INTRODUCTION: Mechanical regulation of ligament fibroblasts has traditionally been studied by using degradable substrates to apply strains to cells following their isolation from ligament tissue [1]. In vivo, however, the mechanisms of mechanical regulation of ligament fibroblasts may utilize transduction processes that involve the fluid-filled extracellular matrix or cell-matrix interactions. Isolation of cells from the ligament tissue is likely to alter such processes and, therefore, the microenvironment of fibroblasts during loading. One alternative approach is to use the ligament tissue itself, so that the cell-matrix microenvironment is maintained during an applied load. The entire ligament from an animal (i.e., rat) may be small enough to allow cells to be viable in an entire ligament organ. The objectives of this study were 1) to develop a system in which ligament tissue can be maintained viable ex vivo and subjected to load, and 2) to determine how mechanical stimuli may regulate expression of type I collagen by fibroblasts in ACL tissue.

METHODS: Male Wistar rats (300-400g; 4 mo. old) were obtained within one hour after sacrifice. Each ACL was carefully dissected free while the insertions to the femur and tibia were maintained by preserving ~8 mm² of bone. During surgery all tissues of the knee joint, particularly the ACL, were generously hydrated with PBS containing penicillin (100 U/mL), streptomycin (100 µg/mL), and fungizone (0.25 µg/mL) (PSF). ACL cell viability. In one set of experiments, viability of ACL fibroblasts in the tissue was assessed immediately after and 24 hours after this preparatory treatment. Briefly, ACLs were excised from bony attachments and incubated in PBS containing 25 µg/mL fluorescein diacetate (FDA) and 20 µg/mL propidium iodide (PI) for 20 minutes. Tissues were then frozen by liquid nitrogen, sectioned on a cryostat into 30 µm slices, and immediately fixed with 2% glutaraldehyde. Sections were then visualized under epifluorescence microscopy. Percentages of cell viability were determined by manual cell counts of live and dead cells. Response of ACL to loading: In tissue loading experiments, bone portions were embedded in Osteobond bone cement (Zimmer, Inc., Warsaw, IN) using a mold. Bone cement pieces were then placed in specially designed polycarbonate clamps in a loading and culture vessel. Each assembly was filled with 70 mL Dulbecco’s Modified Eagle Medium supplemented with 0.8%, n=5, immediately after sample harvest, and 89.6 ± 1.0%, n=6, after 24 hour culture of harvested ACLs. There was no significant difference in cell percentage cell viability immediately after and 24 hours after the harvest procedure. At both time points, a high percentage of cells were alive (89.2 ± 0.8%, n=5, immediately after sample harvest, and 89.6 ± 1.0%, n=6, after 24 hour culture of harvested ACLs). There was no significant difference in cell viability between the 0 hour and the 24 hour groups (p = 0.71).

A representative autoradiograph of type I collagen and corresponding rRNA bands is shown in Figure 2a. Normalized expression levels of mRNA encoding type I collagen is illustrated in Figure 2b. For specimens subjected to a 2 hour constant load increment of 5 N, expression of type I collagen significantly decreased to 52 ± 10% (n=5; p<0.01) of control values.

DISCUSSION: This study introduces a novel bone-ACL-bone ex vivo preparation for the investigation of the effects of mechanical load on gene expression in the rat ACL. Cell viability assays indicated that fibroblasts in the ACL were viable after the tissue harvest procedure. Furthermore, with culture of the tissue for 24 hours, cell viability did not decrease, demonstrating the feasibility of maintaining the bone-ACL-bone complex for the duration of mechanical loading experiments. Application of a physiologic level of load on the ACL demonstrated a significant decrease in mRNA levels of type I collagen. Since the failure load of rat ACL is 31 N [2], it is estimated that the rat ACL likely operates at loads of ~6 N [3]. Thus, the observed inhibition of type I collagen mRNA levels is likely to be a physiological type of response and may be different from responses by isolated ligament fibroblast monolayers strained by an elastomer membrane. The time course of the gene expression response to the onset of steady or dynamic load remains to be determined. The development of this experimental system allows the study of ligament fibroblasts in their native extracellular matrix environment. Such a system may be valuable in future studies on the biology and healing processes of the ACL.


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Fig 1 - (a) Epi-fluorescence micrograph of cells in the rat ACL stained with FDA (live; green) and PI (dead; red) after the ACL harvest procedure. (b) Cell viability (%) of fibroblasts in the rat ACL immediately after and 24 hours after the ACL harvest procedure (± SEM).

Fig 2 - (a) Representative autoradiograph showing the decrease in type I collagen mRNA after 2 hour 5 N constant load increment. (b) Type I collagen mRNA levels decrease after a 2 hour application of a constant 5 N load increment (± SEM).