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Repetitive loading damages healing ligaments more than sustained loading demonstrated by reduction in modulus and residual strength

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ABSTRACT

Healing ligaments have decreased strength compared to normal ligaments, leaving healing ligaments vulnerable to damage accumulation during normal daily activities at functional stresses. Rabbit medial collateral ligament gap scars after 14 weeks of healing were exposed to long-term creep and fatigue loading over a range of functional stresses. In addition to the 58 healing ligaments that underwent in vitro creep and fatigue testing, seven healing ligaments underwent only monotonic failure tests for comparison with residual strength tests that followed creep and fatigue testing. When exposed to repetitive loading during fatigue testing, healing ligaments exhibited modulus reduction earlier than when exposed to sustained loading during creep testing that was occasionally interrupted with unloading/reloading cycles to measure modulus. In other words, after the same loading duration, repetitive loading was more damaging than sustained loading. At modulus reduction, the increase in strain during fatigue was greater than or similar to that during creep. Healing ligaments that were damaged during long-term loading exhibited decreased strength and increased toe-region strain during subsequent residual strength tests. Normal daily activities that result in repetitive loading of a ligament healing from an injury will likely cause damage to accumulate faster than activities that result in sustained loading.

1. Introduction

During normal daily activities, ligaments are subjected to repetitive and sustained loading at 5–10% of their ultimate tensile strength (UTS) (Butler et al., 2003). Ligaments healing from an injury have decreased UTS compared to normal ligaments (Chimich et al., 1991; Thornton et al., 2003, 2005). Daily activities at functional stresses for normal ligaments have the potential to cause damage to healing ligaments, which may have clinical implications when designing rehabilitation protocols following ligament injury. In addition to decreased UTS, healing ligaments have decreased modulus during repetitive loading compared to normal ligaments (Thornton et al., 2002, 2003). In healing ligaments, we propose to track modulus reduction during long-term creep and fatigue loading even though the modulus of a healing ligament is, at the outset, already less than a normal ligament.

In normal ligaments, modulus reduction was a marker of damage during long-term creep and fatigue loading as evidenced by decreased strength and increased toe-region strain in residual strength tests (Thornton et al., 2007a). Damage accumulation was tracked by evaluating the modulus ratio which was the modulus at a time-point normalized to the maximum modulus achieved during loading (Thornton et al., 2007a). During creep and fatigue in normal ligaments, modulus ratio versus time curves had three characteristic regions (Thornton et al., 2007a): increasing; constant; and decreasing modulus. These three regions of modulus were related to three regions of strain (Thornton et al., 2007a, 2007b): decreasing; steady-state; and increasing strain rate. Modulus reduction occurred at the transition from constant to decreasing modulus and was related to the transition from steady-state to increasing strain rate (Thornton et al., 2007a). In these normal ligaments, fatigue caused damage in less time and with less strain than creep (Thornton et al., 2007a).

Our objective was to investigate whether healing ligaments were capable of accumulating damage during long-term loading. Our first hypothesis was that modulus reduction in healing ligaments would occur at earlier times and smaller strains during fatigue than creep, like in normal ligaments (Thornton et al., 2007a). Our second hypothesis was that damage during long-term loading would result in decreased strength and increased toe-region strain in residual strength tests.

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2. Methods

Female one-year-old New Zealand White rabbits underwent bilateral medial collateral ligament (MCL) gap surgeries in this study approved by the University of Calgary Animal Care Committee. Removal of a small (2.0 ± 0.5 mm) section from the MCL midsubstance resulted in a 4.0 ± 0.5 mm gap due to retraction of the ligament ends (Thornton et al., 2000). After 14 weeks of healing with unrestricted cage activity and immediately following euthanasia, hindlimbs were harvested by disarticulation at the hip and ankle, and frozen at -20 °C.

Seven 14-week healing MCLs from seven rabbits underwent monotonic failure testing to determine UTS and failure force (FF) to normalize stress/force levels of creep and fatigue tests, and to compare against residual strength of healing MCLs after creep and fatigue testing. Fifty-eight 14-week healing MCLs from 32 rabbits were subjected to either creep or fatigue, with paired MCLs assigned to each group. Because the UTS of healing MCLs was decreased compared to normal MCLs (Chimich et al., 1991; Thornton et al., 2003, 2005), stress/force levels were relative to UTS or FF of healing MCLs: 30%UTS (creep $n=4$; fatigue $n=8$), 45%UTS (creep $n=4$; fatigue $n=4$), 60%UTS (creep $n=11$; fatigue $n=10$), 60%FF (creep $n=8$; fatigue $n=9$).

2.1. Mechanical testing

After thawing, the hindlimb was dissected, leaving the menisci and collateral and cruciate ligaments intact. The femur was cut 30 mm proximal to the MCL insertion, and the tibia was cut at the fibula-tibia junction. The tibia was cemented with polymethylmethacrylate in the upper grip which was then mounted in series with a 500 N load cell. Force was zeroed after ensuring the healing MCL was aligned with the load axis of the actuator (MTS Systems Corporation, Minneapolis, MN). The femur was then cemented in the lower grip, ensuring approximately 70° of knee flexion, before force and displacement were zeroed.

After two compression–tension cycles from -5 N to $+2$ N, the healing MCL was isolated by removing the lateral collateral ligament, medial and lateral menisci, and anterior and posterior cruciate ligaments. After completing two additional compression–tension cycles and stopping at $+1$ N to establish “ligament zero”, displacement was zeroed. Then, MCL length was measured using digital calipers and MCL midsubstance cross-sectional area (CSA) was measured using area calipers (Shrive et al., 1988). After environment chamber equilibration (37 °C and 99% relative humidity), the healing MCL underwent two additional compression–tension cycles to re-establish “ligament zero”.

