INTRODUCTION: Myofibroblasts are specialized fibroblasts that express the contractile protein α-smooth muscle actin (α-SMA). Increased numbers of myofibroblasts in joint capsules have been associated with human elbow joint contractures. A recently reported rabbit model of post-traumatic knee joint contractures has been characterized biomechanically for motion loss. Our objective is to evaluate myofibroblast numbers in the joint capsule in this model and to determine whether myofibroblast numbers are elevated in the knees with contractures.

METHODS: After approval from the institutional animal care committee, eighteen skeletally mature (12-15 months old, 5.3 ± 0.5 kg) New Zealand White (NZW) female rabbits had five-mm-squares of cortical bone removed from the nonarticular portions of both femoral condyles of the right knee. An extraarticular 1.6-mm-diameter Kirschner wire (K-wire) immobilized the knee in maximum flexion. The left knee served as an unoperated control. The rabbits were equally divided into 3 groups; Group 1 had 4 weeks of immobilization, Group 2 had 8 weeks of immobilization and Group 3 had 8 weeks of immobilization followed by K-wire removal and 32 weeks of remobilization.

Biomechanical measures of joint motion were performed in groups 2 and 3 immediately after sacrifice. The posterior joint capsule was harvested immediately after sacrifice in group 1. The joint capsules were frozen in OCT using N; Standard immunohistochemical methods were used. Double labeling was used to distinguish myofibroblasts from blood vessels using α-SMA (clone asm-1, Roche) monoclonal antibodies and laminin affinity conjugated antibody was used for laminin. Nuclei (cells) were labeled with DAPI (Vector Laboratories). Nuclei (cells) associated with a-SMA and laminin colabeling were considered blood vessels while cells that were α-SMA positive but laminin negative were considered myofibroblasts (Figure 1). Counts of myofibroblasts and total cells were present in groups 2 and 3 as previously reported.

Myofibroblasts were significantly increased in the posterior knee joint capsules of the experimental knees of all 3 groups when compared with the contralateral knees (Table 1). This applied to both absolute counts (p < 0.001) and the percentage of myofibroblasts to total cells (p < 0.001). There were no significant differences (p > 0.05) in the total number of cells between experimental and control sides. When comparing between the experimental knees in each group, there were no significant differences in the total number of cells or the absolute numbers of myofibroblasts (p > 0.05). However, there were significant differences between the groups when considering percentage of myofibroblasts to total cells. Posthoc analysis (student’s t-test with Bonferroni correction) showed significantly elevated values in group 2 when compared to the other 2 groups (Table 1).

DISCUSSION: Our laboratory investigates the pathogenic mechanisms underlying the changes in the joint capsule in post-traumatic contractures. It has been determined that increased myofibroblast numbers in joint capsules are associated with human elbow joint capsules. A model of post-traumatic joint contractures where a long-standing stable loss of motion develops has been characterized. The present study now evaluates myofibroblast numbers in the posterior knee joint capsules in this rabbit model. It was found that myofibroblast numbers and percentages of myofibroblasts to total cells were significantly elevated in the rabbit knees that were injured and immobilized when compared with the contralateral unoperated knees. This was true for all stages investigated, including more acute stages (Group 1) and chronic stages (Group 3) in the contracture process. The work in human elbow contractures studied more chronic stages of the process since all individuals had the surgical releases performed between 5-24 months after the injury. The parallels in elevated myofibroblast numbers chronically and joint motion loss with time when comparing the rabbit knee model of joint contractures with the human clinical condition further validates the animal model.

This unique rabbit model of post-traumatic contractures is promising but further work is required. Future work will include further characterization of myofibroblast regulators and growth factors in the human condition and the rabbit model. Investigation will also focus on the matrix changes within the joint capsules.

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REFERENCES: