The Impact of Mechanotransduction of macroscale forces on fibrosis in degenerative spinal disease

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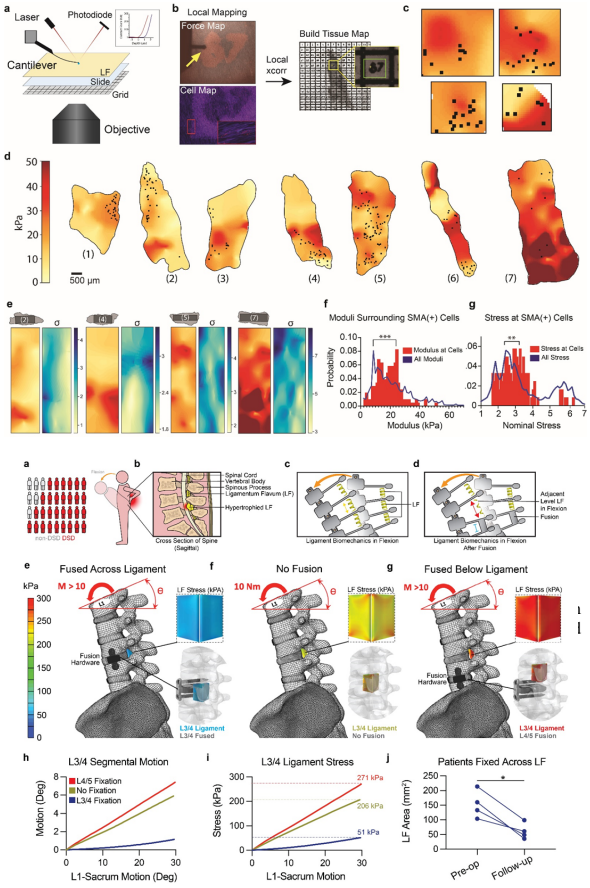
INTRODUCTION: Prolonged and repetitive stresses on the spinal column contribute to the progression of degenerative spinal disease (DSD). However, the precise connection between larger-scale mechanical forces and tissue hypertrophy remains unclear. In the case of degenerative spinal disease (DSD), mechanical stress induces fibrotic alterations in ligaments and bones, ultimately leading to the compression of neural components. [1, 2].
Global studies project that 84% of individuals will experience degenerative spinal disease (DSD) [3], often necessitating surgical decompression and fusion as the ultimate treatment. The combined costs of multimodal treatments in the United States alone amount to $13 billion annually [4]. To develop non-surgical disease-modifying therapies for DSD, it is imperative to comprehend the impact of larger-scale forces on fibrotic changes within the spine. Our study reveals heightened Rho-associated kinase (ROCK) signaling and an increased density of smooth muscle actin alpha (SMAα) myofibroblasts in regions of fibrosis within the human ligamentum flavum (LF), which is naturally exposed to high stress. Using Atomic Force Microscopy (AFM), we pinpoint myofibroblasts in areas of heightened stiffness and microstress within the LF. Employing an innovative loading device, we observe stress-dependent accumulation of LF myofibroblasts in a ROCK-dependent manner. Lastly, we demonstrate a stress-induced transcriptional response in human LF, partially mitigated by ROCK inhibition. These findings justify further in-depth exploration of ROCK inhibitors as a potential class of compounds for non-surgical DSD treatment.



