“Differential Expression of Fibrogenic Biomarkers in Naïve Human Synoviocytes Cultured in Synovial Fluid from Knee Osteoarthritis Patients” to “Fibrogenic Responses of Naïve Synoviocytes to Synovial Fluid from Patients with Different Grades of Osteoarthritic Knee Fibrosis”.

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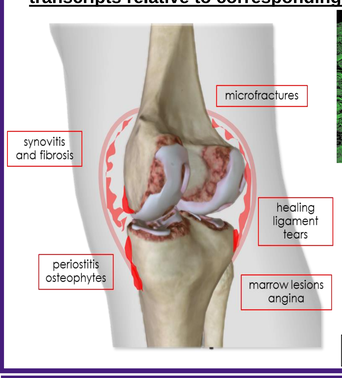
Introduction

- Arthritis is the leading cause of disability in the US, and knee osteoarthritis (kOA) is the most common form of arthritis.[1]
- kOA affects the normal pathophyhsiology of articular cartilage, subchondral bone, synovium, meniscus, and peri-articular muscle.[2,3]
- The synovium maintains normal knee joint function by secreting the functional components of synovial fluid (SF) but is also susceptible to inflammation and fibrosis (SFb) that contribute to kOA symptomology.[4]
- Preliminary data from our lab suggest that SFb severity measured by picrosirius fluorescence (green) correlates with patient reported Knee injury and Osteoarthritis Outcome Scores (KOOS) (i.e., symptoms, pain, and activities of daily life; ADL). (Fig.1)
- Published evidence by our lab also suggest SFb presents heterogeneously in a large sample of patients with end-stage kOA in association with deficits in active range of motion (ROM) that is race-dependent.[5]
- During kOA, diseased chondrocytes and synoviocytes undergo a dynamic crosstalk that influences severity of cartilage degradation and synoviopathy.[4,6]
- We aim to test the responses of validated commercial human fibroblast-like synoviocytes (HFLS) after stimulation with SF from high and low SFb patients that has been pre-screened for levels of key fibrogenic biomarkers known to regulate SFb and compare the HFLS responses to the fibrogenic transcript expression in patient fibroblastic synoviocyte cells (FSC).
- We hypothesize that HFLS challenged with kOA patient SFs will upregulate fibrogenic transcripts relative to corresponding histological measures of SFb severity.



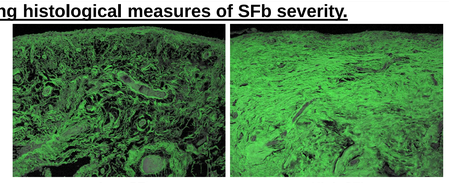
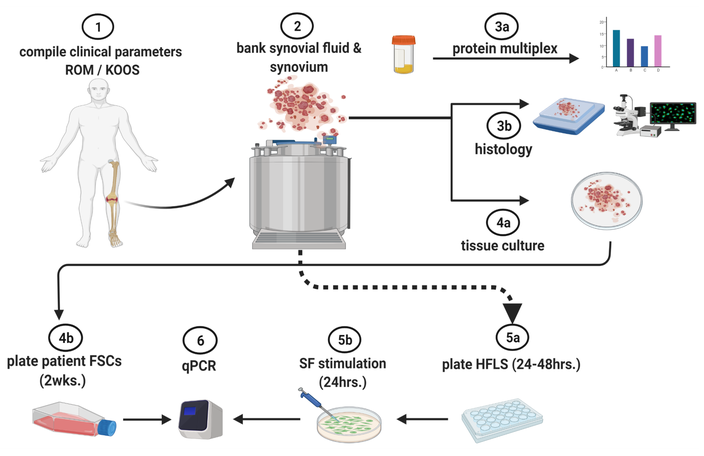


Fig. 1. Higher SFb moderately correlates with lower (worse) KOOS.

Methods



Results

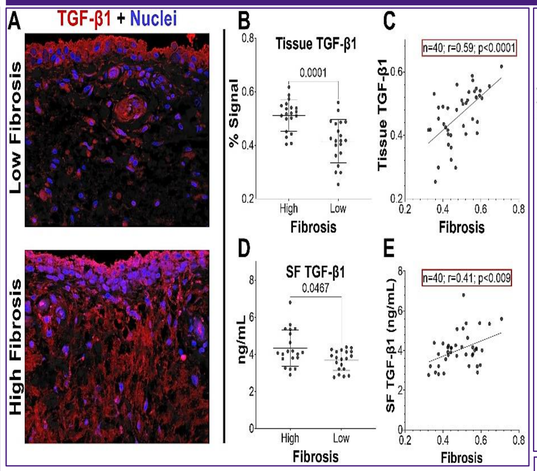


Fig.2. (A-B) TGF-β1 (red) mean±SD is significantly elevated in high (0.51±0.06) vs. low (0.41±0.08) SFb tissues stained by immunohistochemistry with blue nuclear counterstain in (C) association with histological measures of SFb. (D) Free TGF-β1 ng/mL in SFs from high (4.33±0.98) vs. low (3.68±0.55) SFb groups show similar trend and (E) association.

