducers (IFTs). However, the *in-vivo* forces transmitted by the rabbit PT and AT, our animal and tissue models, have not been measured. Consequently to obtain the safety factors for rabbit PT and AT, the *in-vivo* forces for this model should be determined. These data will allow to establish design parameters for tissue engineered implants containing mesenchymal stem cells (MSCs) that are to be used to repair injured tendons like the rabbit patellar and Achilles tendons. These parameters need to be determined within the working ranges of *in-vivo* forces for each tissue type. The next two chapters in this master thesis address these gaps in knowledge.

Chapter 3: *In-Vivo* Forces In The Rabbit Patellar Tendon

3.1 Introduction

Tendon injuries are among the most common orthopaedic injuries experienced by patients. The most common tendon disorders are observed in the rotator cuff (in the shoulder), Achilles tendon (in the ankle) and patellar tendon (in the knee). Each year 44,000 patients undergo Achilles tendon repair and 52,000 patients have surgery to repair rotator cuff tears [Praemer et al. 1992] These disorders include not only rupture of the tendon, but inflammation (tendinitis) and degeneration (tendinosis) as well. All these conditions are responsible for significant disability in patients. Soft connective tissue injuries account for 33% to 47% of all musculoskeletally related hospitalizations, work and school loss days reported [Praemer et al. 1992].

Tendon disorders have traditionally been treated by reducing inflammation, restoring flexibility, and if necessary, performing surgical repair [McCaroll et al. 1995, Kuwada 1995, Winter et al. 1998, Ahmad et al. 2000, Cofield et al. 2001]. More recently new approaches have been evaluated to treat tendon disorders. Functional Tissue Engineering is one of these new approaches. Functional Tissue Engineering ideally seeks to regenerate and substitute damaged tissue and replicate the mechanical performance of normal tissue by measuring *in-vivo* forces and strains to which these tissues will be exposed and using these results to develop design criteria for the tissue engineered constructs [Butler et al. 2000]. By applying the concepts of Functional Tissue Engineering is possible to develop new generations of reparative tissue constructs to regenerate or at least functionally replace damaged tissue.

Our group has experience measuring *in-vivo* forces in the goat [Holden et al 1994, Korvick et al 1996] and rabbit models [Malaviya et al 1998]. These investigators have shown

that peak forces and the rates of rise and fall increase with increasing levels of activity. Tendons typically develop forces earlier than joint ligaments, primarily because muscles must first transmit their developed force to an in series tendon to effect large enough motions before ligaments can develop loads [Korvick et al 1992].

The long-term goal of this study is to establish design parameters for tissue engineered implants containing mesenchymal stem cells (MSCs) that are to be used to repair injured tendons like the rabbit patellar and Achilles tendons. These parameters need to be determined within the working ranges of *in-vivo* forces for each tissue type. In order to achieve this goal, patterns of *in-vivo* force were measured in normal rabbit patellar tendon during various activities. Corresponding safety factors (calculated as the ratio of tendon failure force to tendon *in-vivo* force) were also established for threshold values of these forces. Tendons typically develop much larger forces than ligaments, reaching 30 to 40 percent of ultimate strength, whereas ligaments produce forces that rarely exceed 10 to 12 percent of failure force [Cummings et al 1991, Korvick et al 1996, Malaviya et al 1998]. Consequently, ligaments maintain a safety factor of 8 to 10 whereas tendons have a factor of only 2.5 to 3. Based on these prior in vivo force studies, we sought to test three hypotheses as part of this study: 1) peak *in-vivo* forces and the rates of rise and fall in these forces will increase significantly with increasing levels of activity; 2) the safety factor for the patellar tendon will remain above 2.5 for all activities tested; 3) rabbits undergoing these in vivo measurements will apply the same vertical ground reaction force in both limbs.

In this study patterns and levels of *in-vivo* tensile forces were measured in the patellar tendons of eight one-year-old female New Zealand White rabbits, weighing 4.7 ± 0.3 kg (mean ± SEM) for five different activity levels. The activity levels were chosen to encompass inactive to

vigorous active behavior. For each activity, the peak and minimum forces during repeated cycles were quantified. To determine the dynamic nature of the *in-vivo* force signal, the rates of rise and fall of tensile force were recorded during a series of gait cycles. These *in-vivo* force parameters were correlated with activity level to establish if these signals change significantly with different levels of activity.

3.2 Experimental Design

The overall hypothesis tested, stated in the null form, was that **varying the animal's activity level would not significantly affect peak, minimum, and rates of rise and fall of *in-vivo* tensile force in the rabbit PT.** Activity effects were evaluated using ANOVA to test for significant differences in all response measures for the five activities (α=0.05). If significant differences were observed due to altered activity, the least significant difference method [Montgomery 1984] was used to compare individual treatment means.

In-vivo forces (IVF) were measured in the PT of eight one-year-old female, New Zealand White rabbits. The rabbit PT was selected as the tissue model based on prior experience with repair of this tendon and *in-vivo* measurement of flexor tendon forces. Understanding the *in-vivo* loads in this tendon will permit our group to then tissue engineer repairs of this tissue after injury and graft harvesting for ACL reconstruction.

The primary treatment variable was the **level of activity** imposed on the rabbit. Five levels of activity were selected, including: **quiet standing** (QS) to simulate *in-vivo* forces (IVFs) during disuse or inactivity, **level hopping** (LH) to mimic "in-cage" movements at 0.1 mph and 0.3 mph, and **inclined hopping** (IH) to simulate exercise at 0.1 mph and 0.3 mph. These

treatment types are frequently used in *in-vivo* studies to try and control IVF levels on tissues [Frank et al. 1992, Woo et al. 1981, Malaviya et al, 1998].

Two classes of response measures were evaluated for each activity. 1) **Peak and minimum *in-vivo* tensile forces** were selected to determine the range of loads acting on the PT for each level of activity. 2) **Rates of rise and fall of *in-vivo* forces** were selected to measure the dynamic nature of the *in-vivo* loading signal for each activity. Such dynamic and cyclical signals have been shown to be important for cell function and synthesis in bone [Weinbaum et al. 1994, Rubin et al. 2002] as well as in soft tissues [Almekinders et al. 1993, Evanko et al. 1993, Steinmeyer et al., 1990]. For example, Weinbaum and coworkers [1994] showed that high frequency, low-amplitude postural strains can maintain and even increase bone mass. Rubin and coworkers [2002] demonstrated that extremely low level mechanical stimuli improve both the quantity and the quality of trabecular bone. Evanko and coworkers [1993] found that compressive force can regulate the development of fibrocartilaginous tissue in tendon.

Several variables were controlled during the experiments. 1) Rabbits were trained to hop on the treadmill one week before surgery, five days per week and ten minutes per day. 2) Forces were measured in only the left PT. 3) IVFs were recorded three days post-surgery to permit the animal to partially recover after surgery and to accommodate to the implanted IFT. 4) The resulting 50 trials (5 activity levels/animal x 10 trials/activity level) were randomized to minimize fatigue-related bias in our data.

3.3 Detailed Methods

All animals used in this study were procured, and surgically operated upon, using procedures approved by the University of Cincinnati-Institutional Animal Care and Use

Committee. *In-vivo* forces in the tendon were measured using implantable force transducers (IFTs) that were designed [Xu et al, 1991], tested [Glos et al 1993], analyzed [Herrin 1993], and extensively used [Cummings et al 1991, Korvick et al 1996, Korvick et al. 1992 (new), Ray et al. 1993, Malaviya et al. 1998] in the Noyes-Giannestras Biomechanics Laboratory. The IFT is a thin, curved beam (2.5 mm wide, 5 mm long) instrumented with strain gages on both curved surfaces (Fig. 2). On being surgically implanted into the tendon, the voltage output generated by the IFT is proportional to the tissue's tensile force. This relationship between tissue force and IFT voltage must be established using an *in-vitro* calibration method.

Activity levels were controlled using a treadmill. Because no specialized research treadmill for rabbits was available, a new treadmill (True, Model # 350, Cincinnati, Ohio) was purchased and adapted for safe use by animals. The dimensions of the treadmill-walking surface were 18"x 55" and its speed could be lowered to as little as 0.1 mph. The treadmill was fitted with a custom-designed run enclosure (Figure 1). The enclosure was constructed of clear polycarbonate sheeting with a wall thickness of 0.25". All joints were welded with acrylic solvent cement to assure durable construction, with no sharp edges on the inside of the run. The enclosure was designed to be 48" long and 16" high to prevent the rabbits from jumping off the treadmill surface. The interior width of the enclosure was selected to be 10", which was narrow enough to encourage forward motion of the animal while also discouraging the animal from turning around in the run. The width was also designed to be large enough to prevent the rabbit from rubbing against the sides during normal exercise [Oyen-Tiesma et al. 1998]. A feeding trough was incorporated into the front panel of the run, which was 2" above the tread, and 1" deep. The front and end panels of the run were as wide as the treadmill body, so that the edges could be secured to the stationary frame on either side of the moving tread. The end panel was notched about 0.5"

off the tread to allow fecal material to pass through the run and off the tread. At the conclusion of testing, urine and feces on the treadmill were removed by scrubbing with a dilute Nolvasan solution (Fort Dodge Laboratories Inc., Fort Dodge, Iowa).

The animals were acclimated to the noise of the running treadmill early in the training process, one week before IFT implantation. Small-sized toasted oat cereal was used as an exercise catalyst. Prior to putting the animals on the treadmill, they were introduced to the cereal, which was hand-fed by the handler while the rabbit remained in the safety of its cage [Oyen-Tiesma et al. 1998]. After the exercise room was set up and the rabbits had been introduced to the cereal, they were placed on the belt of the treadmill for four minutes. The treadmill was stationary initially while the trough was filled with cereal. When the rabbit appeared comfortable with the surroundings, the first step in the training process was completed. This took approximately five minutes. The next day the treadmill was turned on at its lowest possible speed (0.1 mph) as the animal was eating the cereal. This process acclimated the animal to the moving tread and the noise from the treadmill motor. The rabbit could then be left on the treadmill as long as it remained interested in eating (typically 2-3 minutes during the early stages of training). If an animal became uneasy, the treadmill was turned off and the rabbit was permitted to rest (approximately five minutes) until she calmed down. The rabbits ran at 0.3 mph for 10 min daily for five days per week, for one week. A veterinarian was present during the exercising program. The length of the treadmill was such that the rabbits could hop three times from the back to the front of the treadmill while in the enclosure. The authors extracted the signal parameters from the second hop of the rabbit, in a set of three hops, because the second hop was the most natural (Fig. 3). The first and third hops were not characteristic since the animal movement was affected by the back and front of the enclosure, respectively.

To implant the IFT, each rabbit was first anesthetized by an intramuscular injection in the psoas major muscle of a mixture of ketamine hydrochloride (0.5 ml/kg) and acepromazine (0.01 ml/kg), and maintained under anesthesia during surgery using isoflurane gas (1.5%-2.5%). The hind limb and shoulder region were shaved and sterilely prepped using betadine solution and alcohol. The PT was exposed by a 2.5 cm longitudinal incision on the medial aspect of the knee. The IFT was placed in the PT between the patella and tibial tubercle by making a 4 mm sagittal slit in the tendon's lateral edge. The slit was extended across the width of the tendon, separating the tendon into anterior and posterior halves. The IFT was placed into the slit and was fully contained within the tendon. The IFT was oriented such that its long axis was parallel to the tendon fibers and its convex surface faced anteriorly. The IFT was stabilized in position and the incision was closed with interrupted 5.0 proline sutures. The lead wires were secured to the knee's lateral fascia and tunneled subcutaneously up the limb, across the flank to exit near the top of the shoulder blades in an area inaccessible to the rabbit. The skin incisions were closed with 5-0 proline sutures.

Immediately after surgery, the rabbit was given buprenorphine hydrochloride (0.05 mg/kg), and its recovery was monitored every 20 minutes until the rabbit was able to independently maintain sternal recumbency. Over the next three days an Elizabethan collar was placed on the rabbit to prevent access to the instrumentation and the suture site was evaluated to ensure absence of complications.

In order to determine in a qualitative way if the rabbits were placing equal weight on both limbs, a pressure measurement system (model 5250, Tekscan Inc., South Boston, MA) was employed with a sensor sheet on which the animal could be positioned. The rabbit was encouraged to stay on the sheet and the balance between the left (operated) and right hind limb

forces was monitored after IFT implantation, on the third postoperative day. The TEKscan sensor sheet consists of an extremely thin (~0.1 mm), flexible tactile force sensor. These sensors are capable of measuring pressures ranging from 0-15 kPa to 0-175 MPa. The standard sensor consists of two thin, flexible polyester sheets that have electrically conductive electrodes deposited in varying patterns. When the two polyester sheets are placed on top of each other, a grid pattern is formed, creating a sensing location at each intersection. By measuring the changes in current flow at each intersection point, the applied force distribution pattern can be measured and displayed on the computer screen.

