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Wound healing differences between Yorkshire and red Duroc porcine medial collateral ligaments identified by biomechanical assessment of scars

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ABSTRACT

Background: Currently, there are no large animal models to assess potential genetic contributions to ligament biomechanics during an injury repair response. Yorkshire and red Duroc pigs display phenotypically and genetically different skin wound healing responses; red Duroc skin scars were hyper-contracted and hyper-pigmented, whereas Yorkshire skin scars were not. Such findings raise the question whether connective tissues of synovial joints display a similar differential healing response in these pig breeds. This study assessed medial collateral ligament healing in Yorkshire and red Duroc pigs at the functional (biomechanical) level.
Methods: Surgical injury was created in the right hind limb medial collateral ligament of Yorkshire and red Duroc pigs. After 10 weeks of healing, low-load (laxity and creep) and high-load (failure) mechanical properties were measured.
Findings: Large, complete ligament scars formed by 10 weeks post-injury. A differential healing response was observed between the breeds, where red Duroc ligament scars had larger cross-sectional areas, exhibited greater static and total creep responses, failed at greater deformations and strains ($P \leq 0.05$), and failed with strong trends for higher loads and lower moduli ($P = 0.06$) than Yorkshire ligament scars.
Interpretation: The ligament healing response of red Duroc pigs differs from Yorkshire pigs. Previously observed breed differences in dorsal skin wound healing are not restricted to skin. Such findings support a genetic basis for breed differences in response to connective tissue injury. Since this animal model is physiologically comparable to humans, these findings could provide further insight into identification of specific genetic contributions to ligament repair in human populations.

1. Introduction

1.1. Clinical relevance

While it is clear from the literature that genetic factors, mostly related to matrix proteins or their regulation, play a significant role in risk for injuries to ligaments and tendons (Grahame, 2000; Gwilym et al., 2009; Khoschnau et al., 2008; September et al., 2007), there is little information regarding genetic influences on endogenous repair processes when such tissues are injured (Gwilym et al., 2009). However, there is some evidence to support that gene intervention enhances ligament and tendon repair (Dahlgren et al., 2002; Hildebrand et al., 2004; Nixon et al., 2007). While some experiments in knockout and transgenic mice have provided insights into possible genetic correlations (Ansorge et al., 2009; Lin et al., 2006; Mikic et al., 2009), there is no evidence regarding genetic influences on ligament healing in a larger, pre-clinical animal model with a physiology similar to humans (e.g. the domestic pig) (Germscheid et al., 2011). Because of this similarity, investigation of porcine breed differences in ligament healing represents a first step towards identifying specific genetic contributions to ligament repair and subsequent identification of these genetic factors in human populations.

1.2. Purpose

Following injury to connective tissues like skin, joint capsule, and ligament, the injury site in species such as humans and pigs heal by matrix deposition, contraction, and remodeling (Gallant-Behm and Hart, 2006; Hildebrand and Frank, 1998). Why it is that some individuals are more susceptible to pathologic scar formation than others is not well understood, but recent studies have indicated a role for genetic factors in both human (Clark et al., 2009) and porcine (Gallant-Behm and Hart, 2006; Gallant-Behm et al., 2006; 2007) fibroproliferative conditions.

Previously, a skin wound pig model was used to investigate the genetic, cellular, and molecular basis for abnormal skin wound contracture and scarring (Gallant-Behm and Hart, 2006; Gallant et al., 2004; Gallant-Behm et al., 2006, 2007; Wang et al., 2001) between

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dorsal dermal healing of Yorkshire (YK) and red Duroc (RD) pigs. RD pigs formed abnormal skin wounds characterized as fibrotic, hypercontracted, and hyper-pigmented (Gallant et al., 2004). This scarring was a unique healing phenotype which is specific to RD pigs and did not occur in YK dorsal skin scars. The mechanisms governing hypercontraction in RD pigs are unknown; however, further studies have indicated that there is a genetic component (Gallant-Behm and Hart, 2006; Gallant-Behm et al., 2006, 2007). Interestingly, the healing of oral mucosal wounds in the RD pig occurred fairly normally (Eslami et al., 2009; Mak et al., 2009; Wong et al., 2009); therefore, some genetic aspects associated with abnormal wound healing may be tissue-restricted.

As mucosal wound healing may be unique (Eslami et al., 2009; Mak et al., 2009; Wong et al., 2009), it would be important to assess wound healing in YK and RD pigs where another tissue that more closely resembles skin was injured. Knee ligaments are similar to skin in that they are mainly comprised of a collagen type I-rich matrix (Amiel et al., 1987; Frank et al., 1983a,b; Murphy et al., 1994) and work in a low-load biomechanical environment (Thornton et al., 2000). Following injury to the medial collateral ligament (MCL) of the knee, a scar response is initiated which undergoes a defined series of events (Frank et al., 1999a, 1999b) similar to skin. Similarly, the original structural organization and functional properties are not restored within these tissues after healing (Corr et al., 2009; Frank et al., 1999a). Recent studies have revealed that the biomechanical properties of the normal YK and RD MCL are similar (Germscheid et al., 2011). Thus, the apparent similarities in the normal MCL tissues would allow for assessment of whether the response to injury was unique when the MCL was injured.

