Histological Analysis of Structural Alterations Towards Innervation Changes in Human Knee Osteoarthritis Patients

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DISCLOSURES: Y. Cruz-Almeida: 8; Associate Editor for Journal of Pain. 9; Treasurer of the US Association for the Study of Pain. K. Allen: 8; Associate Editor for Osteoarthritis and Cartilage.

INTRODUCTION: Knee osteoarthritis (OA) is a degenerative joint disease that affects the entire joint as an organ, warranting a holistic assessment of structural and innervation changes along with the patient-reported symptoms to have a clearer understanding of the disease.[1] Innervation changes associated with OA severity are not well understood. While most joint tissues, excluding hyaline cartilage, are richly innervated, the nerve density can vary based on tissue type and location. To characterize innervation in knee OA, regions of interest must be identified to observe where innervation changes may be occurring. In this work, we have performed histology to understand the pathological and structural changes in osteoarthritic joints. In addition, we have developed a pipeline for future work to systematically map joint alterations, using both 2D structural histology and 3D innervation analyses.

METHODS: Patients (N=15, n=5 males, median age=71; n=10 females, median age=71.5) from the University of Florida Shands Hospital underwent chronic pain assessment followed by total knee arthroplasty (TKA). Immediately following TKA, the following tissues were collected and transferred to our dissection team on ice in phosphate-buffered saline (PBS): tibia, femur, synovial lining, infrapatellar fat pad (IPFP), anterior collateral ligament (ACL), and meniscus. These tissues were photographed and mapped on a dissection map to accurately preserve the spatial information before dissection and allocation for histological analysis. Samples were fixed in 4% paraformaldehyde (48 hrs, 4ºC). and washed with PBS to remove excess fixative. Hard tissues (tibia and femur) were decalcified in STAT Immunocal (7-9 days, room temperature), and the extent of decalcification was confirmed via CT imaging. For 2D analysis, following decalcification and dehydration with xylene, tissues were infiltrated and embedded with paraffin wax for microtome sectioning (5-10 µm). Slides were either stained with Safranin-O/Fast-Green (Saf-O/FG) (tibia, femur, meniscus) or Hematoxylin and Eosin (H&E) (synovial lining, IPFP, ACL, meniscus) and coverslipped. Brightfield images were taken using a Zeiss Axio Observer microscope with either the 10X or 20X objective and stitched using the Zeiss Zen Blue software. For 3D imaging, samples were cleared using the polyethylene glycol (PEG)-associated solvent system (PEGASOS)-based tissue clearing protocol[2], immunostained with anti-neurofilament antibody (anti-NF, 1:400, Abcam, ab215903) followed by secondary antibody (goat anti-mouse IgG, Dylight 633, ThermoFisher, cat # 35512), and imaged using an UltraMicroscope BLAZE (Miltenyi Biotec).

RESULTS: Tibia and femur samples showed signs of tidemark multiplication at the osteochondral junction, bone marrow adipose tissue and fibrosis, and superficial fibrillation of the cartilage **(Fig. 1A).** Proteoglycan content was evident in the interior of the lateral middle body meniscus sample, with intensity varying across patients **(Fig. 1B,C)**. Further, synovium obtained from the lateral aspect of the joint had evidence of immune cell infiltration **(Fig. 2)** and presence of vasculature and lymphatic structures **(Fig. 2B,C)**. Tissues from TKA (meniscus, bones) were cleared with the PEGASOS protocol and were optically transparent **(Fig. 3)**. Immunostaining with anti-NF antibody revealed the presence of NF+ fibers of the cleared samples, which were largely localized at the meniscal attachment sites **(Fig. 3)**.