All data collection was performed at UC's Laboratory Animal Medicine (LAMS) facility three days post-surgery, where the investigators recorded IFT voltage for all five activities. On the day of data collection, a computer (Model Micron Transport Trek2, MicronPC, LLC, Nampa ID) was interfaced to the connector exiting the skin between the ears. A data acquisition card (12 bit National Instruments DAQ Card-500, National Instruments, Austin, TX) was employed for data collection. For quiet standing (QS), data was collected while the animal was sedentary. For level hopping (LH), the rabbit hopped on a level treadmill (True, Model # 350, St. Louis, MO) at 0.1 mph and 0.3 mph. For inclined hopping (IH) the treadmill was raised to give a 12° incline and rabbit hopped up this incline at 0.1 mph and 0.3 mph. For each animal, ten trials (hops from back to front of enclosure) were performed for level and inclined hopping. Trial order was randomized, using a random number generator, to minimize bias due to any animal fatigue.

Rabbits were then euthanized (sodium pentobarbital at 100 mg/kg) to perform *in-vitro* calibration. Calibration was performed within 20 minutes of sacrifice. The limb was dissected above the knee to expose the quadriceps muscle and the quadriceps tendon was attached to a scale (Remington Arms, Madison, NC) using a #5 Ethicond suture (Ethicon, Somerville, NJ). A

series of loads, ranging from 1 lb to 15 lb, was applied using the scale in 1 lb increments to obtain the voltages associated with these loads. The results of this analysis were applied to data collected during the *in-vivo* phase of the project to find actual force values for the rabbit. The calibration revealed a nearly linear relationship between the voltages and muscle force:

$$F = a \cdot V + b$$

where F is the actual force, V is the voltage output, a is the slope of the calibration curve and b the Y intercept. Typical calibration factors for all tests were 25.7 ± 2.3 N/v (mean ± SEM) for slope and 4.8 ± 0.7 N (mean ± SEM) for Y intercept. The calibration curve was linear over the range of voltages measured *in-vivo*, with a correlation coefficient of 0.98. The tabulated force data in Microsoft Excel was scanned to determine peak and minimum values and the range or difference between local peak and minimum values. The rates of rise and fall of force were obtained using the same software. Endpoints were chosen visually and the slope was automatically computed.

Failure tests were performed on the contralateral right patellar tendons to determine *in-vivo* safety factors for the tissue. The patella-patellar tendon-tibial tuberosity unit was first removed from the limb. The specimen's bone ends were potted in aluminum box grips using methylmethacrylate (Dentsply Inc., York, PA). Cross sectional area (CSA) was then measured at three equidistant locations along the tendon using an area micrometer (accurate to 0.1 mm^2) that applied a uniform plunger pressure of 0.12 MPa for 2 minutes [Butler et al., 1984]. The PT was then permitted to equilibrate in a phosphate buffered saline (PBS, 0.138 M NaCl, 0.0027 M KCl, pH 7) bath at 37ºC that was mounted in a materials testing system (model 8501, Instron Corp., MA). The PT was preconditioned 50 times in tension between 0 and 100 N (or about 12.5% of PT failure force) at 1 Hz prior to failure testing. Tensile force and displacement were then

collected simultaneously during failure testing using a Micron Transport Trek2 computer (MicronPC, LLC, Nampa ID).

The statistical analysis was performed using Analysis of Variance (ANOVA), in which the effect of activity level on *in-vivo* force generation was tested. The design structure was a randomized block design in which the experimental units, rabbit patellar tendons (PT), served as the blocks. The within-block experiment was a 2^2 factorial treatment structure in a completely randomized design in which the two factors corresponding to activity level were the amount of inclination (0º and 12º) and the speed (0.1 and 0.3 mph). A mixed-model analysis method was used with specimens as the random factor, all other factors being fixed. This experimental design blocked for inter-animal differences by separating the variance due to inter-animal differences from the measurement error variance. The 'F' statistic was obtained and significance was judged using the reported p-values in which a Bonferroni's adjustment was made to account for multiple comparisons among the contrasts that were examined. Thus, all conclusions regarding the significance of activity on *in-vivo* force measurements were made at the $\alpha = 0.05$ experiment-wise level.

3.4 Results

Figure 3 shows typical *in-vivo* tensile force values from one animal. *In-vivo* forces in the PT always remained greater than zero. Forces during **quiet standing** were nearly constant for each animal, averaging 14.9 ± 1.7 N across all 8 animals (mean ± SEM). By contrast, the **peak maximum forces** for level and inclined hopping averaged 67.9 ± 0.8 N (mean ± SEM) and 82.3 ± 1.8 N (mean ± SEM), respectively, for the 10 trials for all animals. Patellar tendon peak force significantly increased with increasing activity level (Table 1), being significantly greater for

inclined hopping (IH, 12° inclination) than for level hopping (LH, 0° inclination) (Fig. 4, $p<0.001$). The forces for both of these activities were significantly greater than the forces for quiet standing (QS) ($p<0.001$). These peak forces were not significantly different across speeds (0.1 mph and 0.3 mph) for either level or inclined hopping ($p>0.05$). The PT always maintained a minimum level of force throughout each trial across all activities (Fig. 5). These **minimum forces** were 13.8 ± 1.3 N (mean ± SEM) and were not significantly different across activities ($p>0.1$).

The **rates of rise and fall in tendon force** were also affected by activity level (Figs. 6 and 7). Note that the *in-vivo* force rise times for both level and inclined hopping were nearly identical, being approximately 0.3 seconds and independent of peak force achieved. The rates of rise for level hopping at 0.1 mph were 166.9 ± 3.6 Ns^{-1} (mean ± SEM), for level hopping at 0.3 mph were 177.2 ± 6.5 Ns^{-1} (mean ± SEM), for inclined hopping at 0.1 mph were 205.2 ± 8.4 Ns^{-1} (mean ± SEM), and for inclined hopping at 0.3 mph were 214.9 ± 11.7 Ns^{-1} (mean ± SEM). The rates of fall for level hopping at 0.1 mph were 166.7 ± 3.8 Ns^{-1} (mean ± SEM), for level hopping at 0.3 mph were 176.7 ± 6.7 Ns^{-1} (mean ± SEM), for inclined hopping at 0.1 mph were 204.9 ± 7.4 Ns^{-1} (mean ± SEM), and for inclined hopping at 0.3 mph were 216.2 ± 11.8 Ns^{-1} (mean ± SEM). The rates of rise and fall in PT force also significantly increased with increasing level of inclination and speed ($p<0.001$ for both).

Peak *in-vivo* forces and stresses in the patellar tendon were on average within 10% of the tendon's ultimate load and ultimate stress capacities (Table 2). Thus, the safety factor (ratio of tendon failure force to *in-vivo* force) never declined below 10.

Figure 8 shows rabbit patellar tendon TEKscan data. These data demonstrated that the rabbits were placing equal forces in both limbs. During standing, the ratio of the ground reaction force applied by the operated limb vs. the unoperated limb averaged 0.98 ± 0.04 (mean ± SEM).

3.5 Discussion

The results of this study show that increasing the intensity of activity results in a significant increase in peak patellar tendon force. Other structures like the soleus and tibialis anterior tendons in the cat do not show this behavior [Gregor et al., 1988; Herzog et al., 1993; Prilutsky et al., 1994]. But our findings are consistent with *in-vivo* measurements from the human Achilles tendon [Komi 1990; Komi et al., 1992], goat patellar tendon [Korvick et. al, 1996], cat gastrocnemius tendon [Herzog et al., 1993; Prilutsky et al., 1994], and rabbit flexor tendon [Malaviya et al. 1998].

The rates of rise and fall were also found to correlate with the level of activity. Linked to the increase in peak maximum force with increasing activity were increases in the rate of rise and fall in muscle force. As more muscle fibers are recruited, not only is a new peak force generated, but the rate at which this peak is achieved, and the rate at which this force is dissipated back to a baseline value are increased as well. This event is closely related with the increase in peak force with activity since the time of rise and fall were roughly constant (0.3 seconds approximately) for both LH and IH activities. As a consequence, an increase in peak force is associated with a proportional increase in the rate of force generation.

Finally, even for IH, the most vigorous activity, the PT developed *in-vivo* forces and stresses that, on average were no more than 10% of the tendon's ultimate force and stress values. These values corresponded to a minimum, that safety factor (ratio of tendon failure force to *in-*

vivo force) of ten for all activity levels. The average safety factor for quiet standing was 53.3, for level hopping was 11.6 and for inclined hopping was 9.9. The magnitude of the loads measured during these tests was considerably smaller than was expected in the study design. But this is explained by the expectation that the activity modeled in this study is considerably less rigorous than might be demanded in the wild.

The results of this study may have been affected by several factors, both at surgery and after sacrifice. 1) Installing the implantable force transducer and making measurements 3 days after surgery may have affected the output of the muscle tendon unit. However, there was no abnormal gait in the limb implanted with the transducer. And, by making early measurements, the IFT's continued to function as designed, and that any tissue reaction to the presence of the device would be minimal. 2) With the treadmill the speed or linear velocity of the animal during each activity could not be controlled. It is possible that by better controlling the speed of the animal's activity, even more significant effect of altering activity level could have been obtained. 3) The joint flexion angles during the five activities and calibration were not monitored. A knowledge of how tensile forces relate to join kinematics would have being useful in better understanding recruitment patterns for different muscle groups. 4) While our post-mortem calibration may not have fully replicated all the *in-vivo* loading situations, the tendon was handled carefully to ensure that the IFT did not move within the tissue before calibration. Even if the transducer had relocated, however, a previous *in-vitro* study has shown that after intentionally misaligning the IFT, the device realigned itself and provided identical outputs when loads were reapplied to the tissue [Glos et al., 1993]. 5) Using TEKscan system the balance between the forces applied by the operated and unoperated limbs during quiet standing (QS)

were measured. It would be useful to perform these measurements while the animals were hopping.

The long-term goal of this study was to establish design parameters for tissue engineered implants containing mesenchymal stem cells (MSCs) that are to be used to repair injured tendons like the rabbit patellar tendon. The *in-vivo* force parameters recorded in this study will be employed to mechanically stimulate tissue engineered implants in culture. It is expected that the application of mechanical stimulation to the tissue engineered implants containing mesenchymal stem cells (MSCs), will result in implants that will more effectively repair surgical defects in this same tissue.

Table 1. Summary of rabbit PT *in-vivo* force data for different activity levels

Subject #	Peak Force (N)			Minimum Force (N)			Rate of Rise (Ns^{-1})		Rate of Fall (Ns^{-1})	
	QS	LH[a]	IH[b]	QS	LH	IH	LH	IH[b]	LH	IH[b]
	10.1[c]	68.3	82.6	10	15.1	16.2	162.6	200.1	161.8	205.8
1	0.1[d]	1.9	2.9	0.1	1.1	1.4	4.6	5.3	4.4	5.8
	10[e]	10	10	10	10	10	10	10	10	10
	14.2	67.1	81.0	14	12.7	15.1	167.3	205.9	167.2	202.9
2	0.2	2.2	2.7	0.1	1.3	1.5	3.9	4.9	5.1	5.5
	10	10	10	10	10	10	10	10	10	10
	18.2	66.7	83.8	18	12.3	13.2	164.3	219.7	166.2	223.6
3	0.1	1.1	1.9	0.1	1.1	1.4	3.7	5.4	5.4	5.8
	10	10	10	10	10	10	10	10	10	10
	20.1	69.1	84.7	20	13.2	16.2	164.9	207.4	165.1	208.4
4	0.2	2.2	3.3	0.2	1.3	1.8	3.7	5.8	4.7	6.2
	10	10	10	10	10	10	10	10	10	10
	11.2	70.1	82.6	11	13.8	15.1	166.0	222.7	164.5	221.6
5	0.1	1.7	3.8	0.1	0.8	1.2	3.5	5.7	5.2	6.8
	10	10	10	10	10	10	10	10	10	10
	20.1	66.4	84.5	20	13.3	14.8	166.9	213.5	167.6	217.5
6	0.1	1.9	3.5	0.1	0.6	0.7	4.3	5.9	5.2	6.5
	10	10	10	10	10	10	10	10	10	10
	8.2	67.4	82.9	8	12	13.9	171.2	226.1	172.1	226.6
7	0.2	1.1	2.2	0.2	0.8	1	4.5	6.1	3.8	4.2
	10	10	10	10	10	10	10	10	10	10
	18.1	66.2	81.5	18	12.6	14.3	168.9	223.4	169.4	224.9
8	0.1	0.7	1.6	0.1	0.7	1.1	3.9	6	3.6	5.4
	10	10	10	10	10	10	10	10	10	10

[a] Significantly greater than QS. [b] Significantly greater than LH.
[c] Average.
[d] Standard error of measurement
[e] Number of replications.

Table 2. Maximum *in-vivo* force and stress in rabbit PT during inclined hopping, compared to the ultimate force and stress.