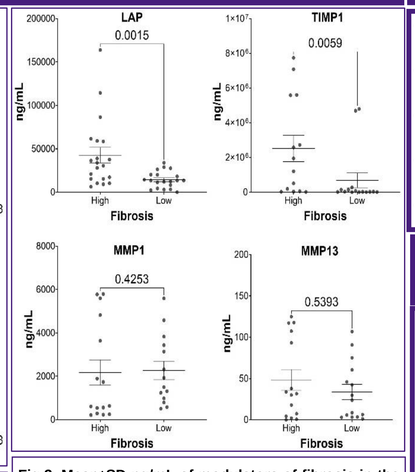
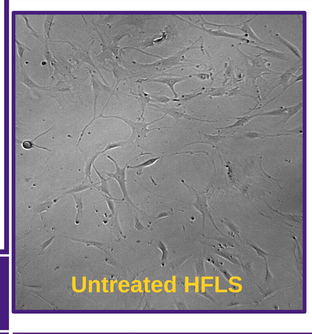
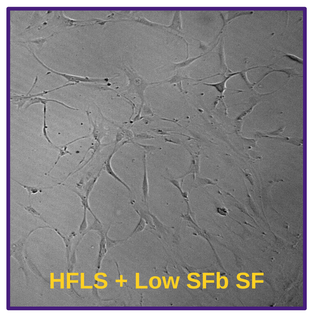
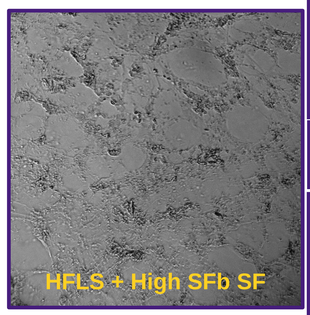


Fig.3. Mean±SD ng/mL of modulators of fibrosis in the SF of high vs. low SFb groups can help explain differences in SFb turnover: LAP (42716±39930 vs. 14317±9617); TIMP-1 (2521130± 2818396 vs. 683938± 1649579); MMP1(2172± 2271 vs. 2267±1604); and MMP13(48.43±48.65 vs. 33.82± 35.22). Student t-test.







Tgfb1	Upregulated throughout kOA and is the key factor contributing to a high fibrotic environment.
Plod2	Encodes for LH2b, which is responsible for pyridinoline crosslinking between procollagen fibrils.
Timp1	Upregulated in a *Tgfb-1* dependent manner, and its inhibition enhances severity of SFb.
Ctgf	Can induces fibrosis in a *Tgfb-1* independent manner triggering fibroblast proliferation.
Col1a1	Encodes for pro-1*alpha* chain of collagen type 1.
Acta2	Marker for myofibroblast differentiation.
Mmp1	Regulates ECM integrity and composition, and targets collagen 1 degradation.
Mmp13	Regulates ECM integrity and composition, and targets collagen 1 degradation.
Smad7	Negatively regulates *Tgfb-1*.
Urotensin2	Increases collagen synthesis and deposition in renal and cardiovascular fibrosis.
PGFalpha2	Upregulates the *Col1a1* promoter.
Hprt1	Housekeeping gene.
Actb	Housekeeping gene.

Expected Outcomes

- Our overarching goal is to expand on how the SF secretome of the kOA SF upkeeps SFb severity through a dynamic crosstalk between synoviocytes and chondrocytes in vivo.
- Naïve HFLS cells will respond to SF from high SFb patients with increased collagen synthesis, proliferation, and myofibroblast differentiation.
- HFLS stimulated with SF from high SFb patient will have a more robust fibrogenic gene expression response than those stimulated with SFs from low SFb patients.
- We expect FSCs from high SFb OA patients to express higher levels of fibrogenic factors *Plod2, Timp-1, Tgfb-1, Ctgf, and Col1a1.*
- High SFb kOA FSCs will express lower *Smad7* transcripts compared to a low SFb cohort.

Discussion and Limitations

- This is the beginning of a multifaceted analysis of SFb as a structural component of kOA that significantly contributes to functional limitations and pain of the joint.
- Since the synovium is highly vascularized and innervated and has been linked to kOA pain and limited ROM, it serves a primary target for further evaluation of kOA symptomology.[3,5,7]
- In this study we were able potentially to confirm Tgfb-1 as a reliable marker of stiffness, since levels in tissue and SF increased in relation to histological SFb severity.
- Levels of unbound latency-associated peptide (LAP), responsible for binding *Tgfb-1* in its latent form, were elevated in high SFb SF, confirming elevated levels of free/active *Tgfb-1*.
- Levels of Timp1 were elevated in high SFb, but without differences in MMP-1/13 between cohorts, which can be attributed to our aging patient population. L.B Kim et. al have published data suggesting Timp1 levels in blood serum increase with aging, while Mmp-1 serum levels decrease.[8]
- Further exploring the synovium and its role during kOA will bring us closer to a more complete understanding of kOA and anti-fibrotic interventions to attenuate stiffness and improve function before and after TKA.
- Studies on the crosstalk between synovium and articular cartilage using the SF as a medium will aid us in refining evaluation of kOA severity status non-invasively by screening inflammatory and fibrogenic factors in the SF linked to structural features of kOA.

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Acknowledgments

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