TGF-β–Mediated Arthrofibrosis Following Septic Arthritis in a Porcine Stifle Model

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Introduction: Arthrofibrosis (AF) is triggered by inflammation after trauma, infection, surgical procedures, or prolonged joint immobilization[1]. AF manifests as the accumulation of fibrotic collagen within the synovium and connective tissues of diarthrodial joints, including the shoulder, elbow, wrist, hip, knee, and ankle[1]. Like other fibrotic conditions, AF pathophysiology is driven by inflammation-induced activation of Transforming Growth Factor Beta (TGF-β) in the synovium leading to elevated deposition of fibrotic type-I collagen[2]. Clinically, AF presents as painful restricted joint motion[1,3]. Current therapeutic options remain limited and largely symptomatic treatments, which include physical therapy, intra-articular corticosteroid injections, NSAIDs, and nerve blockers[3]. These provide only marginal or temporary symptomatic relief. Other treatments center around inflammation reduction by using COX-1, COX-2 inhibitors[3]. However, none of these pharmacological therapies address the dysregulated deposition of collagen in synovial tissues[2]. Surgery, including manipulation under anesthesia, arthroscopic synovectomy, and/or capsulotomy, restores some joint range of motion but is associated with risks, such as further exacerbating AF[2]. Importantly, septic arthritis (SA) is a common clinical precursor to AF, with poor patient – reported outcome rates of 20-30% despite appropriate antimicrobial and surgical management[4,5]. This is why establishing a reproducible animal model of SA – induced AF provides a critical platform to evaluate both surgical and pharmacologic treatment strategies and to assess the efficacy of potential therapies. **Methods:** *Animal Model:* Three healthy, 3-month-old castrated male Yorkshire pigs were assigned to 72-hour, 2-week, and 12-week groups. Under general anesthesia, the left stifle joint of each pig was inoculated with 0.1 mL of *Staphylococcus aureus* (2.5×10^6 CFU/mL) at 2-hour intervals (9:20 a.m., 11:20 a.m., and 1:20 p.m.), while the right stifle served as the control. Clinical monitoring included daily physical examinations and pain assessments, including weight-bearing, to confirm the development of SA during the first week after inoculation. Weekly ultrasonography of both stifles (Terason™, Teratech Corporation, Burlington, MA, USA) and synovial fluid (SF) collection from the left stifle were performed to evaluate total protein (TP), nucleated cell count (NCC), and cytology **(Fig. 1A1–3)**. Additional SF samples were stored at −80 °C for ELISA assays (DuoSet, R&D Systems) to quantify active and latent TGF-β isoforms (1, 2, and 3). Animals were euthanized at predetermined time points (72 hours, 2 weeks, and 12 weeks; n = 1 per time point) for tissue collection, and SF from the right stifle (control) was collected at that time. Synovial and cartilage tissues were subjected to histological and immunohistochemical (IHC) evaluation of pSmad2/3 and α-smooth muscle actin (α-SMA) expression to assess TGF-β–mediated signaling and myofibroblast differentiation. Raman spectroscopic imaging was used to evaluate biochemical composition and collagen deposition within the synovium and cartilage. *Inoculum Preparation: S. aureus* (ATCC 25923) was cultured overnight in tryptic soy broth at 36.5°C with agitation (235 rpm). A 15 mL aliquot was centrifuged (1200×g, 10 min), and the pellet was resuspended in 10 mL sterile Phosphate-Buffered Saline. After measuring Optical Density (OD) at 600 nm, the suspension was adjusted to OD 0.3 and serially diluted to 2.5×10^6 CFU/mL. Inoculum concentration was confirmed by colony counting. **Results:** ELISA assay data were obtained from stifle SF samples collected from the 72-hour and 2-week models until their endpoints, and from the left stifle SF of the 12-week model up to week 3 after inoculation. Histological, IHC, and Raman spectroscopic data were obtained from stifle tissues of the 72-hour and 2-week models **(Fig. 3)**. The day after inoculation, all three pigs exhibited visible lameness in the inoculated joints, which were warm and painful on palpation. Three days after inoculation, pain scores of all models decreased over the week following inoculation. In terms of synovial fluid NCC and TP, the 72-hour model showed markedly increased values consistent with an acute inflammation response, with serosanguineous fluid supporting an active synovial response. The 2-week model demonstrated mildly elevated NCC and TP with a hemorrhagic appearance, compatible with a mild post-inflammatory or traumatic reaction. In the 12-week model, both parameters gradually decreased from week 1 to 5, with fluctuating NCC around 1,000 cells/uL indicating a transition from active inflammation toward improvement or low-grade inflammation **(Fig. 1B1-2)**. Ultrasonographic evaluation of synovial capsule thickness revealed distinct differences between the left (inoculated) and right (control) stifle joints. Thickening was observed in all inoculated stifles, while the 12-week model exhibited reduced differences between the left and right stifle joints beginning from week 9 to week 12 after inoculation **(Fig. 2)**. SF analysis of the left stifle joint (inoculated) demonstrated a large increase in active TGF-β3 **(Fig. 3A)**, relative to right stifle joint (control) 3 days after induction of SA. Further, latent TGF-β1 and latent TGF-β2 isoforms were elevated in the inoculated joints at days 3 and 7 **(Fig. 3B)**, indicating sustained TGF-β activation in the infected joint tissues. Histology showed elevated p-smad2/3 and alpha-Smooth Muscle Actin (α-SMA) expression at days 3 and 14, demonstrating TGF-β activation and conversion of synovial fibroblasts to myofibroblasts **(Fig. 3C)**. Raman imaging showed elevated protein content **(Fig. 3D)**, consistent with collagen deposition and capsular fibrosis. **Discussion:** This study successfully established a porcine model of SA that progresses toward TGF-β–mediated AF. The acute inflammatory response observed in the 72-hour model was characterized by markedly increased synovial fluid NCC and TP, indicating an intense inflammatory phase. In contrast, the 2-week and 12-week models demonstrated gradual resolution of inflammation, reflected by declining cell counts and reduced differences in synovial capsule thickness over time. These findings suggest a transition from acute synovitis to chronic, low-grade inflammation associated with early fibrotic remodeling. Consistent with these observations, molecular analyses revealed significant activation of the TGF-β signaling pathway. The early increase in active TGF-β3 following infection supports its role as an upstream regulator of tissue remodeling, while the sustained elevation of latent TGF-β1 and TGF-β2 isoforms suggests ongoing activation of pro-fibrotic signaling in the joint environment[6]. The upregulation of p-Smad2/3 and α-SMA confirms downstream TGF-β pathway activation and the differentiation of synovial fibroblasts into myofibroblasts, which are key mediators of AF[7]. Furthermore, Raman imaging demonstrated increased protein content within the joint capsule, consistent with collagen accumulation and fibrotic tissue formation. Collectively, these findings indicate that SA initiates a self-sustaining fibrotic response driven by persistent TGF-β signaling, leading to capsular thickening and potential loss of joint mobility. **Significance**: This study established a reproducible porcine model that shows the natural transition from SA to TGF-β–mediated AF. By combining clinical, histologic, and molecular analyses, it confirms the transition from acute synovial inflammation to chronic fibrotic remodeling within the joint capsule. The demonstrated activation of latent and active TGF-β isoforms, along with downstream Smad2/3 signaling and myofibroblast differentiation, provides direct mechanistic evidence linking infection-induced inflammation to fibrosis. This model offers a relevant platform for investigating the molecular drivers of post-infectious joint contracture and for testing targeted anti-fibrotic therapies aimed at modulating the TGF-β/Smad axis.