In vivo IH data	Subject number	Peak force (N)	CSA (mm^2)	Peak Stress (MPa)
	1	82.6	11.13	7.4
	2	81.0	9.57	8.4
	3	83.8	8.11	10.3
	4	84.7	7.98	10.6
	5	82.6	11.16	7.4
	6	84.5	11.18	7.5
	7	82.9	10.15	8.2
	8	81.5	7.5	10.9
	Mean	82.9	9.6	8.8
	SEM	0.5	0.55	0.5
Tensile failure data	Contra lateral tendon	Ult Force (N)	CSA (mm^2)	Ult Stress (MPa)
	1	728.7	11.34	64.3
	2	730.1	9.77	74.7
	3	896.7	8.41	106.6
	4	889.2	8.12	115.9
	5	825.1	11.36	72.6
	6	890.8	11.37	78.3
	7	550.4	10.38	53.0
	8	885.0	7.7	114.9
	Mean	799.5	9.81	85.0
	SEM	43.4	0.55	8.5
In vivo/ ultimate ratio		10.4%		10.4%

Fig. 1 A view showing the treadmill with the polycarbonate cage

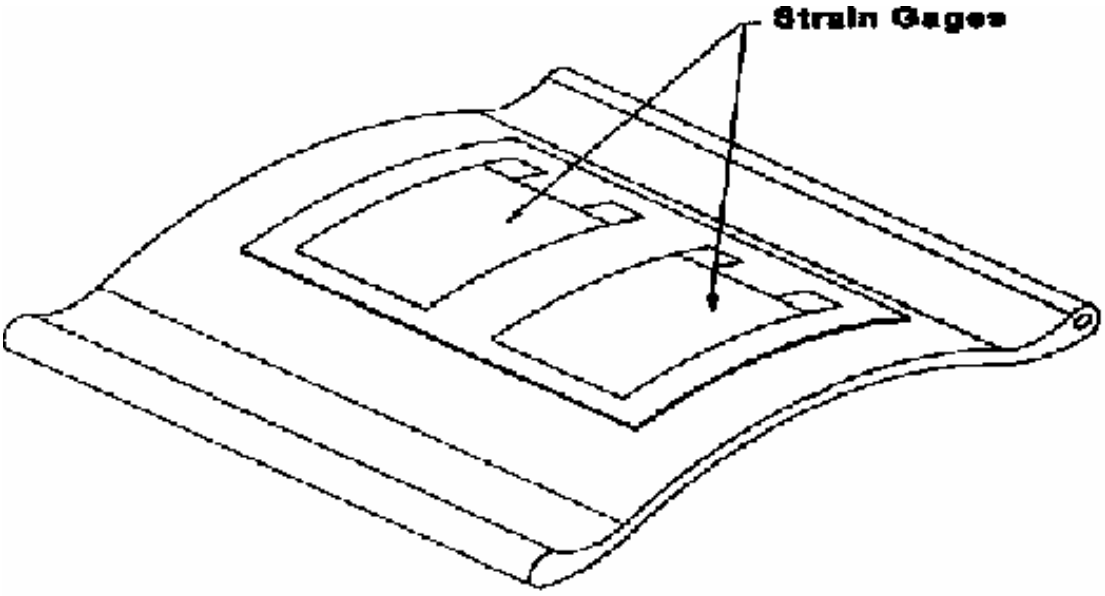

Fig. 2. Schematic of the strain-gaged implantable force transducer (IFT) used in this study.

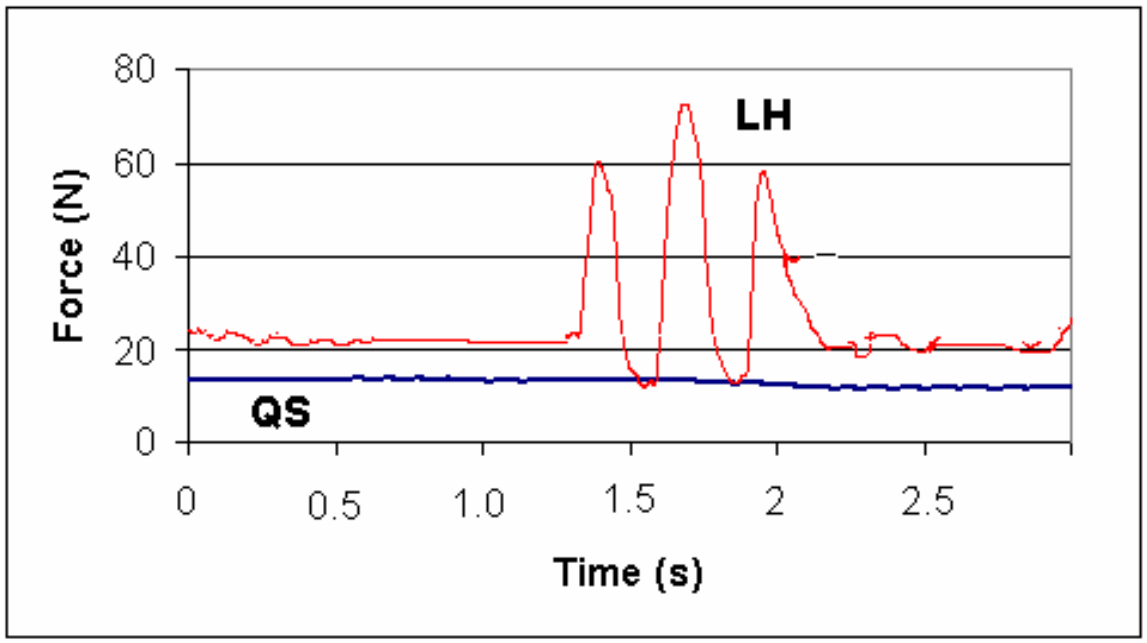

Fig. 3. Typical *in-vivo* forces generated in the rabbit patellar tendon for different activity levels. LH (level hopping) forces vary greatly but always remain greater than zero. QS (quiet standing) forces maintain a steady non-zero value.

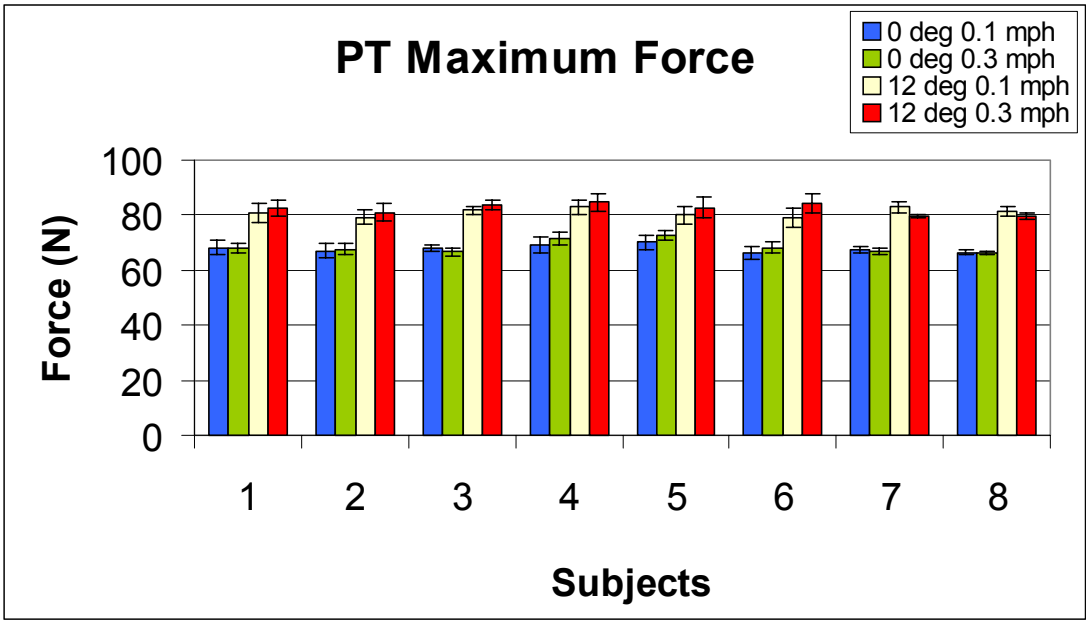

Fig. 4. Rabbit patellar tendon peak force (mean ± SEM) for different levels of activity. Activity has a significant effect on the peak force ($p<0.001$).

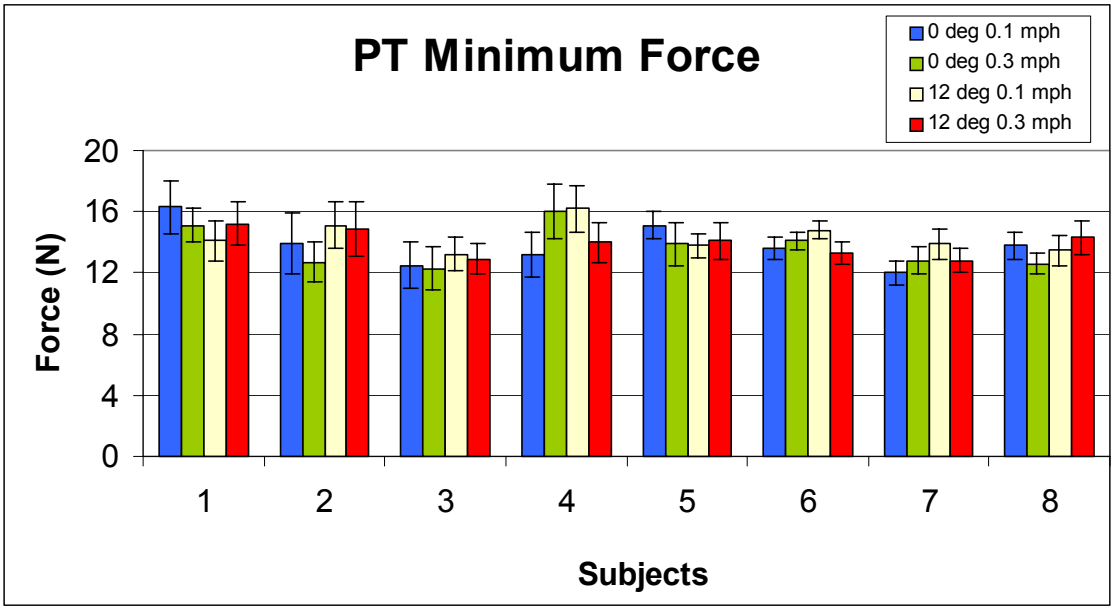

Fig. 5. Rabbit patellar tendon minimum force (mean ± SEM) for different levels of activity. Activity does not have a significant effect on the minimum force ($p>0.1$).

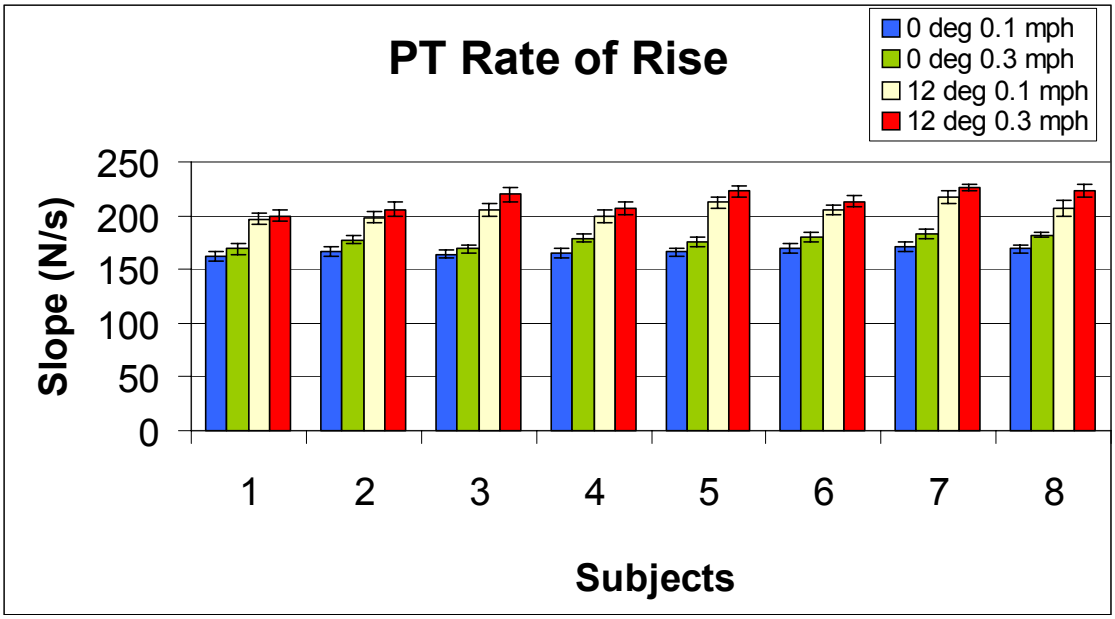

Fig. 6. Rate of rise in the rabbit PT (mean ± SEM) for different levels of activity. Activity has a significant effect on the rate of rise ($p<0.001$).