The present study investigates whether the biomechanical properties of porcine MCLs exhibit a differential healing response between the YK and RD pig breeds. We hypothesized that a differential healing response would exist between the geometric and biomechanical properties of the healing YK and RD pig ligament, where the RD pig would exhibit a greater healing response than the YK pig, thus displaying a unique healing phenotype. Additionally, we hypothesized that the healing porcine MCL would be biomechanically inferior to the uninjured porcine MCL.

2. Methods

2.1. Animals

Adolescent female YK and RD pigs were procured from Pig Improvement Canada Ltd. (Airdrie, AB). Animals were individually housed indoors at the University of Calgary Veterinary Sciences Research Station. They were given food and water ad libitum and cared for by an experienced technician. Prior to receiving a hind limb operation, animals were acclimatized to their surroundings for 7–10 days. All animal protocols were approved by the University of Calgary Animal Care Committee in accordance with the Canadian Council on Animal Care guidelines. All animals were skeletally immature throughout the course of the study.

2.2. Surgical procedure

Excisional wounds were created in the right knee MCLs of eight YK [4–6 months old, mean 55 (SD 11) kg] and six RD [4–6 months old, mean 63 (SD 4) kg] female pigs. Animals were pre-medicated with intramuscular ketamine (15 mg/kg, Bimeda-MTC Animal Health Inc., Cambridge, ON), acepromazine (0.4 mg/kg, Ayerst Veterinary Laboratories, Guelph, ON), and glycopyrrolate (0.2 mg/mL, Sandox Canada, Inc., QC) which was followed by general anesthesia (3–5% isofluorane). All animals required endotracheal intubation. The medial aspect of the right hind limb was shaved and sterilized with betadine solution. Longitudinal skin and fascial incisions were made over the medial side of the right knee. The MCL was exposed and a 4 mm section of the MCL midsubstance was removed. The ends of the MCL retracted, creating an 8 mm gap (Supplementary Fig. 1). The transected ends of the MCL were marked with 4-0 non-absorbable braided sutures (Ethicon, Johnson & Johnson Medical Products, Peterborough, ON). All fascial defects were closed with 2-0 synthetic absorbable monofilament sutures (Ethicon, Johnson & Johnson Medical Products, Peterborough, ON). Pain control was achieved by administering butorphanol (0.2 mg/kg, i.m., Wyeth Animal Health, Guelph, ON) and metacam (5 mg/mL, i.m., Boehringer Ingelheim, Ltd., Burlington, ON) for 24 and 72 h, respectively. Excenel (5 mg/kg, i.m., Pharmacia Animal Health, Orangeville, ON) was the administered antibiotic. Within a few hours after surgery, all animals were able to stand. The left knee served as the contralateral control. All of the surgeries were conducted by the same personnel.

Animals resumed cage activity for a 10-week healing period. At 5 weeks post-surgery, dermal sutures were removed and the knees were examined for possible abnormalities. At 10 weeks post-surgery, animals were sacrificed [YK: mean 107 (SD 14) kg; RD: mean 110 (SD 12) kg] with an intracardiac overdose of sodium pentobarbital (Euthanyl, 0.5 mL/kg, Bimeda-MTC Animal Health Inc., Cambridge, ON). Both hind limbs were disarticulated at the hip and ankle, wrapped in saline soaked gauze, sealed in plastic bags, and frozen at −20 °C. Freezing has been shown to not affect the biomechanical properties of rabbit ligament (Woo et al., 1986).

2.3. Specimen preparation

Limbs were thawed at 4 °C for 24 h prior to biomechanical testing. The tibia and femur were transected approximately 15 cm from the joint line. Muscle, fascia, and capsule were removed from the knee joint while the menisci and collateral and cruciate ligaments remained intact. No samples showed signs of degenerative joint disease or previous ligamentous injuries. Since the superficial aspect of the anterior border of the MCL was attached to the anterior joint capsule, scars not only encompassed the ligament midsubstance, but also formed on the capsule's anterior border. Therefore, ligament scars were carefully dissected based on the tibial and femoral insertion sites and the suture markers (Fig. 1).

The tibia was securely cemented with polymethylmethacrylate (Westan Dental Products Group, Calgary, AB) into the upper clamp of a custom-built apparatus designed for the MTS system (MTS Systems Corporation, Minneapolis, MN) (Germscheid et al., 2011). After curing, the upper clamp was mounted to a 5 kN load cell on the hydraulic actuator of the MTS. Once the joint was positioned at approximately 80° of flexion and the ligament was aligned with the load axis of the actuator, the load was zeroed and the femur was cemented to the lower clamp. A humidity-blower was used throughout the testing procedure to ensure the knee joint and MCL were maintained at physiological conditions (~37 °C, 99% relative humidity) (Germscheid et al., 2011).

2.4. Biomechanical testing protocol

A previously developed porcine MCL testing protocol conducted on normal YK and RD porcine MCLs was used (Germscheid et al., 2011). Compression–tension cycles consisting of 2 cycles of 20 N of compression and 8 N of tension at 1 mm/min were performed before isolating the MCL. Whole joint laxity was defined as the displacement recorded when the entire joint was loaded between 20 N of compression and 8 N of tension. The femur–MCL–tibia-complex (FMTC) was created by sequentially removing the lateral collateral ligament, the medial and lateral menisci, and the cruciate ligaments. Two additional compression–tension cycles were performed and stopped at 0.1 N of tension to establish 'ligament zero'. MCL laxity was defined as the displacement of the isolated MCL between 0.1 N of compression and 0.1 N of tension.