INTRODUCTION: A porcine model of dermal wound healing has recently shown a genetic basis in skin wound healing. The skin of two different pig breeds demonstrated a difference in their healing response where the red Duroc pig developed a unique healing phenotype characterized by hypercontracted, hyperpigmented scars. A genetic influence on healing may pertain to other connective tissues such as ligament. The objectives of this study were: (1) to create a porcine biomechanical model for medial collateral ligament (MCL) healing in order to evaluate initial and tensile properties; (2) to compare the pig MCL scar to uninjured pig MCL, and (3) to compare the MCL healing characteristics between the Yorkshire (YK) and red Duroc (RD) breeds. We hypothesized the creep response to be greater and the tensile properties to decrease in the wounded porcine MCL compared to contralateral and normal porcine MCL and there to be a difference between the YK and RD MCL healing response.

METHODS: Institutional animal care committee approval was obtained. A 4mm section of the MCL midsubstance was removed from the right knees of four RD and four YK female pigs (4-5 months old, 58 ± 8 kg). The transected ends of the MCL were marked with sutures. Following surgery, the animals resumed cage activity for a 10 week healing period. Contralateral limbs served as unoperated controls and normal knee joints were obtained from two size and gender matched pigs of each breed. All animals were skeletally immature throughout the course of the study. At the time of sacrifice (6-8 months old, 95 ± 31 kg), hindlimbs were dissected at the hip and ankle, wrapped in saline soaked gauze, sealed in plastic bags, and frozen at -80°C until tested. Twenty-four hours prior to testing, limbs were thawed at 4°C. Muscle and fascia from the femur and tibia were removed while the menisci, collateral and cruciate ligaments remained intact. Bones were transected approximately 15 cm from the joint line. The knee joint was mounted with polymethylmethacrylate in custom clamps designed for the MTS system and was kept at physiological conditions (37°C, 99% humidity). The knee joint was placed at 70-85° of flexion while ensuring the MCL was aligned with the load axis of the actuator. The whole joint underwent 2 cycles of 20 N of compression to 8 N of tension at 1 mm/min. Menisci and cruciate ligament and collateral ligaments were removed, leaving the femur-MCL-tibia complex (FMTC). Two additional compression-tension cycles were performed in order to establish ‘ligament zero’. ‘Ligament zero’ is the cross-head position at which the ligament begins to take up a load of 0.1 N. This was used as the starting point for all tests. Digital calipers were used to measure the MCL length at the proximal and distal ends of the ligament. The MCL was loaded for 30 cycles at 1 Hz to 100 N for cyclic creep. The MCL was then immediately loaded to 100 N and held at that constant load for 20 minutes for static creep. Subsequently, the MCL was loaded to failure at 20 min/min.

Total creep was defined as the increase in deformation from the peak of the first cycle in cyclic creep to the end of 20 minutes of static creep. Cyclic and static creep were the deformation increases during those respective tests. Experimental and contralateral limbs were compared to each other using a paired t-test. A Student’s t-test was used to compare experimental and contralateral limbs to normal FMTCs and between experimental FMTCs of the two breeds. The data are presented as mean ± standard deviation where p-values were significant if p ≤ 0.05.

RESULTS: After 10 weeks of MCL healing, the pigs formed a large amount of scar tissue around the wound site. A significant difference in cross-sectional area was observed between injured MCL and contralateral and normal MCL (p < 0.05). The injured MCLs were approximately five times larger in size than the contralateral and normal ligaments. A difference in scar tissue production was not observed between the breeds. The typical nonlinear structural behavior of soft tissues was seen in the load-deformation curves. The ultimate tensile strength (UTS) was similar between experimental (786.10 ± 261.59 N), contralateral (870.71 ± 130.70 N), and normal FMTCs (924.81 ± 104.41 N). However, it is important to note that the tensile failure properties of the FMTCs were different. Four different types of failures occurred: tibial avulsion, tibial insertions, femoral condyle fractures, and midsubstance. Tibial avulsion failures occurred when bone broke off from the ligament; whereas, tibial insertion failures were when the ligament detached itself from the tibial insertion site. The majority of the MCL scars failed in the midsubstance (71%) whereas only 38% of the normal and contralateral FMTCs failed in the ligament midsubstance. YK and RD pigs did not differ in UTS or failure mode. A significant increase was observed in the creep response between experimental FMTCs compared to contralateral and normal FMTCs (Table 1). The experimental FMTCs demonstrated significantly greater creep compared to the contralateral and normal FMTCs. The static and total creep deformations of the injured FMTCs were significantly increased in the injured RD pig compared to the injured YK pig (Table 2). The data for one of the YK pigs was excluded because only the anterior border of the MCL was transected during the surgical procedure.

DISCUSSION: This study presents preliminary results obtained from a newly designed porcine model of MCL healing and is the first to biomechanically evaluate and compare YK and RD pig breeds. The present results reveal a significant increase in the creep response (cyclic, static, and total) in injured FMTC compared to contralateral and normal FMTC. The two pig breeds displayed significantly different low load behaviors with static and total creep parameters for RD FMTCs being significantly greater than YK FMTCs. The results also demonstrate that there were no differences in UTS between the two breeds.

The creep data is consistent with previous studies conducted on healing rabbit MCL which have also demonstrated inferior creep behavior of MCL scars compared to normal and contralateral MCLs. A difference in static and total creep was detected between the scars of the two breeds. Since the RD forms more contracted skin wounds scars than the YK pigs, it was surprising to observe the increased creep response in the RD scar compared to the YK scar. We expected the RD MCL scar to form a more contracted ligament scar whereby it would not creep as much as a YK ligament scar. Due to a low sample size (n=3 and 4) for breed comparison, more work needs to be conducted before these results are conclusive. However, these results suggest that there are differences in the ligament healing response of different breeds.

A similarity in UTS between injured FMTCs and normal and contralateral FMTCs was observed. While failure sites differed in injured versus uninjured FMTCs making quantitative comparisons of the MCL properties impossible, it is clear that the porcine ligament scar remains inferior to contralateral and normal ligaments. The larger cross-sectional area of the injured MCLs would imply inferior MCL material properties. The high prevalence of tibial avulsion and insertion failures may be a result of skeletal immature animals.

In summary, a difference in MCL healing biomechanical properties in two pig breeds supports previous work on skin wound healing that suggests a genetic modification of these processes in pigs. Further work is required to understand molecular changes contributing to these processes and contributions of other factors such as age/maturity, sex/gender and tissue (skin/ligament).

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