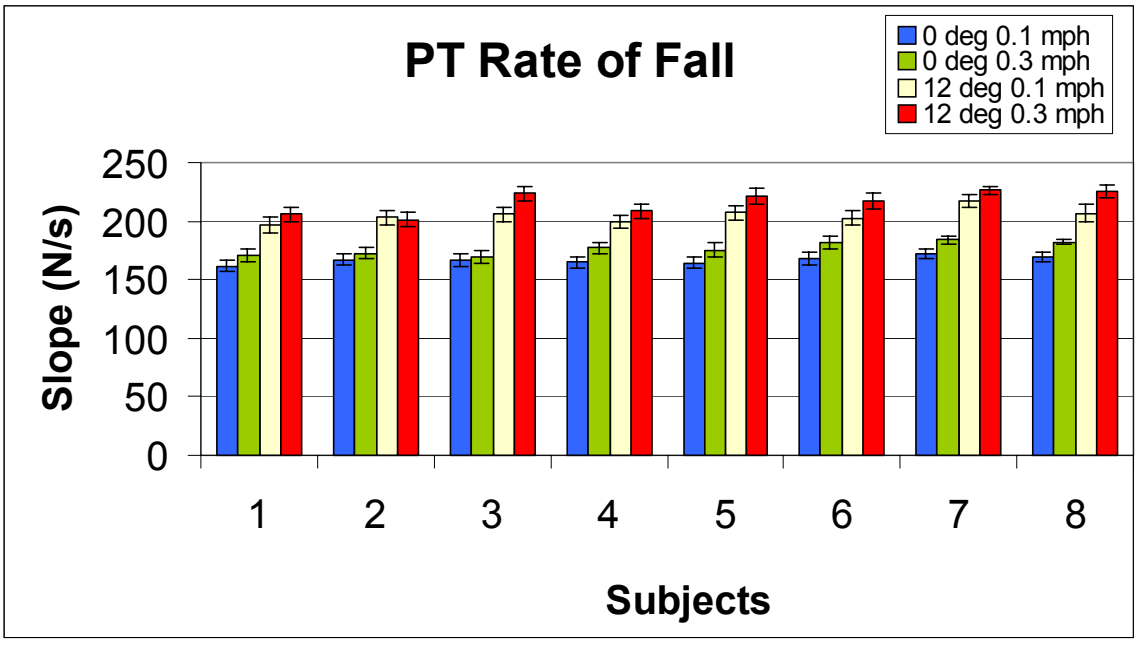

Fig. 7. Rate of fall in the rabbit PT (mean ± SEM) for different levels of activity. Activity has a significant effect on the rate of fall ($p<0.001$).

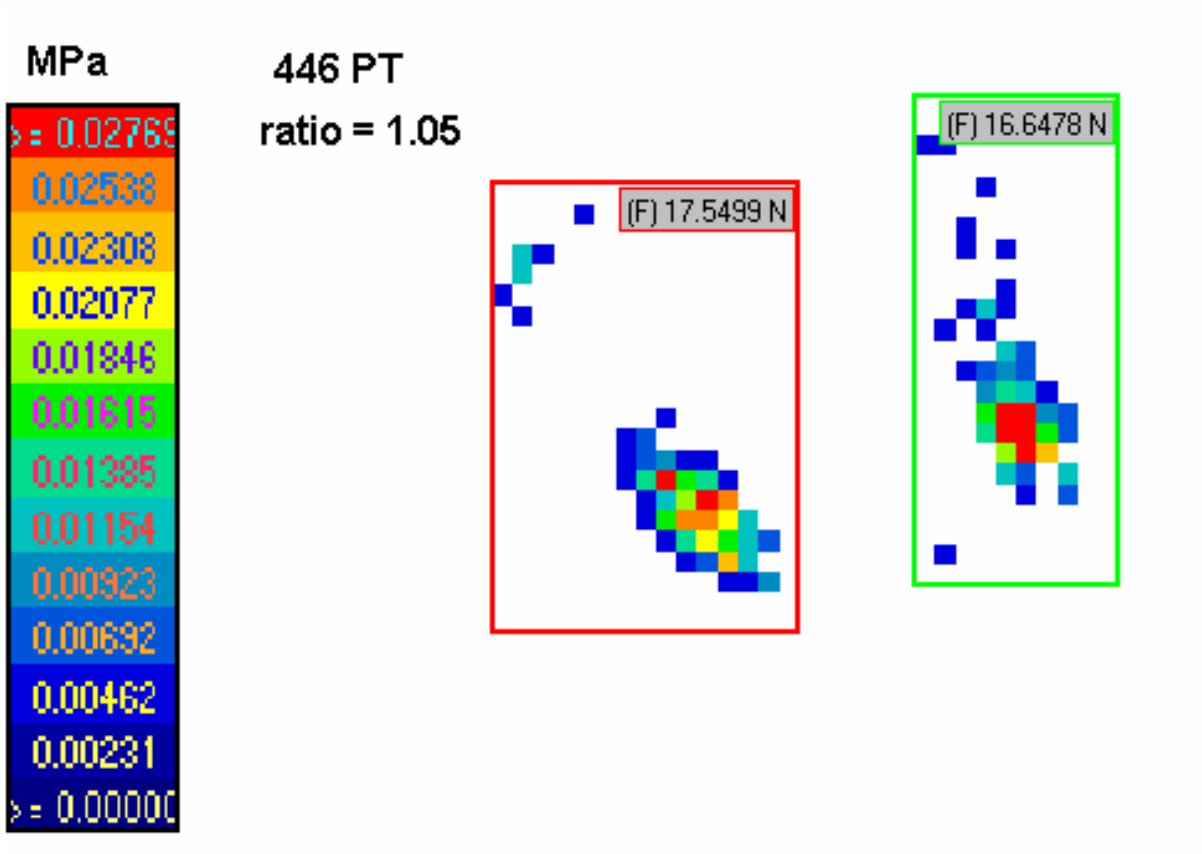

Fig. 8. Rabbit patellar tendon TEKscan data. The ratio between the force applied by the operated limb (in red) and the force applied by the unoperated limb (in green) is shown for a specific animal.

Chapter 4: *In-Vivo* Forces In The Rabbit Achilles Tendon.

4.1 Introduction

Tendon injuries are among the most common orthopaedic injuries experienced by patients. The most common tendon disorders are observed in the rotator cuff (in the shoulder), Achilles tendon (in the ankle) and patellar tendon (in the knee). Each year 44,000 patients undergo Achilles tendon repair [Praemer et al. 1992]. These disorders include not only rupture of the tendon, but inflammation (tendinitis) and degeneration (tendinosis) as well. All these conditions are responsible for significant disability in patients.

Numerous experiments have documented the mechanical function of the Achilles tendon (AT) *in-vitro* [Roberts et al., 1983, Nakagawa et al., 1996, Young et al., 1998], but only limited information has been published related to AT loading *in-vivo*. Studying the Achilles tendons (AT) in humans, Komi and coworkers [Komi 1990; Komi et al, 1992] showed that forces increased with increasing levels of activity, rising to as high as 9000 N. They further argued that these high forces give rise to quite high rates of *in-vivo* force generation and dissipation, making both parameters as clinically important as the peak levels achieved [Komi et al, 1992]

Due to the lack of information about *in-vivo* AT forces, the interpretation of studies of AT function and healing have relied on assumptions regarding the loading or unloading of the tendon. Variables such as range of motion, weight bearing, and speed of activity have been assumed to affect AT forces in various ways, but no direct evidence has validated these assumptions.

The overall goal of this research study was to establish design parameters for tissue engineered implants containing mesenchymal stem cells (MSCs), for rabbit patellar tendon and Achilles tendon models, within the working ranges of *in-vivo* forces for each tissue type. To

achieve this goal, patterns of *in-vivo* force transmitted by normal rabbit Achilles tendon were determined during various activities. Corresponding safety factors (ratio of failure force to in vivo force) were also calculated for threshold values of these forces. Tendons typically develop much larger forces than ligaments, reaching 30 to 40 percent of ultimate strength, whereas ligaments produce forces that rarely exceed 10 to 12 percent of failure force [Cummings et al 1991, Korvick et al 1996, Malaviya et al 1998]. Consequently, ligaments maintain a safety factor of 8 to 10 whereas tendons have a factor of only 2.5 to 3. The following hypotheses were tested: peak *in-vivo* forces and the rates of rise and fall in these forces increase significantly with increasing levels of activity; safety factor for the Achilles tendon remained above 2.5 for all activities tested; rabbits instrumented with an implantable force transducer experience similar ground reaction forces in both limbs during quiet standing.

In this study the patterns and levels of *in-vivo* tensile forces were measured in the Achilles tendon of eight one-year-old female New Zealand White rabbit, weighing 4.7 ± 0.1 kg (mean ± SEM), for five different activity levels. The activity levels were chosen to encompass inactive to very active behavior. For each activity, peak and minimum forces were quantified during repeated cycles. To determine the dynamic nature of the *in-vivo* force signal, the rates of rise and fall of tensile force were recorded during a series of gait cycles. These *in-vivo* force parameters were correlated with activity level to establish if these signals change significantly with different levels of activity.

4.2 Experimental Design

The overall hypothesis tested, stated in the null form, was that **varying the animal's activity level would not significantly affect peak, minimum, and rates of rise and fall of *in-***

***vivo* tensile force in the rabbit AT.** Activity effects were evaluated using ANOVA to test for significant differences in all response measures for the five activities (α=0.05). If significant differences were observed due to altered activity, the least significant difference method [Montgomery 1984] was used to compare individual treatment means.

In-vivo forces (IVF) were measured in the AT of eight one-year-old female, New Zealand White rabbits. The rabbit AT was selected as the tissue model based on prior experience with repair of this tendon and *in-vivo* measurement of flexor tendon forces. Understanding the *in-vivo* loads in this tendon will permit our group to then tissue engineer repairs of this tissue after injury.

The primary treatment variable was the **level of activity** imposed on the rabbit. Five levels of activity were selected, including: **quiet standing** (QS) to simulate *in-vivo* forces (IVFs) during disuse or inactivity, **level hopping** (LH) to mimic "in-cage" movements at 0.1 mph and 0.3 mph, and **inclined hopping** (IH) to simulate exercise at 0.1 mph and 0.3 mph. These treatment types are frequently used in *in-vivo* studies to try and control IVF levels on tissues [Frank et al. 1992, Woo et al. 1981, Malaviya et al, 1998].

Two classes of response measures were evaluated for each activity. 1) **Peak and minimum *in-vivo* tensile forces** were selected to determine the range of loads acting on the AT for each level of activity. 2) **Rates of rise and fall of *in-vivo* forces** were selected to measure the dynamic nature of the *in-vivo* loading signal for each activity. Such dynamic and cyclical signals have been shown to be important for cell function and synthesis in bone [Weinbaum et al. 1994, Rubin et al. 2002] as well as in soft tissues [Almekinders et al. 1993, Evanko et al. 1993, Steinmeyer et al., 1990]. For example, Weinbaum and coworkers [1994] showed that high frequency, low-amplitude postural strains can maintain and even increase bone mass. Rubin and

coworkers [2002] demonstrated that extremely low level mechanical stimuli improve both the quantity and the quality of trabecular bone. Evanko and coworkers [1993] found that compressive force can regulate the development of fibrocartilaginous tissue in tendon.

Several variables were controlled during the experiments. 1) Rabbits were trained to hop on the treadmill one week before surgery, five days per week and ten minutes per day. 2) Forces were measured in only the left AT. 3) IVFs were recorded three days post-surgery to permit the animal to partially recover after surgery and to accommodate to the implanted IFT. 4) The resulting 50 trials (5 activity levels/animal x 10 trials/activity level) were randomized to minimize fatigue-related bias in our data.

4.3 Detailed Methods

All animals used in this study were procured, and surgically operated upon, using procedures approved by the University of Cincinnati-Institutional Animal Care and Use Committee. *In-vivo* forces in the tendon were measured using implantable force transducers (IFTs) that were designed [Xu et al, 1991], tested [Glos et al 1993], analyzed [Herrin 1993], and extensively used [Cummings et al 1991, Korvick et al 1996, Korvick et al. 1992 (new), Ray et al. 1993, Malaviya et al. 1998] in the Noyes-Giannestras Biomechanics Laboratory. The IFT is a thin, curved beam (2.5 mm wide, 5 mm long) instrumented with strain gages on both curved surfaces (Fig. 2). On being surgically implanted into the tendon, the voltage output generated by the IFT is proportional to the tissue's tensile force. This relationship between tissue force and IFT voltage must be established using an *in-vitro* calibration method.

