The effects of Pathogen Associated Molecular Patterns on synovial fibroblast mechanosensitivity

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## Introduction

In the knee joint, fibrosis of the synovial membrane is associated with symptoms of osteoarthritis progression such as joint pain and stiffness.(1) Under homeostatic conditions, synovial fibroblasts produce and maintain the ECM as well as secrete nutrients like hyaluronan to lubricate and nourish joint cartilage(1). Upon activation, synovial fibroblasts increase collagen production and contractility while downregulating production of joint lubricants,(1) resulting in the production of fibrotic tissue. This fibrotic tissue is characterized by the upregulation of ECM, presence of contractile myofibroblasts, and tissue stiffening.(2) Both chemical and physical cues are responsible for synovial fibroblast activation, however the pathways which link the two are not fully understood. From a biophysical perspective, increased ECM density and stiffness are sensed by the fibroblast cytoskeleton(3) and relayed via transcriptional regulators YAP (yes-associated protein) and TAZ (transcriptional co-activator with PDZ-binding motif).(4) This results in formation of mature focal adhesions and α-SMA stress fiber polymerization resulting in the heightened cell contractility associated with myofibroblasts.(5) From a biochemical perspective, presence of pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) are sensed by toll-like receptors (TLRs) and trigger an inflammatory response that activates fibroblasts to myofibroblasts.(6) While the link between cell mechanoactivation and physical environment is widely studied, the impact of PAMP-mediated inflammatory response on cell mechanoactivation is not well characterized.

Here, we utilized a culture system mimicking ECM stiffening during fibrosis and introduced lipopolysaccharide (LPS), a canonical PAMP that activates TLR signaling, to explore how biochemical cues synergize with mechanical cues to alter synovial fibroblast phenotype.

## Materials & Methods

*Primary synovial fibroblast isolation*
Synovial fibroblasts were isolated from supra-patellar synovium of bovine knee joints. Tissue was cultured overnight at 37°C in high-glucose DMEM (Dulbecco's Modified Eagle Medium) supplemented with 1X antibiotics and antimycotics and 0.1% Type IV collagenase. A 70 μm strainer was used to filter cells before expanding to confluence at P0 and storage in liquid nitrogen. Cells were utilized following two passages on tissue culture plastic.

*Synovial fibroblast mechanoactivation*
Synovial fibroblasts were cultured on either fibronectin-coated polyacrylamide (PAA) or glass. PAA substrates were fabricated as reported(7) with 5 kPA and 55 kPa moduli. PAA substrates were functionalized with Sulfo-SANPAH and coated overnight at 4°C with 20 μg/mL fibronectin. 30,000 cells were seeded on PAA and glass substrates. Culture media consisted of

high-glucose DMEM supplemented by 10% fetal bovine serum and 1% penicillin/streptomycin. Lipopolysaccharide (LPS) was utilized at 1 µg/mL and added at the time of cell seeding.

*In vitro imaging and analysis*

Immunofluorescence imaging for α-SMA was conducted on synovial fibroblasts after three days on various substrates. Counterstaining with phalloidin (actin) and Hoechst (nuclei) were used before imaging with Zeiss Axio Scan.X1. Images were analyzed with CellProfiler. Statistics were carried out in GraphPad Prism with two-way ANOVA analysis.

