Alterations in Fibrous Network Topography Regulate Onset of Fibrotic Phenotypes in Annulus Fibrosus Cells

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Introduction

- Swelling of the nucleus pulposus of the intervertebral disc results in large residual strains in the outer annulus fibrosus [1,2].
- Mechanical microenvironments guide cell phenotype [3], but it is unknown if the prestressed environment of a healthy annulus dictates mechano-perception and phenotype.
- Here, we combined in vivo and in vitro systems to understand how residual strains in the disc facilitates contact guidance and how loss of residual strains initiates an aberrant response.

Methods

- In vivo/ex vivo studies were performed on New Zealand White rabbit lumbar spine discs. Annular puncture was conducted in the anterior AF [4]. Discs were assessed mechanically, histologically, and via second harmonic generation imaging (SHG).
- In vitro studies were conducted using electrospun PCL scaffolds seeded with bovine caudal annulus fibrosus cells. Cells were assessed in both free swelling and prestrained (9%) scaffolds to mimic the residual strains of the disc (or loss thereof).

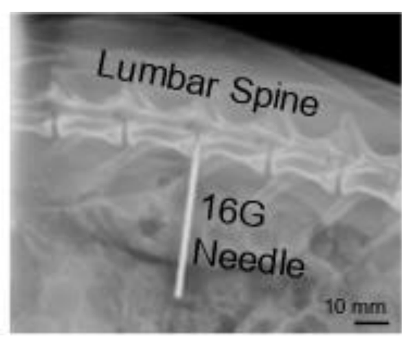
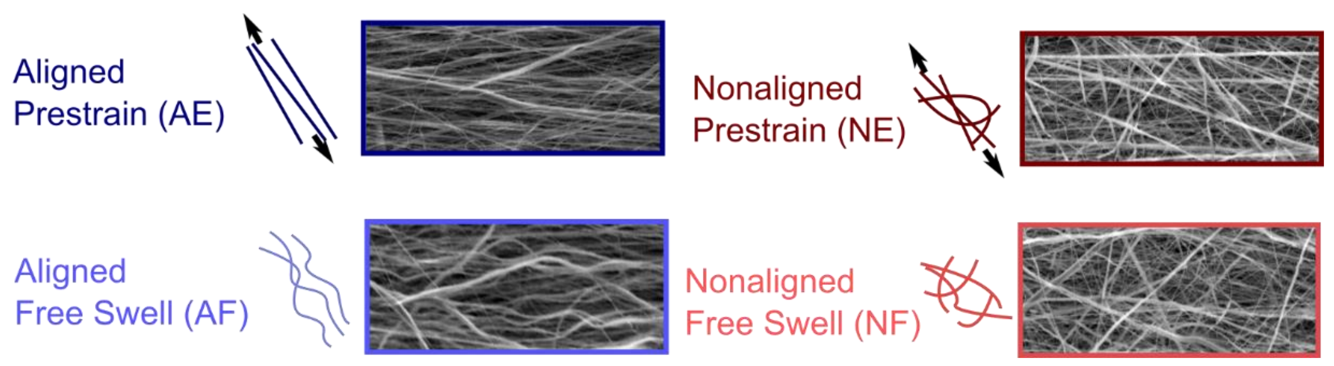
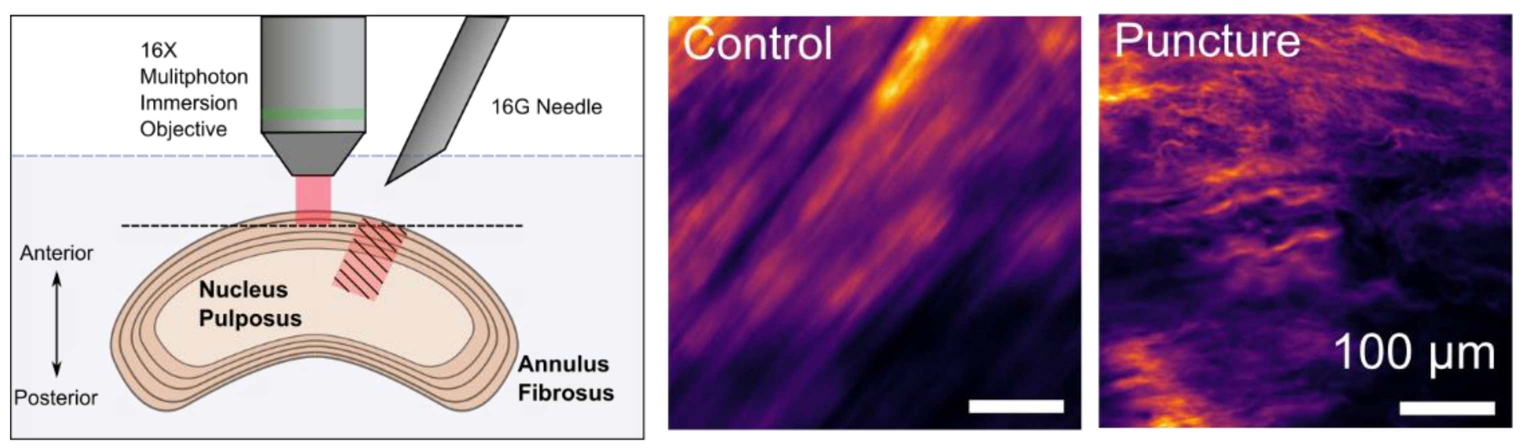