To implant the IFT, each rabbit was first anesthetized by an intramuscular (the psoas major muscle) injection of a mixture of ketamine hydrochloride (0.5 ml/kg) and acepromazine

(0.01 ml/kg), and maintained under anesthesia during surgery using isoflurane gas (1.5%-2.5%). The hind limb and shoulder region were shaved and sterilely prepped using betadine solution and alcohol. The surgeon exposed the AT through a lateral skin incision above the ankle. The IFT was placed in a slit created in the conjoined tendons of the gastrocnemius and soleus midsubstance 1 cm above the calcaneus. The transducer was oriented such that its long axis was parallel to the tendon fibers and its convex surface faced anteriorly. The incision was closed with interrupted 5.0 sutures. The lead wires were secured to the knee's lateral fascia and tunneled subcutaneously up the limb, across the flank to exit near the top of the shoulder blades in an area inaccessible to the rabbit. The skin incisions were closed with 5-0 proline sutures.

Immediately after surgery, the rabbit was given buprenorphine hydrochloride (0.05 mg/kg), and its recovery was monitored every 20 minutes until the rabbit was able to independently maintain sternal recumbency. Over the next three days an Elizabethan collar was placed on the rabbit to prevent access to the instrumentation and the suture site was evaluated to ensure absence of complications.

In order to determine in a qualitative way if the rabbits were placing equal weight on both limbs, a pressure measurement system (model 5250, Tekscan Inc., South Boston, MA) was employed with a sensor sheet on which the animal could be positioned. The rabbit was encouraged to stay on the sheet and the balance between the left (operated) and right hind limb forces was monitored after IFT implantation, on the third postoperative day. The TEKscan sensor sheet consists of an extremely thin (~0.1 mm), flexible tactile force sensor. These sensors are capable of measuring pressures ranging from 0-15 kPa to 0-175 MPa. The standard sensor consists of two thin, flexible polyester sheets that have electrically conductive electrodes deposited in varying patterns. When the two polyester sheets are placed on top of each other, a

grid pattern is formed, creating a sensing location at each intersection. By measuring the changes in current flow at each intersection point, the applied force distribution pattern can be measured and displayed on the computer screen.

All data collection was performed at UC's Laboratory Animal Medicine (LAMS) facility three days post-surgery, where the investigators recorded IFT voltage for all five activities. On the day of data collection, a computer (Model Micron Transport Trek2, MicronPC, LLC, Nampa ID) was interfaced to the connector exiting the skin between the ears. A data acquisition card (12 bit National Instruments DAQ Card-500, National Instruments, Austin, TX) was employed for data collection. For quiet standing (QS), data was collected while the animal was sedentary. For level hopping (LH), the rabbit hopped on a level treadmill (True, Model # 350, St. Louis, MO) at 0.1 mph and 0.3 mph. For inclined hopping (IH) the treadmill was raised to give a 12° incline and rabbit hopped up this incline at 0.1 mph and 0.3 mph. For each animal, ten trials (hops from back to front of enclosure) were performed for level and inclined hopping. Trial order was randomized, using a random number generator, to minimize bias due to any animal fatigue.

Rabbits were then euthanized (sodium pentobarbital at 100 mg/kg) to perform *in-vitro* calibration. Calibration was performed within 20 minutes of sacrifice. The limb was dissected above the knee and the proximal portion of the medial and lateral heads of the gastrocnemious tendon were attached to a scale (Remington Arms, Madison, NC) using a #5 Ethicond suture (Ethicon, Somerville, NJ). A series of loads, ranging from 1 lb to 15 lb, was applied using the scale in 1 lb increments to obtain the voltages associated with these loads. The results of this analysis were applied to data collected during the *in-vivo* phase of the project to find actual force values for the rabbit. The calibration revealed a nearly linear relationship between the voltages and muscle force:

$$F = a \cdot V + b$$

where F is the actual force, V is the voltage output, a is the slope of the calibration curve and b the Y intercept. Typical calibration factors for all tests were for slope 22.3 ± 1.5 N/V (mean ± SEM) and for Y intercept 6.9 ± 0.9 N (mean ± SEM). The calibration curve was linear over the range of voltages measured *in-vivo*, with a correlation coefficient of 0.98. Using Microsoft Excel, force data was scanned to determine peak and minimum values and the range or difference between local peak and minimum values. The rates of rise and fall of force were obtained using the same software.

The right (contralateral) tendon was failed in tension to determine the safety factor for the Achilles tendon. Cross sectional area (CSA) was then measured at three equidistant locations along the tendon using an area micrometer (accurate to 0.1 mm^2) that applied a uniform plunger pressure of 0.12 MPa for 2 minutes [Butler et al., 1984]. The AT was then failed in tension using a materials testing system (Model 8501, Instron Corp., MA). The calcaneus was rigidly fixed to the base plate of the testing system in a methylmethacrylate (Dentsply Inc., York, PA) block. The tendinous portion of the gastrocnemius was then secured to an actuator through a freeze clamp. The AT was preconditioned with 50 axial cycles from 0 to 100 N (or about 20% of AT failure force) at 1 Hz prior to failure testing. Tensile force and displacement were then collected simultaneously during failure testing using a Micron Transport Trek2 computer (MicronPC, LLC, Nampa ID).

The statistical analysis was performed using Analysis of Variance (ANOVA), in which the effect of activity level on *in- vivo* force generation was tested. The design structure was a randomized block design in which the experimental units, rabbit Achilles tendons (AT), served as the blocks. The within-block experiment was a 2^2 factorial treatment structure in a completely

randomized design in which the two factors corresponding to activity level were the amount of inclination (0º and 12º) and speed (0.1 and 0.3 mph). A mixed-model analysis method was used with specimens as the random factor, all other factors being fixed. This experimental design blocked for inter-animal differences by separating the variance due to inter-animal differences from the measurement error variance. The 'F' statistic was obtained and significance was judged using the reported p-values in which a Bonferroni's adjustment was made to account for multiple comparisons among the contrasts that were examined. Thus, all conclusions regarding the significance of activity on *in-vivo* force measurements were made at the $\alpha = 0.05$ experiment-wise level.

4.4 Results

Figure 9 shows typical *in-vivo* tensile force values from one animal. In that figure it can be observed that AT had a baseline level of force, that just before the rabbit hopped, the force dropped and reached a minimum value. When the rabbit had her toe off the force increased up to a peak value, then decreased and reached a minimum value. At the end of the hop the force increased from the minimum force to the baseline level force. The initial portion of the signal, where the drop in force was produced, was called initial slope and the final portion of the signal, where the force again reached baseline values, was called final slope.

In-vivo forces in the AT always remained greater than zero. Forces during quiet standing remained steady at 16.3 ± 2.2 N (mean ± SEM). By contrast, the **peak maximum forces** for level and inclined hopping were 57.6 ± 1.7 N (mean ± SEM) and 77.8 ± 2.2 N (mean ± SEM) respectively. Achilles tendon peak force significantly increased with increasing activity level (Table 3), being significantly greater for inclined hopping (IH, 12° inclination) than for level

hopping (LH, 0° inclination) (Fig. 10, $p<0.001$), both of which were significantly greater than for quiet standing (QS) ($p<0.001$). These peak forces were not significantly different across speeds (0.1 mph and 0.3 mph) ($p>0.05$). Also note that the *in-vivo* force rise times for both level and inclined hopping were nearly identical, being approximately 0.3 seconds and independent of peak force achieved. The AT always maintained a minimum level of force throughout each trial across all activities (Fig. 11). These **minimum forces** were 8.9 ± 0.9 N (mean ± SEM) and were not significantly different across activities ($p>0.1$).

The **rates of rise and fall in tendon force** were also affected by activity level (Fig. 12, Fig. 13). The rates of rise for level hopping at 0.1 mph were 161.5 ± 5.7 Ns^{-1} (mean ± SEM), for level hopping at 0.3 mph were 166.9 ± 6.4 Ns^{-1} (mean ± SEM), for inclined hopping at 0.1 mph were 193.6 ± 2.4 Ns^{-1} (mean ± SEM), and for inclined hopping at 0.3 mph were 199.6 ± 3.1 Ns^{-1} (mean ± SEM). The rates of fall for level hopping at 0.1 mph were 160.5 ± 5.5 Ns^{-1} (mean ± SEM), for level hopping at 0.3 mph were 165.6 ± 5.8 Ns^{-1} (mean ± SEM), for inclined hopping at 0.1 mph were 195.2 ± 3.1 Ns^{-1} (mean ± SEM), and for inclined hopping at 0.3 mph were 200.4 ± 3.3 Ns^{-1} (mean ± SEM). The rates of rise and fall in AT force also significantly increased with increasing level of inclination and speed ($p<0.001$ for both). QS data is not presented here because QS forces remain steady during a trial. The parameters of baseline force, initial slope and final slope were not significantly different across activities ($p>0.1$, Fig. 14, Fig. 15, Fig. 16). The baseline force was 24.3 ± 1.2 N (mean ± SEM), the initial slope was 52.6 ± 2.1 Ns^{-1} (mean ± SEM) and the final slope was 52.1 ± 2.3 Ns^{-1} (mean ± SEM).

Peak *in-vivo* forces and stresses in the Achilles tendon were on average within 19% of the tendon's ultimate load and ultimate stress capacities (Table 4). Thus, the safety factor (ratio of tendon failure force to *in-vivo* force) never declined below 5.

Figure 17 shows rabbit Achilles tendon TEKscan data. These data show that the rabbits placed nearly equal forces in both limbs. The ratio between the force applied by the operated limb and unoperated limbs were 1.03 ± 0.1 (mean ± SEM).

4.5 Discussion

The results of this study show that increasing the intensity of activity results in a significant increase in peak Achilles tendon force. Other structures like the soleus and tibialis anterior tendons in the cat do not show this behavior [Gregor et al., 1988; Herzog et al., 1993; Prilutsky et al., 1994]. But our findings are consistent with *in-vivo* measurements from the human Achilles tendon [Komi 1990; Komi et al., 1992], goat patellar tendon [Korvick et. al, 1996], cat gastrocnemius tendon [Herzog et al., 1993; Prilutsky et al., 1994], and rabbit flexor tendon [Malaviya et al. 1998].

The rates of rise and fall were also found to correlate with the level of activity. Linked to the increase in peak maximum force with increasing activity were increases in the rate of rise and fall in muscle force. As more muscle fibers are recruited, not only is a new peak force generated, but the rate at which this peak is achieved, and the rate at which this force is dissipated back to a baseline value are increased as well. This event is closely related with the increase in peak force with activity since the time of rise and fall were roughly constant (0.3 seconds approximately) for both LH and IH activities. As a consequence, an increase in peak force is associated with a proportional increase in the rate of force generation.

Finally, even for IH, the most vigorous activity, the AT developed *in-vivo* forces and stresses that, on average were no more than 19% of the tendon's ultimate force and stress values. These values corresponded to a minimum, that safety factor (ratio of tendon failure force to *in-*

vivo force) of five for all activity levels. The average safety factor for quiet standing was 24.5, for level hopping was 6.9 and for inclined hopping was 5. The magnitude of the loads measured during these tests was considerably smaller than was expected in the study design. But this is explained by the expectation that the activity modeled in this study is considerably less rigorous than might be demanded in the wild.

In chapter 3 *in-vivo* data for the rabbit patellar tendon (PT) was discussed. The force signal for PT did not show the initial drop in the force level. We hypothesize that the initial drop in the AT force level was due to the release of the muscle force in preparation for the jump. The values of minimum force, maximum force, rate of rise and rate of fall for patellar tendon and Achilles tendon were compared. These four parameters were significantly different ($p<0.001$) between the tendons across the same four levels of activity: Level Hopping at 0.1 mph and at 0.3 mph, and Inclined Hopping at 0.1 mph and at 0.3 mph. Moreover the values of the mentioned parameters were higher for patellar tendon than for Achilles tendon.

The results of this study may have been affected by several factors, both at surgery and after sacrifice. 1) Installing the implantable force transducer and making measurements 3 days after surgery may have affected the output of the muscle tendon unit. However, there was no abnormal gait in the limb implanted with the transducer. And, by making early measurements, the IFT's continued to function as designed, and that any tissue reaction to the presence of the device would be minimal. 2) With the treadmill the speed or linear velocity of the animal during each activity could not be controlled. It is possible that by better controlling the speed of the animal's activity, even more significant effect of altering activity level could have been obtained. 3) The joint flexion angles during the five activities and calibration were not monitored. A knowledge of how tensile forces relate to join kinematics would have being useful in better

understanding recruitment patterns for different muscle groups. 4) While our post-mortem calibration may not have fully replicated all the *in-vivo* loading situations, the tendon was handled carefully to ensure that the IFT did not move within the tissue before calibration. Even if the transducer had relocated, however, a previous *in-vitro* study has shown that after intentionally misaligning the IFT, the device realigned itself and provided identical outputs when loads were reapplied to the tissue [Glos et al., 1993]. 5) Using TEKscan system the balance between the forces applied by the operated and unoperated limbs during quiet standing (QS) were measured. It would be useful to perform these measurements while the animals were hopping.