DISCUSSION: Characterizing the structural and innervation changes in human OA will be important for future work to help characterize the disease pathology and create effective treatments. Our work demonstrated clear OA pathology in patients with TKA, including synovial inflammation, immune cell infiltration, and bone changes at the osteochondral junction, among others. Increased proteoglycan content has been demonstrated in the meniscus of patients with knee OA, corroborating our observations **(Fig. 1B,C)**.[3] The main meniscal body was primarily acellular; however, cellularity was increased at the meniscal attachment sites compared to the middle body, as expected (results not shown). Signs of immune infiltration **(Fig. 2B)** and lymphatic vessels **(Fig. 2C)** were observed in the synovium of the patients. Synovitis, or inflammation of the synovium, was observed in our samples, which is a common symptom of OA.[4] The initial characterization of bone, meniscus, and synovium will be utilized to identify regions of interest for immune and innervation staining. The clearing of post-fixed surgical tissues, including hard and soft tissues (meniscus and bone), demonstrates a significant advance towards innervation mapping in clinical tissues. The meniscus and bone had the presence of NF+ fibers (Fig. 3). The work will culminate in the characterization of 15 patients who underwent TKA to identify innervation and structural features of OA. Future work aims to process more samples and quantify results.

SIGNIFICANCE/CLINICAL RELEVANCE: Through the development of 2D and 3D imaging pipelines, we can further assess structural and innervation changes to develop a deeper understanding of osteoarthritic degradation.

REFERENCES: **(1)** Loeser RF, *et al*., *Arthritis and Rheum,* 64, 1697-1707(2012). **(2)** Jing D, *et al., Cell Research*, 28, 803-818 (2018). **(3)** Sun Y, *et al*., *Rheumatol J*., 6, 24-32 (2012). (4) Eitner A, et al., Osteoarthritis and Cartilage, 21(9), 1383-1391 (2013).

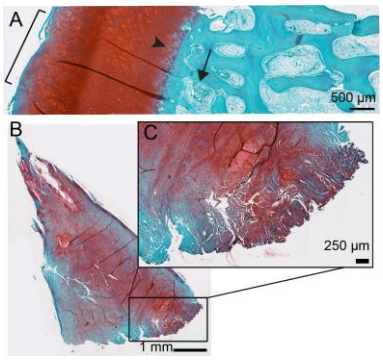

Figure 1: A) Medial tibia stained with Saf-O/FG (20X). Lateral meniscus stained with Saf-O/FG at 10X **(B)** and 20X **(C)**. Bracket indicates surface fibrillation; Arrowhead denotes the osteochondral junction; Arrow denotes the bone marrow space.

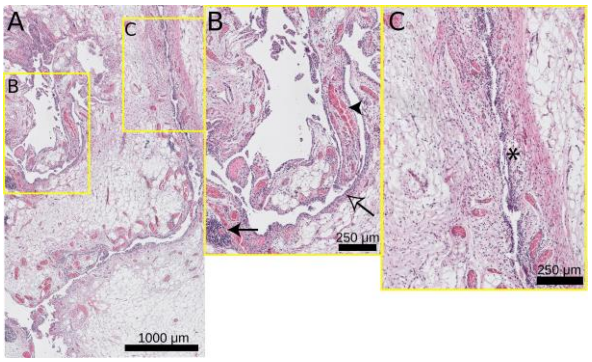

Figure 2: Lateral synovium stained with H&E at 10X **(A)**, and 20X **(B,C)**. **B)** Region of synovium with increased immune cell activity (arrow), vasculature (arrowhead), and synovial lining (empty arrow), **C)** Region of the synovium with a lymph structure (*).

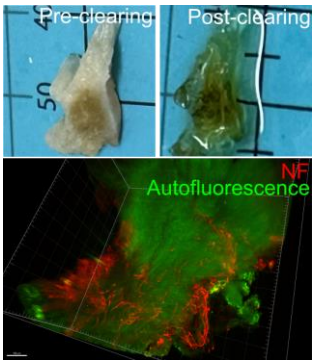

Figure 3: PEGASOS-based tissue clearing renders meniscus optically transparent. Detection of NF+ nerve fibers (red) at the meniscal attachment site. Green represents autofluorescence. Scale bar: 500 µm.