References: [1] Walocha A et al. *Folia Med Cracov*. 2022;62(1):55–70. [2] Blessing WA et al. *Trends Pharmacol Sci*. 2021;42(5):398–415. [3] Chen AF et al. *Connect Tissue Res*. 2019;60(1):21–28. [4] Leroy R et al. *Ann Rheum Dis*. 2016;75:851. [5] Abram SGF et al. *Lancet Infect Dis*. 2020;20(3):341–349. [6] Hinz B, Lagares D. *J Exp Med.* 2019;216:1823-1845. [7] Watson RS et al. Arthritis Res Ther. 2010;12(2):R35.

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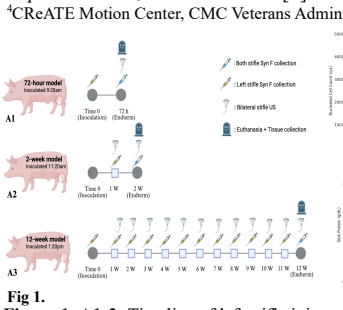

Fig 1.

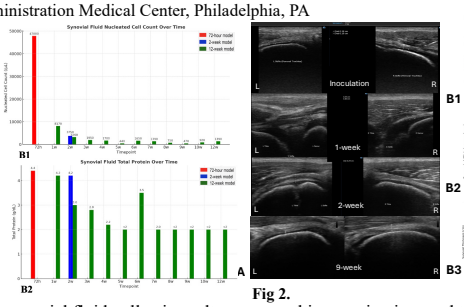

Fig 2.

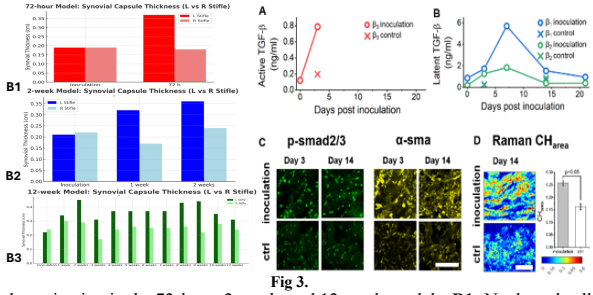

Fig 3.

Figure 1. A1-3: Timeline of left stifle joint synovial fluid collection, ultrasonographic examination, and termination in the 72-hour, 2-week, and 12-week models. **B1:** Nucleated cell counts in left stifle synovial fluid across all models. **B2:** Total protein levels in left stifle synovial fluid across all models. **Figure 2. A:** Ultrasonographic images of left and right stifle joints at inoculation, and 1-, 2-, and 9-weeks post-inoculation. **B1-3:** Bar graphs showing weekly bilateral changes in synovial capsule thickness across all models. **Figure 3. :** Based on synovial fluid collected from stifle joints up to 3 weeks after inoculation in all models and synovial tissue collected from the stifle joints in the 72-hour and 2-week models. Elevation of (**A)** active TGF-β3 and (**B)** latent TGF-β 1/2 in porcine stifle SF post bacterial inoculation. (**C**) Elevated expression of pSmad2/3 and α-SMA in synovium post inoculation. Bar: 100 μm (**D)** Raman images of synovium show elevated collagen deposition (CH_{area} signal) post inoculation. Bar: 250 μm