The long-term goal of this study was to establish design parameters for tissue-engineered implants containing mesenchymal stem cells (MSCs) that are to be used to repair injured tendons like the rabbit Achilles tendon. The *in-vivo* force parameters recorded in this study will be employed to mechanically stimulate tissue engineered implants in culture. It is expected that the application of mechanical stimulation to the tissue engineered implants containing mesenchymal stem cells (MSCs), will result in implants that will more effectively repair surgical defects in this same tissue.

Table 3. Summary of AT *in-vivo* force data for different activity levels

Animal #	Peak Force (N)			Minimum Force (N)			Rate of Rise (Ns^{-1})		Rate of Fall (Ns^{-1})	
	QS	LH[a]	IH[b]	QS	LH	IH	LH	IH[b]	LH	IH[b]
	18.2[c]	57.4	78.6	18	7.9	11.2	165	202.1	160.6	199.7
1	0.2[d]	3.1	4.2	0.2	1.1	1.6	9.4	10.1	5.8	7.6
	10[e]	10	10	10	10	10	10	10	10	10
	18.1	57	74.9	18	5.9	7.3	162.5	197.7	161.6	198.6
2	0.1	2	2.8	0.1	0.7	0.9	10.3	10.6	7.3	8.8
	10	10	10	10	10	10	10	10	10	10
	24.2	58.6	78.7	24	7	8.6	166.6	198.4	165.2	195.9
3	0.2	3.4	4.3	0.2	0.6	1.3	9.2	11.3	10.2	11
	10	10	10	10	10	10	10	10	10	10
	18.1	57.6	75.5	18	7.1	10.5	158.7	199.6	157.9	203
4	0.1	2.4	3.4	0.1	0.9	1.4	5.5	10	5.6	9.4
	10	10	10	10	10	10	10	10	10	10
	24.2	53.9	76.2	24	8.5	9.3	154.8	196.5	155.2	199.4
5	0.2	1.6	1.7	0.2	0.9	1.2	4.1	6.6	3.4	8.3
	10	10	10	10	10	10	10	10	10	10
	8.2	60.6	79.5	8	8.5	9.3	170.3	197.2	170.9	203.7
6	0.1	1.1	1.8	0.1	0.5	0.6	3.6	5.1	3.6	4.9
	10	10	10	10	10	10	10	10	10	10
	10.2	54.6	78.9	10	8.3	8.4	154	199.3	153.7	198.6
7	0.2	0.8	2.1	0.2	0.4	0.5	2.1	4.6	1.9	5.2
	10	10	10	10	10	10	10	10	10	10
	10.1	56.2	80.2	10	8.2	8.5	160.1	205.9	159.5	205.6
8	0.1	1.1	3.2	0.1	0.5	0.7	2.9	6.2	3.5	5.8
	10	10	10	10	10	10	10	10	10	10

[a] Significantly greater than QS. [b] Significantly greater than LH.
[c] Average.
[d] Standard error of measurement
[e] Number of replications.

Table 4. Maximum *in-vivo* force and stress in AT during inclined hopping, compared to the ultimate force and stress.

In vivo IH data	Subject number	Peak force (N)	CSA (mm^2)	Peak Stress (MPa)
	1	78.6	12.57	6.2
	2	74.9	9.53	7.9
	3	78.7	9.75	8.1
	4	75.5	10.47	7.2
	5	76.2	14.47	5.3
	6	79.5	13.4	5.9
	7	78.9	11.29	7.0
	8	80.2	13.13	6.1
	Mean	77.8	11.8	6.7
	SEM	0.7	0.6	0.3
Tensile failure data	Contra lateral tendon	Ult Force (N)	CSA (mm^2)	Ult Stress (MPa)
	1	403.3	12.77	31.6
	2	307.0	9.33	32.9
	3	285.9	9.95	28.7
	4	146.8	10.67	13.8
	5	523.0	14.27	36.7
	6	377.3	13.6	27.7
	7	536.1	11.49	46.6
	8	641.2	13.33	48.1
	Mean	402.6	11.9	33.3
	SEM	56.4	0.6	3.9
In vivo/ ultimate ratio		19.3%		20.1%

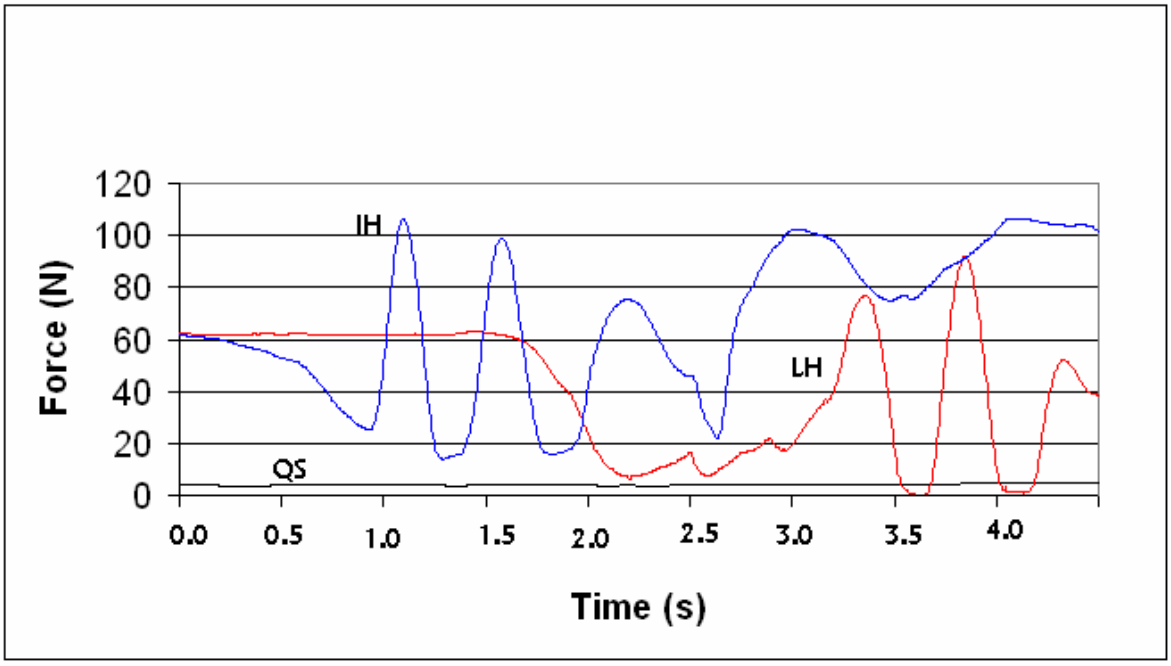

Fig. 9. Typical *in-vivo* forces generated in the rabbit Achilles tendon for different activity levels. LH (level hopping) and IH (inclined hopping) forces vary greatly but always remains greater than zero. QS (quiet standing) forces maintain a steady non-zero value.

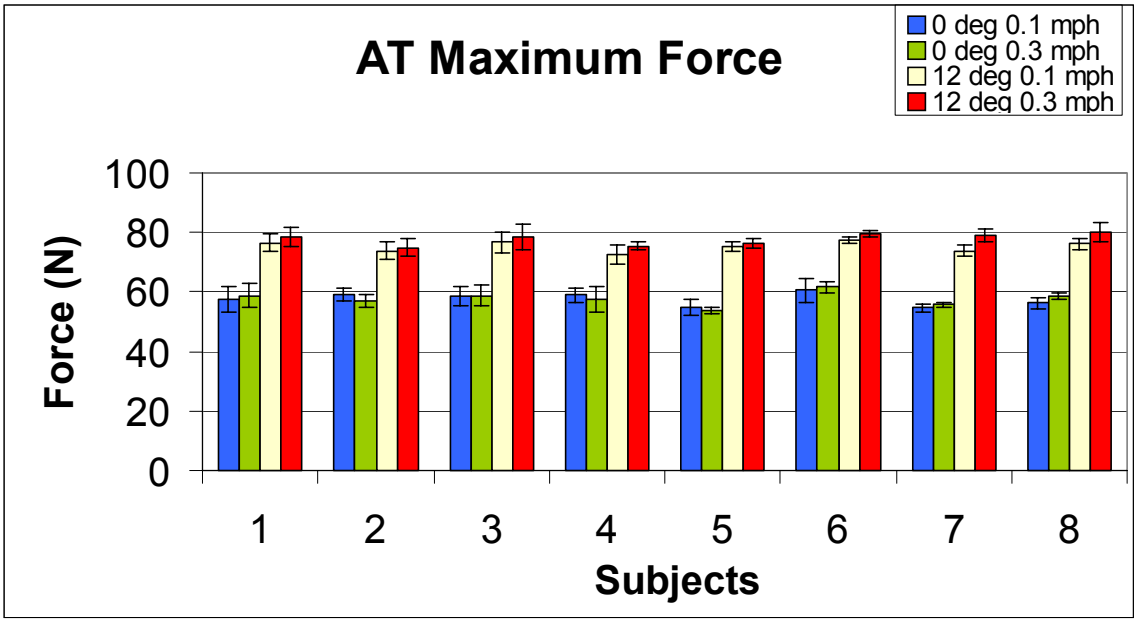

Fig. 10. Rabbit Achilles tendon peak force (mean ± SEM) for different levels of activity. Activity has a significant effect on the peak force ($p<0.001$).

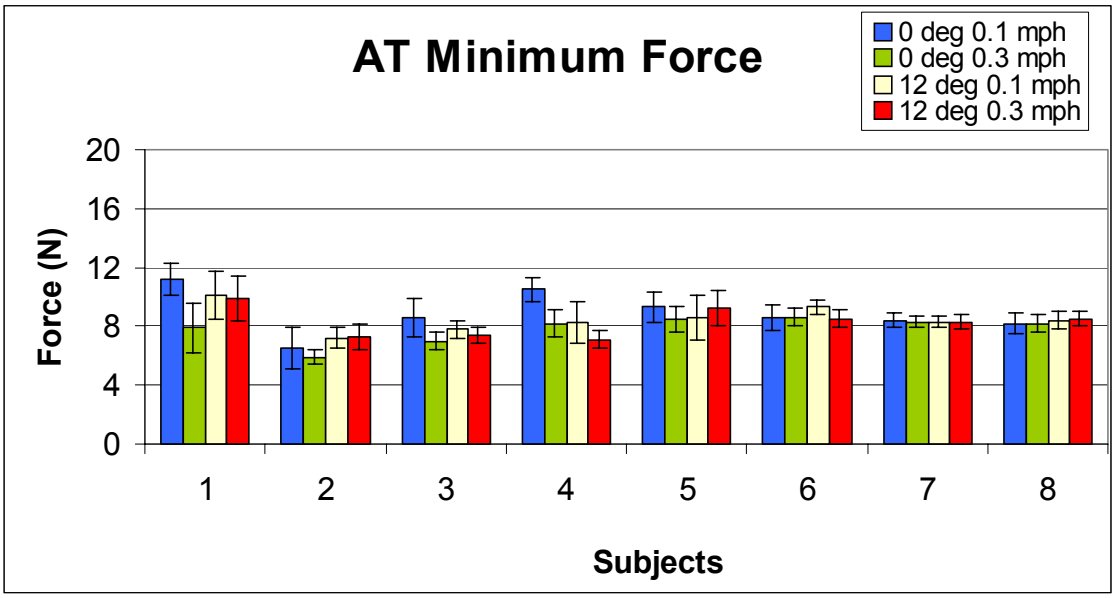

Fig. 11. Rabbit Achilles tendon minimum force (mean ± SEM) for different levels of activity. Activity does not have a significant effect on the minimum force ($p>0.1$).

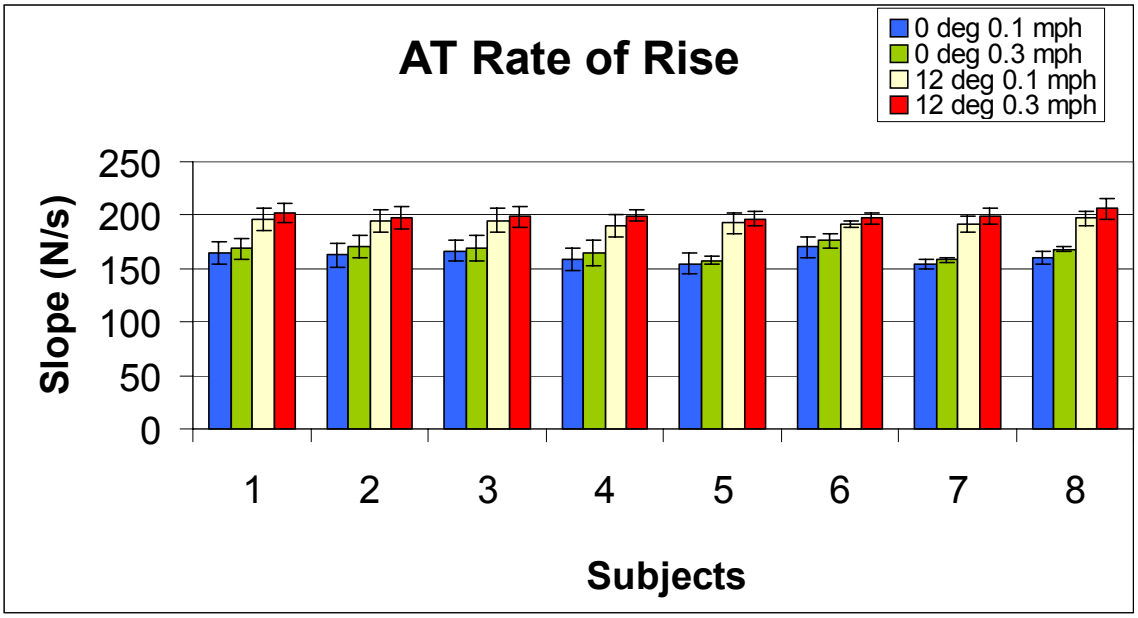

Fig. 12. Rate of rise in the rabbit AT (mean ± SEM) for different levels of activity. Activity has a significant effect on the rate of rise ($p<0.001$).