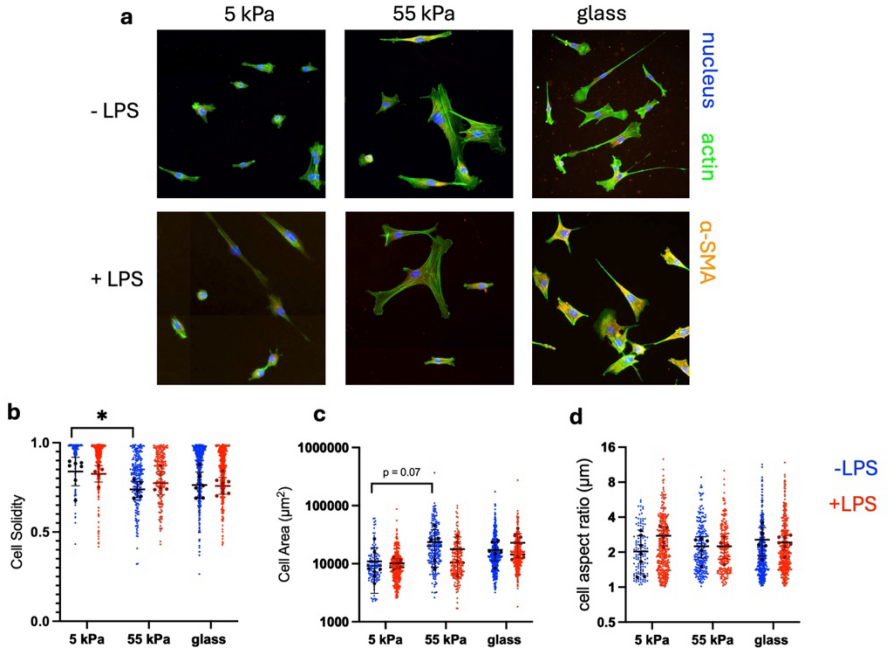


**Figure 1:** *Biophysical cues alter synovial fibroblast phenotype.* (a) Synovial fibroblast morphology after three days on various substrates with exposure to LPS. Quantification of (b) Cell solidity (c) cell area and (d) cell aspect ratio (cell major axis : cell minor axis) between treatment groups and substrate stiffness. * p<.05

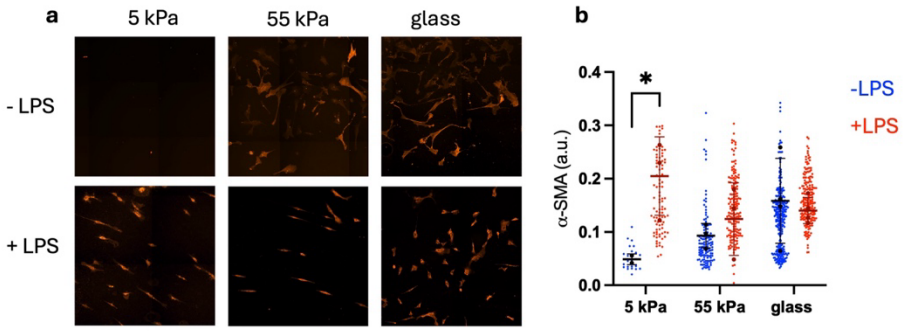


**Figure 2:** *Biochemical cues alter synovial fibroblast phenotype.* (a) Representative images of α-SMA in synovial fibroblasts after three days on varied stiffness substrates and inflammatory treatment. (b) Quantification of α-SMA mean fluorescence intensity. * p<.05

**Results, Conclusions, Discussion**

Synovial fibroblast phenotype can be altered by exposure to both physical and inflammatory stimuli. Cell solidity and cell area decreased 5 kPa and 55 kPa substrates. However, no significant difference in cell aspect ratio between media conditions and substrate stiffness was seen, likely due to low sample number.

Across substrate stiffnesses, there was a trend of upregulation of α-SMA in cells. Additionally, cells cultured on 5kPa with exposure to LPS increased significantly increased α-SMA expression, indicating mechanoactivation triggered by biochemical cues. With this, we see that chemical cues can alter fibroblast mechano-phenotype, but further research is needed to verify this relationship. Here, cells were exposed to PAMPs for three days, which may not be the optimal timepoint for significant changes in cell morphology to manifest due to mechanoactivation. Future studies utilizing various other PAMPs that target different TLRs as well as the use of TLR inhibitors would further explore the link between inflammatory response and fibroblast activation.

**References**


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