Introduction: The clinical outcome of combined ligament injuries is typically worse than that for isolated ligament tears [1]. In particular, combined injury to the medial collateral and the anterior cruciate ligaments (ACL) of the knee inevitably leads to increased joint laxity and instability.

Both biological and mechanical causes for the inferior healing response in combined injuries have been proposed. For example, combined injury may increase the initial inflammatory response leading to elevated levels of inflammatory mediators and altered expression of key matrix molecules in the healing tissue [2]. Alternatively, in vivo mechanical stresses on a healing ligament may exceed the tissue’s ability to transmit load leading to persistent re-injury.

The aim of this study was to measure the effect of one week vs. two weeks of ACL deficiency on the mechanical behaviour of the healing medial collateral ligament (MCL). We hypothesized that healing MCLs from ACL-deficient joints would demonstrate increased creep strain and laxity when compared with MCLs from ACL-intact joints. We further hypothesized that both creep strain and laxity would be greater after two weeks of combined injury than after one week.

Methods: Twenty skeletally mature, female NZW rabbits (average mass: 5.5 ± 0.4 kg) were utilized in this study approved by our institutional animal care committee. Experimental animals were divided into two groups (A and B), with both groups undergoing bilateral surgical transection of the MCL at the joint-line. Four weeks following transection, animals in group A (n = 8) underwent ACL transection in one hindlimb, while the contralateral limb received a sham operation (capsulotomy). The limb undergoing ACL transection was randomly assigned. Animals in group B (n = 8) underwent the same protocol as group A, with ACL transection occurring at five weeks post MCL injury. At six weeks, all animals were sacrificed. Both hindlimbs were dissected rapidly (musculature removed, ligaments and menisci intact) and prepared for mechanical testing. Age-matched, uninjured animals served as controls (n = 4).

In vitro mechanical testing: Limbs were potted and mounted at approximately 70° of flexion in custom-designed clamps in a servohydraulic testing machine (MTS Systems, Eden Prairie, MN, USA). An alignment jig was utilized for mounting all joints to ensure consistent alignment of ACL-intact vs. ACL-deficient joints. Laxity: The whole joint was subjected to two cycles of 5N compression and 2N tension at 1mm/min. The ACLs of ACL-intact joints were then transected and the joint subjected to another two compression-tension cycles. Finally, the LCL and PCL were sectioned in all joints, compression-tension cycles were repeated and stopped at 0.1N to establish “ligament zero”. MCL length and cross-sectional area were measured. Creep testing: Following installation of an environment chamber (99% relative humidity, 37°C), “ligament zero” was established. The MCL was then cyclically loaded between “ligament zero” and 30N for one hour at 1 Hz. Following creep testing, ligaments were allowed to recover at zero load for one hour and were then fail at an extension rate of 20mm/min. Outcome measures: MCL laxity was defined as the distance between “ligament zero” (+0.1N) and the point where the tibial and femoral condyles began to transmit compressive load across the joint (40N). Cyclic creep strain was defined as the strain at the peak of the 3600th cycle minus the strain at the peak of the first cycle. Data were analyzed using paired and unpaired Student’s t-tests.

Results: Data from one animal in group A was excluded due to a power failure during mechanical testing. One MCL scar in group A (ACL-deficient) and four scars in group B (1 ACL-deficient/3 ACL-intact) failed during cyclic loading. Exclusion of failed samples did not alter the results of the statistical tests for any of the parameters investigated here. The results of the paired t-tests are shown (group A, n = 6; group B, n = 4).

MCL scars from ACL-deficient knees had significantly greater cross-sectional areas than scars from ACL-intact legs, with all scars having larger cross-sectional areas than uninjured controls (data not shown). Sectioning the ACL before or after mounting had no significant effect on measures of MCL laxity or creep strain in the uninjured controls (p > 0.05). Normal values were therefore pooled for all subsequent analyses.

Discussion: MCL scars in an ACL-deficient environment (2 or 6 weeks of deficiency) have previously been shown to be biomechanically inferior to scars from ACL-intact joints [3]. In the current study, we have demonstrated that scars from ACL-deficient joints had greater ligament laxity after one or two weeks of ACL deficiency. Interestingly, there was no difference in laxity or creep strain between MCL scars from joints subjected to one or two weeks of ACL deficiency, suggesting that alterations to these mechanical properties occurs within one week of ACL injury. The high incidence of failure in group B scars, in particular in MCLs from ACL-intact joints, may be attributable to inflammation-induced weakening or may represent alterations in loading of the joint – weight-bearing on the ACL-intact limb, placing higher stresses on the healing MCL. Overall, this study demonstrates that alterations in the material properties of ligament scars occur within one week of injury to the ipsilateral ACL. The rapidity with which these changes occur points to altered loading patterns in vivo, rather than “remodelling”, as the probable cause of increased laxity and creep strain in ACL-deficient joints. The ‘stretching-out’ of MCL scars with loading in this combined injury model may help to explain the poor outcome that is seen clinically in patients with combined injuries.

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