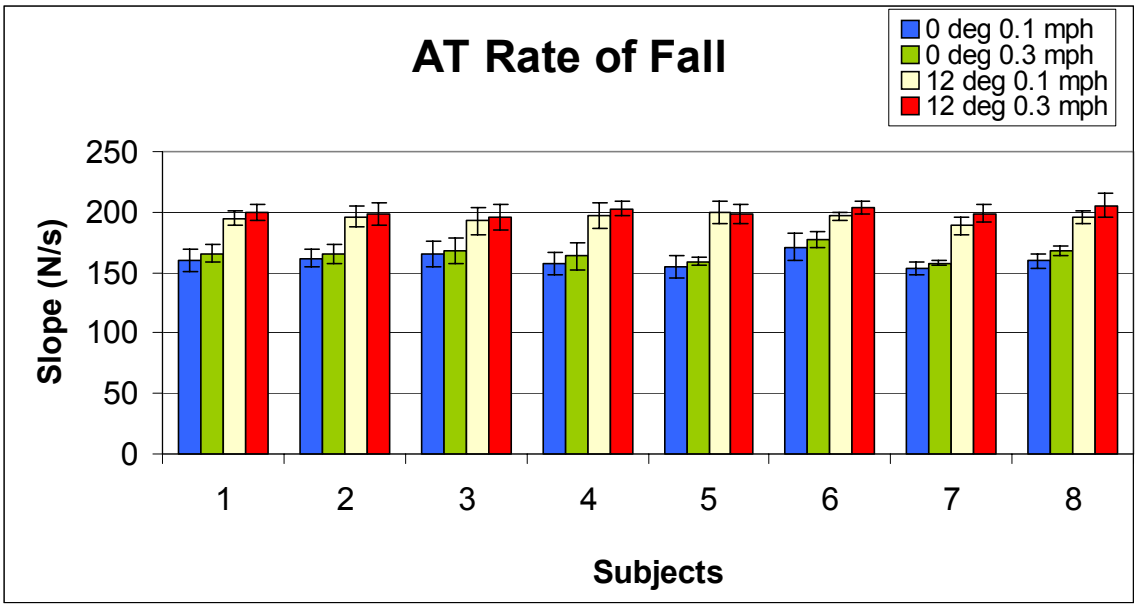

Fig. 13. Rate of fall in the rabbit AT (mean ± SEM) for different levels of activity. Activity has a significant effect on the rate of fall ($p<0.001$).

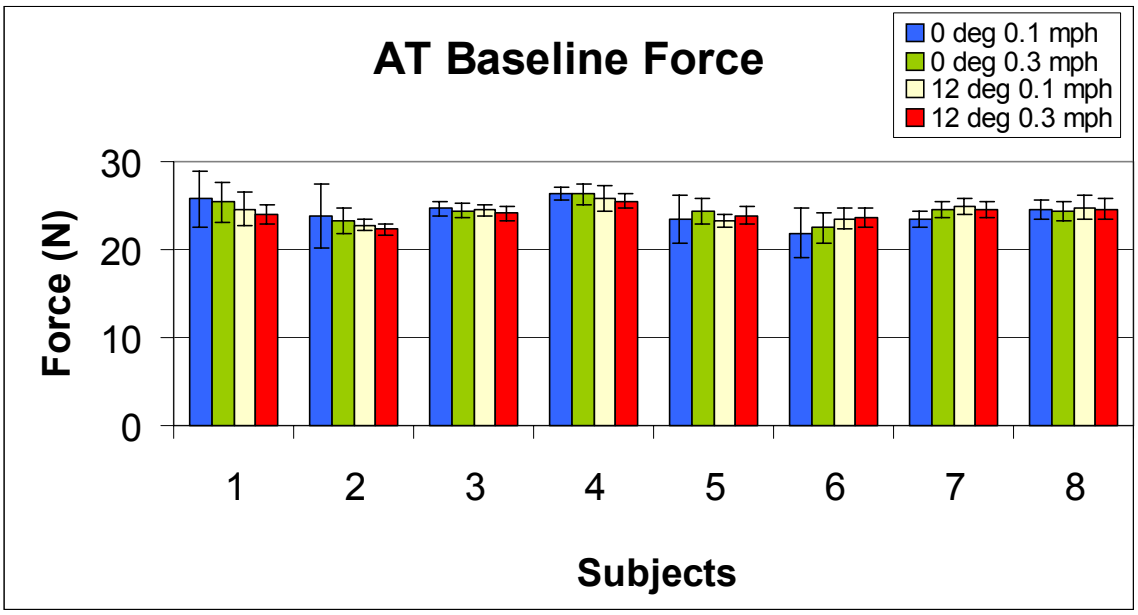

Fig. 14. Baseline force in the rabbit AT (mean ± SEM) for different levels of activity. Activity does not have a significant effect on the baseline force ($p>0.1$).

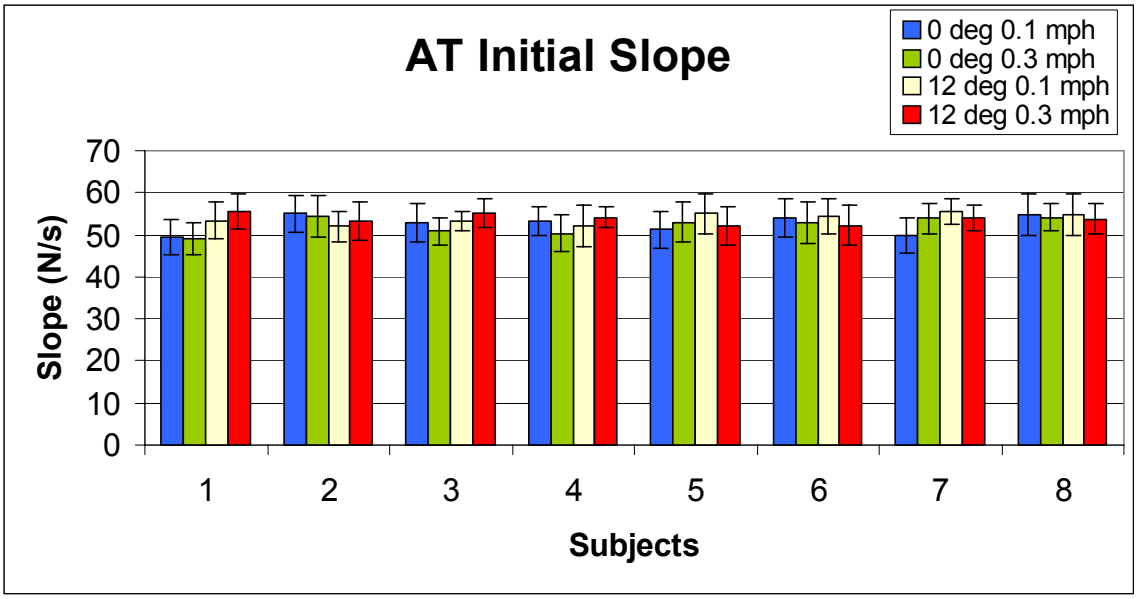

Fig. 15. Initial slope in the rabbit AT (mean ± SEM) for different levels of activity. Activity does not have a significant effect on the initial slope ($p>0.1$).

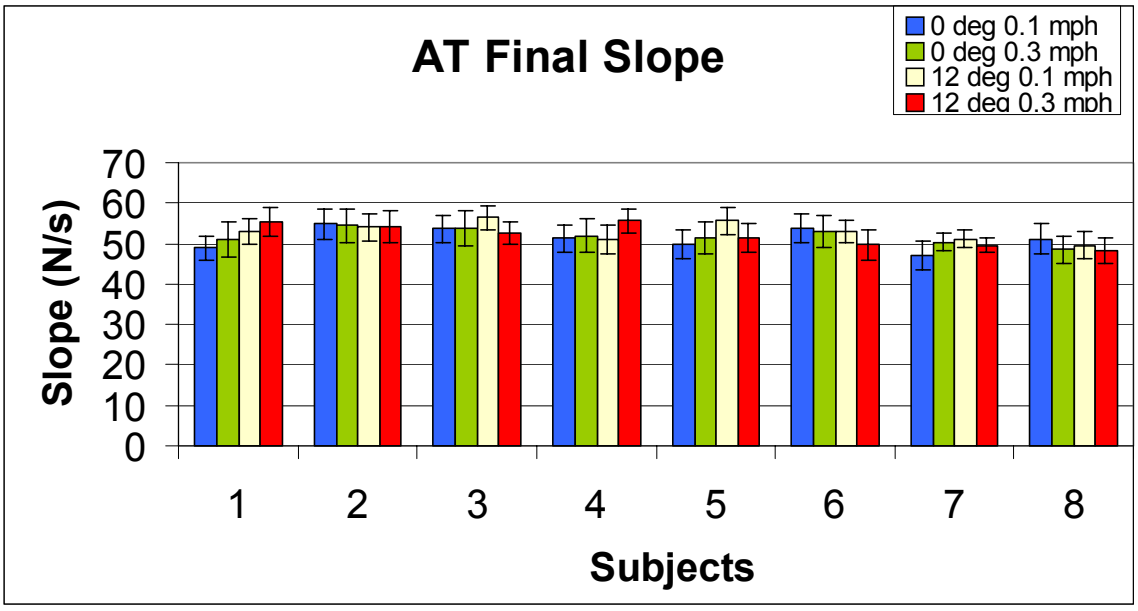

Fig. 16. Final slope in the rabbit AT (mean ± SEM) for different levels of activity. Activity does not have a significant effect on the final slope ($p>0.1$).

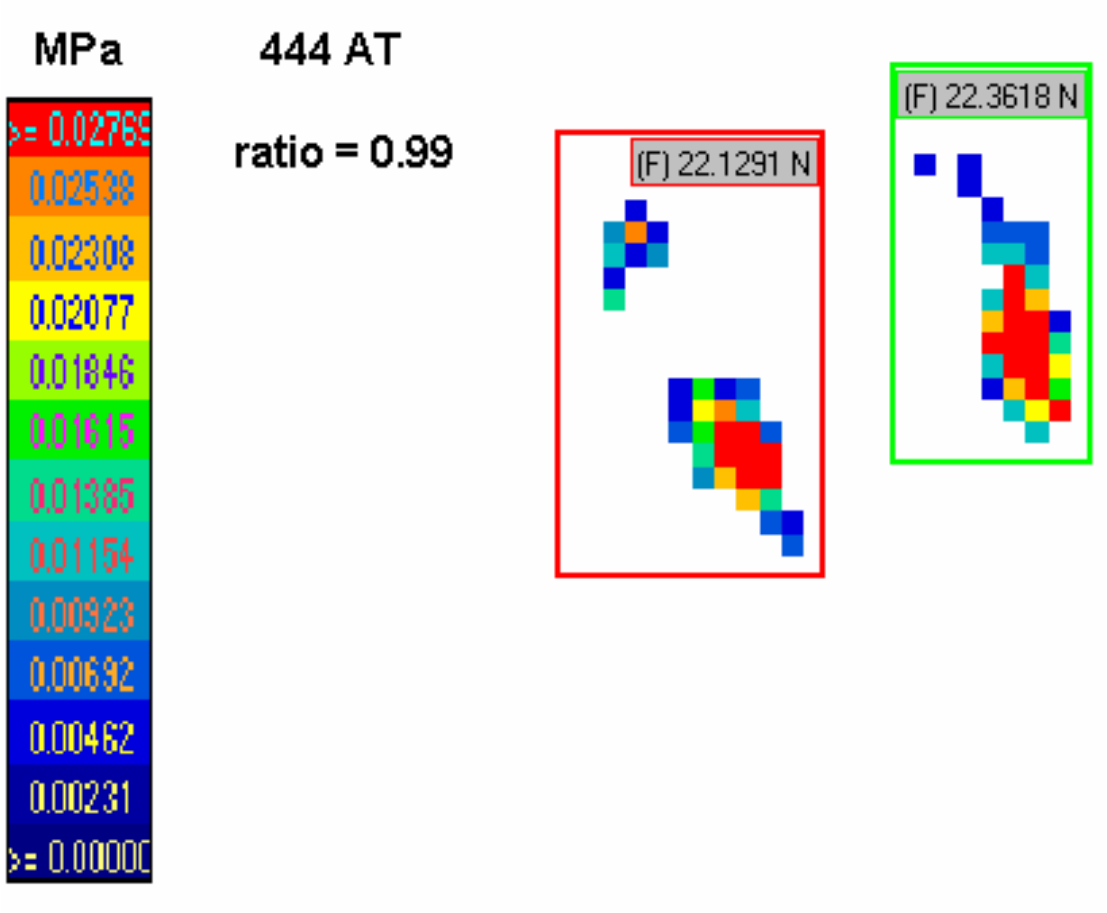

Fig. 17. Rabbit Achilles tendon TEKscan data. The ratio between the force applied by the operated limb (in red) and the force applied by the unoperated limb (in green) is shown for a specific animal.

