

Viscoelastic properties of collateral ligaments in a rabbit model eight weeks after anterior cruciate ligament transection

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INTRODUCTION: Injuries to the anterior cruciate ligament (ACL) are the most prevalent knee injuries among individuals engaged in sports activities. ACL injuries lead to alterations in load distribution across other knee joint tissues. However, the impact of ACL ruptures on the mechanical properties of the collateral ligaments remains underexplored. We hypothesize that eight weeks after an ACL injury, there are alterations in the viscoelastic properties and microstructure of the collateral ligaments in both the injured knee and the uninjured contralateral knee.

METHODS: Unilateral anterior cruciate ligament transection (ACLT) surgery was performed on the knees of skeletally mature female New Zealand White rabbits (N = 6, age 12 months at the time of surgery). Eight weeks after the ACLT, the lateral collateral (LCL) and Medial collateral ligaments (MCL) were collected from both ACLT, and the contralateral (CL) knees. In addition, ligaments were collected from both knees of four unoperated age-matched rabbits for control purposes (CNTRL). Three ligaments were damaged during the harvest, leaving the following number of ligaments in the different groups: nLCL = 5, nMCL = 6 in ACLT group; nLCL = 6, nMCL = 5 in CL group; and nLCL = 8, nMCL = 7, in control group. All procedures were conducted under the guidelines of the Canadian Council on Animal Care and were approved by the Animal Ethics Committee at the University of Calgary. The cross-sectional area of samples was measured using micro-computed tomography by placing them in sealed tube to prevent drying. Subsequently, ligaments were subjected to a stress-relaxation test up to 8 % strain, and let to relax for 30 min. Then, at 8 % strain, a sinusoidal loading test was conducted using 0.5 % strain amplitude for 5 cycles at frequencies of 0.01, 0.05, 0.1, 0.5, 1, and 2 Hz. After biomechanical testing samples were histologically processed and cut to 5µm thick sections that were deparaffinized and imaged unstained for quantitative polarized light microscopy to analyze collagen orientation (0° = parallel to ligament axis). The storage and loss moduli of the collateral ligaments were compared between groups using a linear mixed model. In the model, group, ligament type, and test frequencies were defined as categorical fixed effects, while ligament within animal and knee within animal were defined as random effects with random intercept. Log transformation was applied to non-normal data and normality of residuals and heterogeneity of the models were checked.

RESULTS: The storage modulus of the LCL CL group knees was significantly higher than that of the ACLT group across all frequency ranges. The greatest difference was observed at a frequency of 0.1 Hz, with a standardized mean difference of 4.71 MPa (95% confidence interval: 1.62 to 7.80 MPa, $p = 0.01$). The LCL loss modulus of CL group knees was significantly higher than that of the ACLT group knees at all frequencies. The highest difference was at 2 Hz with a standardized mean difference of 4.24 MPa (95% confidence interval: 1.40 to 7.08 MPa, $p = 0.10$). The percentage of parallel collagen fibers was greater in LCL and MCL samples of CNTRL group animals than that of ACLT animals with a mean difference of 6.4 (95% confidence interval: -1.4 to 14.2, $p = 0.12$) and 7.8 (95% confidence interval: -1.8 to 17.3, $p = 0.13$) respectively, however the differences were not statistically significant.

DISCUSSION: The storage modulus of the LCL in ACLT group knees was significantly lower than that of the CL group knees across all frequencies (Figure 1.A). This result suggests that there might be structural and compositional changes in the LCL of ACLT knees that reduce LCL's ability to store elastic energy. The loss modulus, which reflects energy dissipation, increased in the LCL and MCL with increasing frequency in all groups, but the loss modulus of the LCL CL group knees was significantly higher than ACLT group knees at all frequencies (Figure 1.B). PLM results indicated that the collagen fibers in the CNTRL and CL group knees were well aligned compared to those in ACLT group knees (Figure 1.C). In conclusion, collateral ligaments of CL knees get stiffer due to loading alterations in animal knees following transection surgery; however, the relationship between changes in viscoelastic properties and changes in collagen fiber alignment is not straightforward. Considering that the study's findings are constrained by certain limitations, further research could benefit from a larger sample size and the inclusion of a sham-operated knee group to assess surgery-related effects on the mechanical properties of the collateral ligaments.

SIGNIFICANCE/CLINICAL RELEVANCE: The outcomes of this study enhance our understanding of the effects of isolated ACL injury on the collateral ligaments of the knee. These findings may be used to investigate the efficacy of graft materials employed in ligament reconstruction surgery.

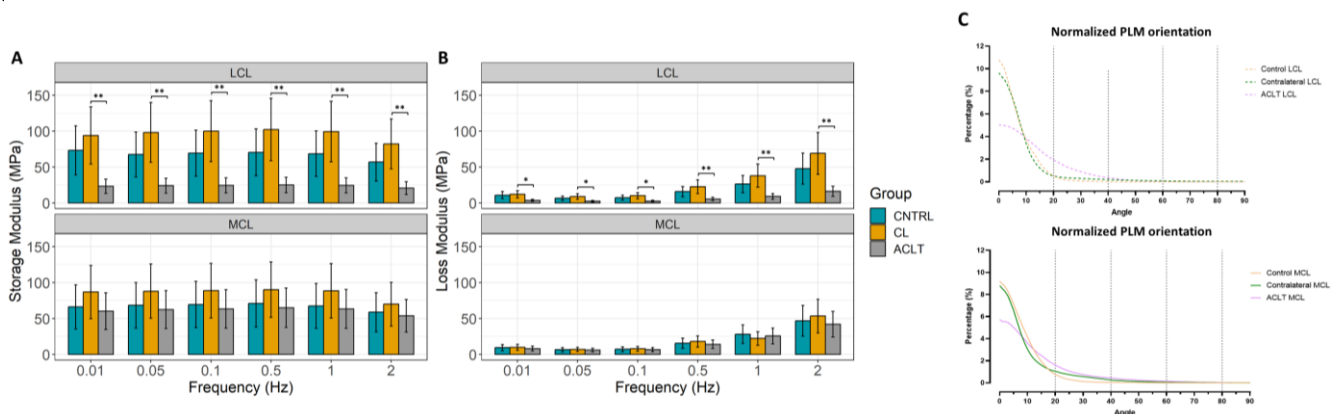


Figure 1 – Storage modulus (A) and loss modulus (B) of lateral collateral (LCL) and medial collateral ligaments (MCL) of control (CNTRL), contralateral (CL), and anterior cruciate ligament transected (ACLT) group animals at loading frequencies of 0.01, 0.05, 0.1, 0.5, 1 and 2 Hz. The bar plot illustrates the estimated marginal mean of each group and the corresponding standard error (whiskers). (* $p \leq 0.05$ and ** $p \leq 0.01$). The collagen fiber angle distribution (C) in the LCL and MCL are shown separately for control (CNTRL), contralateral (CL), and anterior cruciate ligament transected (ACLT) group animals. Collagen fibers with 0° and 90° are considered parallel and perpendicular to the ligament longitudinal axis, respectively.