2.1.1. Monotonic failure

In the monotonic failure group, healing MCLs were preconditioned for 30 cycles using a sine wave at 1 Hz from $+1$ N (“ligament zero”) to a force corresponding to 5% of previously reported UTS of 14-week healing MCLs (23.4 ± 8.9 MPa, $n=6$) (Thornton et al., 2003). After preconditioning, healing MCLs were elongated to failure at 20 mm/min.

2.1.2. Fatigue

Using the UTS of the monotonic failure group, healing MCLs were preconditioned for 30 cycles using a sine wave at 1 Hz from $+1$ N to a force corresponding to 5%UTS. For fatigue testing, healing MCLs were loaded repeatedly using a sine wave at 1 Hz from $+1$ N to a force corresponding to the desired stress/force level. Tests continued until rupture, until the deformation safety limit was reached, or until the allotted test time was reached (24.0 or 48.4 h). If fatigue tests ended before rupture, healing MCLs underwent additional mechanical testing to measure residual strength.

2.1.3. Creep

Using the UTS of the monotonic failure group, healing MCLs were preconditioned for 30 cycles using a sine wave at 1 Hz from $+1$ N to a force corresponding to 5%UTS. For creep testing, healing MCLs were loaded using a sine wave at 1 Hz from $+1$ N to a force corresponding to the desired stress/force level and then held at that force. Interruptions to track modulus in an otherwise static test (Thornton et al., 2007a; Wang and Ker, 1995) occurred initially at 0.8 h and subsequently every 1.16 h. An interruption involved unloading to $+1$ N, reloading to the desired force using a sine wave at 1 Hz, and then holding at that force again. Tests continued until rupture, until the deformation safety limit was reached, or until the allotted test time was reached (24.0 or 48.4 h). If creep tests ended before rupture, healing MCLs underwent additional mechanical testing to measure residual strength.

2.1.4. Residual strength

If healing MCLs did not rupture during long-term loading, they were unloaded to $+1$ N and allowed to recover for 1 h. Following recovery, healing MCLs underwent two compression–tension cycles from -5 N to $+2$ N, and then 30 cycles of preconditioning using a sine wave at 1 Hz from $+1$ N to a force corresponding to 5% of the UTS of the monotonic failure group. After preconditioning, healing MCLs were elongated to failure at 20 mm/min.

Four healing MCLs were specifically allocated for residual strength testing. These tests were stopped at deformation limits based on deformations during long-term loading: initial loading, modulus reduction, and rupture. After reaching the desired deformation limit, the force was decreased to $+1$ N and the healing MCL was allowed to recover for 1 h followed by residual strength testing as described above.

2.2. Analysis

2.2.1. Modulus

Stress was recorded force divided by CSA. Strain was deformation divided by undeformed MCL length. Modulus was the slope of the linear regression of the upper 25% of the cyclic stress–strain curve. Maximum modulus was the maximum calculated modulus during long-term loading. Modulus ratio was the modulus at a time-point divided by the maximum modulus. Rupture modulus ratio was the modulus ratio in the last cycle completed before rupture. To detect the onset of damage, time and increase in strain at modulus reduction were evaluated using the last cycle where modulus ratio was equal to 0.90. Increase in strain was strain relative to initial strain which was the strain when the healing MCL was initially loaded to the desired test force.

2.2.2. Residual strength

Residual strength was residual failure force divided by CSA. Failure strain was deformation at residual failure force divided by undeformed MCL length. Tangent modulus was the slope of the linear regression of the upper 25% of the failure stress–strain curve before discontinuity. Toe-region strain was taken from the failure stress–strain curve as the strain at 5% of the UTS of the monotonic failure group.

2.2.3. Statistics

Data was first tested for normality using a Shapiro-Wilk test. If data followed a normal distribution, then a paired t-test was used for complete pairing or a linear mixed model regression with random effects was used for incomplete pairing. If data did not follow a normal distribution, then a Wilcoxon signed-rank test was used for complete pairing or a Mann–Whitney-U rank-sum test was used for incomplete pairing. For comparison of three groups when data did not follow a normal distribution, a Kruskal-Wallis test was followed by Conover post-hoc analyses.

3. Results

3.1. Modulus ratio

At all stress/force levels, maximum modulus was not different between creep and fatigue. Modulus ratio versus time curves generally had three regions (Fig. 1): increasing modulus; relatively constant modulus; and rapidly decreasing modulus. After the same amount of time exposed to loading, fatigue caused more damage than creep because the modulus decrease was greater.

3.2. Rupture modulus

At all stress/force levels other than 30%UTS, rupture modulus ratio was less for fatigue than creep ($p < 0.04$; Fig. 2). At 30%UTS, only one healing MCL of the four tested had ruptured during 48.4 h of creep loading (Fig. 2). The rupture modulus ratio differences between creep and fatigue may be due to the timing of the creep interruptions used to measure modulus because the true maximum modulus may have occurred between interruptions and/or modulus reduction may have occurred between the last interruption and rupture.

3.3. Modulus reduction

For most fatigue modulus ratio versus time curves, modulus ratio of 0.90 acted as a natural lower boundary for the relatively constant modulus region below which the curves entered the rapidly decreasing modulus region (Fig. 1). The largest fatigue rupture modulus ratio was 0.86 (60%FF). Using a modulus ratio of