IN-VIVO FORCES IN THE RABBIT PATELLAR TENDON
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Introduction
The objective 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.

In designing the MSC constructs the biomechanical function of the tissue being replaced has to be considered. In fact, the biomechanical properties of this tissue are critical to its proper function in-vivo. In order to repair or replace this structure effectively it is necessary to know the patterns and thresholds of force, stress, and strain that normal tissues transmit or encounter and the mechanical properties of the tissue when it is subjected to expected in-vivo stresses and strains, as well as under failure conditions [1]. For rabbit patellar tendon (PT), two hypotheses were tested: first that peak in-vivo forces and the rates of rise and fall in in-vivo forces increase with increasing levels of activity and second that the safety factor (ratio of tendon ultimate force to peak in-vivo force) for the PT will remain above 2.5 for all activities tested [2,3].

Methods
The PT was instrumented in eight one-year-old female New Zealand White rabbits using implantable force transducers (IFTs). These transducers generate a voltage output approximately proportional to the tissue’s tensile force. In-vitro calibration after sacrifice allows forces to be computed from these voltages. The IFT was implanted in the left PT employing sterile surgical procedures approved by the University of Cincinnati Institutional Animal Care and Use Committee (IACUC). The treatment variable was the level of activity. Activity levels were controlled using a treadmill (True, Model 350, St. Louis, MO). Five levels of activity were evaluated in all rabbits, including quiet standing (QS) to simulate in-vivo forces during disuse or inactivity, level hopping (LH) at 0.1 mph and 0.3 mph, to mimic “in-cage” movements, and for 12 degrees of inclined hopping (IH) at 0.1 mph and 0.3 mph, to imitate more vigorous exercise. Ten trials (sets of three hops from back to front of treadmill) were performed for each activity per animal. Trial order was randomized to minimize bias due to fatigue. All in-vivo forces were collected three days post surgery to allow the animal to recover from surgery so as to ensure more normal hopping activities.

In-vitro calibration was performed twenty minutes after sacrifice. The quadriceps tendon was attached to a spring scale in situ (Remington Arms, Madison, NC) using a #5 Ethicon suture (Ethicon, Somerville, NJ). A series of loads, ranging from 1 lb to 15 lb, were applied using the scale in 1 lb increments to obtain the voltages associated with these loads. The calibration revealed a nearly linear relationship between voltage and muscle force. Four parameters (minimum force, maximum force, rate of rise and rate of fall) were recorded from the second hop (since this hop was unaffected by the enclosure used to contain the animal on the treadmill).

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 within-block experiment was a 2 factorial treatment structure in a completely randomized design. A mixed-model analysis method was used with specimens as the random factor, all other factors being fixed. All conclusions regarding the significance of activity on in-vivo force measurements were made at the $\alpha = 0.05$ experiment-wise level.

Results
Forces during quiet standing stayed constant at low values whereas forces for level and inclined hopping were much larger for each trial. In-vivo forces in the PT always remained greater than zero. Forces during quiet standing remained steady for the 8 animals, averaging 14.9 ± 1.7 N (mean ± SEM). The average peak forces for level and inclined hopping were 67.9 ± 0.8 N (mean ± SEM) and 82.3 ± 1.8 N (mean ± SEM), respectively. Patellar tendon peak force significantly increased with increasing activity level, being significantly greater for inclined hopping (IH, 12° inclination) than for level hopping (LH, 0° inclination) (p<0.001) [Fig. 1], 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).

In-vivo force rise times for both level and inclined hopping were nearly identical, requiring approximately 0.3 seconds to complete and independent of peak force achieved. The rates of rise and fall for level and inclined hopping were 166.9 ± 3.6 N s$^{-1}$ (mean ± SEM) and 216.2 ± 11.7 N s$^{-1}$ (mean ± SEM), respectively. The rates of rise and fall in PT force also significantly increased with increasing level of inclination and speed (p<0.001 for both) [Fig. 2].

The PT always maintained a minimum level of force throughout each trial across all four activities. These minimum forces were 13.8 ± 1.3 N (mean ± SEM) and were not significantly different across activities (p>0.1). Peak in-vivo forces and stresses in the patellar tendon were on average about 10% of the tendon’s ultimate load and stress capacities, indicating safety factors of approximately 10 for these activities.

Discussion
The results of this study show that increasing the intensity of activity results in a significant increase in peak tendon force. The rates of rise and fall for LH and IH correlated with peak force since the time (0.36) required to achieve peak force was constant. 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. The magnitude of the loads measured during these tests was considerably smaller than was expected based on prior studies [2,3]. This could be explained by the fact that the activity modeled in this study is considerably less rigorous than might be demanded in the wild. These data will be employed to mechanically stimulate tissue engineered implants in culture to mimic in-vivo force patterns.

References

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