METHODS: Ligamentum Flavum (LF) samples were obtained from more than 250 patients who had undergone spinal surgeries at Massachusetts General Hospital (MGH). These samples were collected within 30 minutes of surgical removal and were promptly transported in a sterile specimen cup on ice, taking less than 15 minutes for transportation. The processing and storage procedures for the samples varied for each experimental method, as described in the subsequent sections. All sample collection procedures adhered to the IRB protocol 2017P000635. While different samples from the same patients were employed for various concurrent experiments, no sample was reused. To facilitate accurate measurements, we created a custom transparent grid at a millimeter scale, numbering each grid, and printed it on transparency film, resulting in a square grid measuring 2 cm x 2 cm (Figure 1). Using cyanoacrylate adhesive, we affixed one grid to the bottom of each microscope slide. The ligament samples of interest were swiftly frozen within cryomolds containing OCT, cross-sectioned to a thickness of 20μm, and placed on the microscope slide's surface, ensuring they were centered on the grid beneath the slide. Utilizing the Evos stitching microscope, we captured images of the entire microscope slide to document the precise positioning of the ligament in relation to the grid.
To determine the modulus of the samples, we utilized a commercial atomic force microscope (AFM) (MFP-3D, Asylum Research, Santa Barbara, CA). Glass probe tips with varying diameters of 20–50μm were affixed to tipless cantilevers with a nominal spring constant k ~ 10 N/m (Budget Sensors, Sofia, Bulgaria). The spring constants for all tips were directly measured using the thermal calibration method. The glass probe tips were attached to the cantilever using the lift-off process. Locations on the slide were imaged and subsequently indented in a systematic manner to map the complete spatial extent of a given slice or sample.After completing AFM, the ligament was gently fixed and immunofluorescent staining was performed for SMAα (as detailed above). Upon completion of the IF staining, the slide was re-imaged on the Evos stitching microscope to ensure the tissue slice did not move in relation to the grid and then a blinded investigator inspected the ligament slice and recorded the exact locations of each SMAα cell. A custom MATLAB code was used to create modulus maps and to superimpose the locations of SMAα cells on the fabricated maps. To understand the changes in load distrbution on LF in fixation construct we used utilized a validated finite element model of the L1 to pelvis spine [5]. The ligamentum flavum was modeled using a fiber-reinforced hyperelastic material law, with collagen fiber orientation captured. Material parameters were calibrated to match experimental data on ligamentum flavum tensile stiffness and mechanical properties. Three cases were analyzed: intact, L4/L5 fusion, and L3/L4 fusion. For the fusion cases, virtual models of lateral PEEK interbody cages and titanium plates/screws were implanted following simulated lateral discectomy. Surfaces were tied to simulate bone-implant fixation. The intact model underwent a 400N follower preload plus flexion, extension, lateral bending, and axial rotation moments up to 10Nm. The fusion cases used a hybrid loading protocol with 400N follower load and moments adjusted to match the intact range of motion. Following simulation, intact and fused segmental range of motion was computed and compared. The ligamentum flavum was analyzed to determine macroscale principal stresses and strains. Ligamentum flavum stress distributions were compared between intact, fused, and adjacent unfused levels to assess biomechanical changes. This validated computational approach enables detailed quantification of how fusion alters load transfer to the ligamentum flavum.

RESULTS: Fusing adjacent vertebrae to the Ligamentum Flavum (LF) led to a significant increase in both peak segmental motion and LF stress when compared to the spine that was not fused, with a respective increase of 31% and 25% (refer to Figure 2). This heightened LF stress was directly associated with the increased segmental motion, which was necessary to compensate for the adjacent immobilized (fused) spinal level in order to maintain the same overall degree of flexion. Conversely, fusion that extended across the LF resulted in a substantial decrease in both peak segmental motion and stress on the immobilized LF in comparison to the normal spine, with a respective decrease of 81% and 75% (refer to Figure 2). A retrospective chart review further revealed that the immobilization of levels across the LF was a key factor in these observations.

DISCUSSION: We observed stress-driven transcriptional response in human LF, partially reduced by ROCK inhibition. These findings warrant detailed future investigation into ROCK inhibitors as a potential class of compounds to treat DSD in a non-operative manner.

SIGNIFICANCE: Clinical stress risers and in vivo cyclic stress drive rescuable, ROCK-dependent myofibroblast accumulation in spinal ligament contributing to degenerative spinal disease.

REFERENCES: [1] Kushchayev + Insight Imaging, 2018. [2] Tschumperlin+ J Clin Invest, 2018. [3] Ravindra + Global Spine, 2018. [4] Austevoll+Engl J Med, 2021. [5] Kiapour+JNS, 2021.