Chapter 5: Conclusion

The uncertainties in the literature regarding the influence of activity level on *in-vivo* muscle-tendon forces provided the primary motivation for our studies. The overall purpose of this project was to determine and establish design parameters for tissue- engineered implants containing mesenchymal stem cells (MSCs) for rabbit patellar and Achilles tendon repairs. The project required that for each tissue model, the working range of *in-vivo* forces be established so that design parameters like maximum force and stiffness could be set for the engineered constructs. Thus the specific objectives were to determine the patterns of *in-vivo* force that the normal rabbit patellar and Achilles tendons transmit during various activities and the corresponding safety factors for threshold values of these forces. Three hypotheses were tested: peak *in-vivo* forces and the rates of rise and fall in these forces increase significantly with increasing levels of activity; the safety factors for the patellar tendon and Achilles tendons would remain above 2.5 for all activities tested; and the rabbits instrumented with an implantable force transducer would exhibit similar ground reaction forces in both limbs during quiet standing. Knowing these patterns and thresholds will permit MSC-based tissue engineered implants to be designed with greater likelihood of success after surgery.

In these studies the levels and dynamics of *in-vivo* forces have been quantified in the New Zealand White rabbit patellar tendon and Achilles tendon, using an implantable force transducer (IFT), for five different levels of activity. It has been established that increasing activity levels significantly increase the peak and the rates of rise and fall for tensile force in PT and AT. Before discussing the implications of these results, it is important to present the limitations to these studies.

5.1 Limitations

The results presented in Chapters 3 and 4 may have been affected by several factors, both at surgery and after sacrifice. 1) Installing the implantable force transducer and making measurements 3 days after surgery may have affected the output of the muscle tendon unit. It is conceivable that introducing the IFT might shorten the tendon and alter its collagen fiber direction. However, ground reaction forces were found to be balanced between the left and right limbs. The video would suggest some abnormal gait during hopping even though the rabbits seemed to bear weight equally during quiet standing. And, by making early measurements, the IFT's continued to function as designed, and that any tissue reaction to the presence of the device would be minimal. Implantable force transducers (IFTs) normally last no more than two to four weeks in the biological environment [Malaviya et al. 1998]. Other researchers have shown encapsulation of IFTs if they are left in too long, which would likely change the calibration factor. 2) Even though the actual speed of the treadmill was well controlled, the animals did not hop at a constant speed. In theses studies the frequency of hopping of the rabbits was not controlled. It is possible that by better controlling the speed of the animal's activity or by directly measuring the speed using trip detectors, even more significant effects of altering activity level could have been obtained. 3) The joint flexion angles during the five activities and calibration were not monitored. A knowledge of how tensile forces relate to join kinematics would have being useful in better understanding recruitment patterns for different muscle groups. Joint flexion angles can be assessed by employing optical or actual contact measurements. Optical techniques provide the flexion angles in one plane (e.g. the sagittal plane) and they are not very precise. Actual contact measurements [Hasler et al. 1998] can be recorded by inserting pins in bone but they affect the gait of the animal. 4) While post-mortem calibration may not have fully

replicated all the *in-vivo* loading situations, the tendon was carefully handled to ensure that the IFT did not move within the tissue before or during calibration. Even if the transducer relocated, however, a previous *in-vitro* study showed that after intentionally misaligning the IFT, the device realigned itself and provided identical outputs when loads were reapplied to the tissue [Glos et al., 1993]. 5) Ground reaction forces were measured between the operated and nonoperated limbs during quiet standing (QS) using the TEKscan system. It would be useful to perform these measurements while the animals were hopping to determine the extent to which the animal favored the operated limb. The ground reaction forces applied by both limbs were not measured before surgery. It would be useful to perform these force measurements before surgery to quantify the effect of IFT implantation on the distribution of loads between limbs.

5.2 Implications of the Work

Several important implications arise from the recognition that muscle forces correlate, in rather complex ways, with animal activity level.

1. The muscle-tendon unit maintains a minimum level of in-vivo force, regardless of activity level. While the muscle-tendon unit is normally subjected to low *in-vivo* forces during quiet standing, these minimum forces are sustained, even as the level of activity increases. Thus, even when the limb is experiencing no ground reaction forces, the muscle is still transmitting a minimum level of force to its in-series tendon component. Furthermore, this minimum force level appears to be independent of the level of activity. The group at the University of Cincinnati has noted similar results in the goat patellar tendon [Cummings et al. 1991, Korvick et al. 1996] and rabbit flexor tendon models [Malaviya et al. 1998], where a level of force, comparable to quiet standing, was always generated during the swing phase of gait. Thus, in all three model

systems, tendon maintains a homeostatic condition in the presence of a small baseline force, augmented by periodic bouts of increased force. By contrast, ligaments like the anterior cruciate [Korvick et al. 1992] become unloading during the swing phase of gait. This period of unloading may explain why ligaments have adapted to become inherently weaker and more compliant than tendons [Butler et al. 1986].

2. Increased activity increases the maximum rather than the minimum muscle force. As shown in Fig. 4 and Fig. 5 for PT, Fig. 10 and Fig. 11 for AT, increasing the level of activity increases peak *in-vivo* force without affecting minimum force. Such data suggests that by monitoring the frequency and level of the specific activity in which the rabbit is engaged, one can estimate the peak forces acting on the tendon. If this is true, one may be able to minimize the use of implantable force transducers currently needed to estimate the peak forces, transmitted to the tendon and to the bone, which culminate in motion. To exhibit this level of confidence in these muscle force predictions will, of course, require similar measurements in other muscle-tendon systems in the rabbit and in other animal models.

3. The rates of rise and fall are proportional to the level of activity. Linked to the increase in peak maximum force with increasing activity are increases in the rate of rise and fall in muscle force (Fig. 6 and Fig. 7 for PT, Fig. 12 and Fig. 13 for AT). As more muscle fibers are recruited, not only is a new peak force generated, but the rate at which this peak is achieved, and the rate at which this force is dissipated back to a baseline value are increased as well.

4. Comparison between in-vivo patellar tendon and Achilles tendon force. In chapter three *in-vivo* data for the rabbit patellar tendon (PT) was presented. In figure 3 one can observe that the force signal for patellar tendon did not show the initial drop in the force level that is present in Achilles tendon (Fig. 9). It was hypothesized that the initial drop in the AT force level

was due to the release of the gastrocnemius muscle force in preparation for the jump and because the Achilles tendon is part of a two joint muscle-tendon unit. The more complex nature of the Achilles tendon may be due to the independent flexion motions of the two joints. In contrast, the patellar tendon is part of a one joint muscle-tendon unit.

The magnitude of minimum force, maximum force, rate of rise and rate of fall were compared for patellar tendon and Achilles tendon. These four parameters were significantly different ($p<0.001$) between the tendons across the same four levels of activity: Level Hopping at 0.1 mph and at 0.3 mph, and Inclined Hopping at 0.1 mph and at 0.3 mph. Moreover the values of the mentioned parameters were higher for patellar tendon than for Achilles tendon. This can be due to the difference in position in the joints.

The levels of speed had little effect on the hopping speed, possibly because the speeds were very low and very close to each other. The surgery could also have affected the hopping speed of the animals. The parameters most sensitive to speed and inclination were maximum force, rate of rise and rate of fall, for both patellar and Achilles tendon.

Peak *in-vivo* forces and stresses in the rabbit patellar tendon were approximately 10% of the tendon's ultimate load and ultimate stress capacities, giving a safety factor (ratio of failure force to *in-vivo* force) of 10, for all activities tested. For rabbit Achilles tendon, peak *in-vivo* forces and stresses were on average approximately 19% of the tendon's ultimate load and ultimate stress capacities, producing a safety factor of 5, for all activities tested. The magnitude of the *in-vivo* loads measured during these tests was considerably smaller than was expected in the study design and less than the results found for tendons from previous studies [Cummings et al. 1991, Korvick et al. 1996, Malaviya et al. 1998]. These differences might be explained by the

fact the activities imposed during the current studies are less rigorous than might be demanded in the wild and less than those from previous investigations.

Rabbit patellar tendon TEKscan data (Fig. 8) showed that the rabbits were imposing nearly equal ground reaction forces on both limbs. The ratio between the force applied by the operated limb and the force applied by the unoperated limb was 0.98 ± 0.04 (mean ± SEM). For the rabbit Achilles tendon (Fig. 17), TEKscan data showed that the rabbits were also imposing equal forces in both limbs. The ratio between the force applied by the operated limb and the force applied by the unoperated limb was 1.03 ± 0.1 (mean ± SEM). These results showed that the rabbits were placing equal ground reaction forces on both limbs.

5.3 Future Research Directions

The current study also supports the long-term goal of establishing structure-function relationships for soft connective tissue. To truly understand how *in-vivo* forces modulate connective tissue structure and chemistry, one must first understand the normal force patterns, which this structure must transmit during daily activities. The resulting homeostatic state of the tissue depends not only upon the magnitude of the forces it experiences, but the entire pattern of input signals. Knowing these dynamic force patterns then permits one to alter these mechanical signals at surgery to determine their subsequent effect. While such measurements have been made in bone, the data reported for soft tissue has been primarily restricted to average or peak values. Hence, this study is one of the first to make appropriate measurements of *in-vivo* forces in a model suitable for studying soft tissue remodeling.

These studies showed that increasing the intensity of activity results in a significant increase in peak tendon force. Other structures like the soleus and tibialis anterior tendons in the

cat do not show this behavior [Gregor et al., 1988; Herzog et al., 1993; Prilutsky et al., 1994]. But the current findings are consistent with *in-vivo* measurements from the human Achilles tendon [Komi 1990; Komi et al., 1992], goat patellar tendon [Korvick et. al, 1996], cat gastrocnemius tendon [Herzog et al., 1993; Prilutsky et al., 1994], and rabbit flexor tendon [Malaviya et al. 1998]. Possibly, the response of the Achilles tendon differs from both the soleus and tibialis anterior tendons because its series muscle has fewer slow-twitch fibers available [Gonyea et al. 1981], leading to a stronger contractile force. These fast-twitch Achilles muscle fibers would generate and transmit forces at a more rapid rate like the gastrocnemius and quadriceps muscle fibers, but this require further study.

It is recommended that new experiments should be performed in order to understand the initial drop in the level of force that it is observed in Achilles tendon. Using electromyography techniques it is possible to identify which group of muscles contracts before the rabbit hops. In electromyography the electrical activity of a skeletal muscle is recorded by means of an electrode inserted into the muscle or placed on the skin. In future studies it is important to assess the duty cycle, the percent of the time in which the animals move. To accomplish that, video recording of rabbit normal activity before surgery should be performed.

The *in-vivo* forces measured using the IFTs should also be correlated with the video to understand precisely what physiologic events are associated with specific points on the load-time pattern for each tendon model. For Achilles tendon the maximum force corresponds with toe-off (plantar flexion of the ankle) and the minimum force with dorsiflexion of the ankle. For patellar tendon the peak force coincides with the flexion of the knee and the minimum force with the extension of the knee.

The long-term goal of this study was to establish design parameters for tissue engineered implants containing mesenchymal stem cells (MSCs) that are to be used to repair injured tendons like the rabbit patellar tendon and Achilles tendon. The results of this study provide critical information for tissue engineers preparing biologic implants. The *in-vivo* force parameters recorded in this study will be employed to mechanically stimulate tissue engineered implants in culture. In modeling the *in-vivo* force pattern for the *in-vitro* stimulation, maximum force is a more relevant parameter to track than quiet standing since maximum force indicates the peak loads that the implants would experience *in-vivo.* In addition, initially low peak strain levels (1%) should be employed to mechanically stimulate the implants. After the implants have been accustomed to these low levels of strain, the peak strain levels can be progressively increased to reach the maximum strain levels (3%) that patellar and Achilles tendon experience *in-vivo.* It is expected that the use of tissue engineered implants containing MSCs, and the application of mechanical stimulation to them, will result in implants that will more effectively repair surgical defects in this same tissue. This research will ultimately help surgeons more efficaciously treat problem tendon injuries.

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