Fig 1. (Left) Intraoperative x-ray of puncture injury using an antero-lateral approach. (Below) Electrospun scaffolds were clamped in a tensioning device and stretched prior to cell seeding. Both aligned and non-aligned scaffolds were fabricated and tested in either free swelling or prestrained states.



Releasing Residual Strain

- **Release of residual strains leads to altered fiber morphology and compromised disc mechanics.**



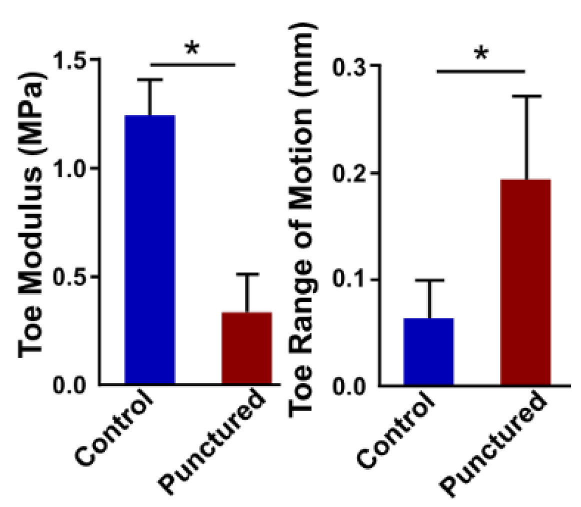
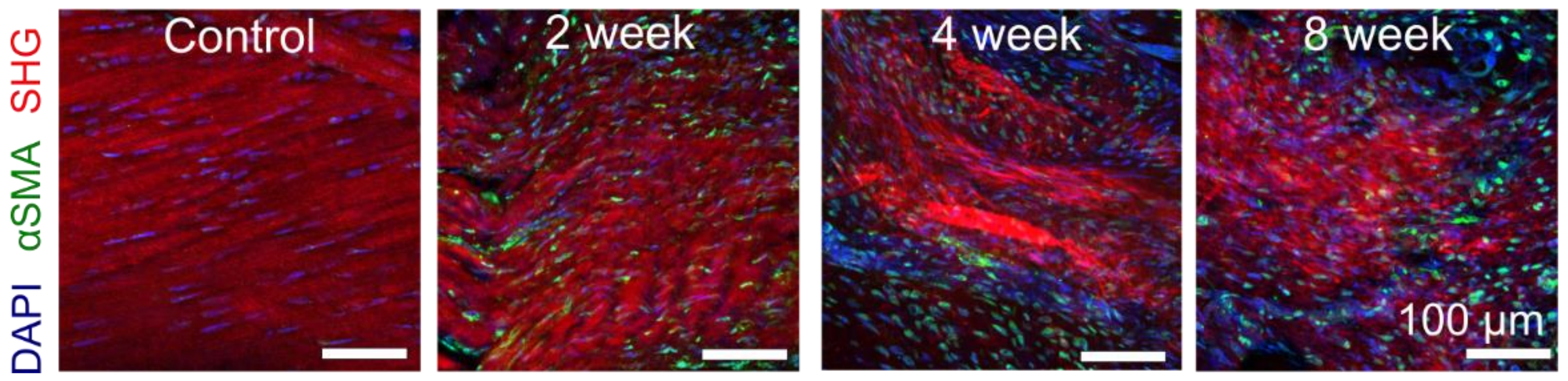


Fig 2. (Top) Ex vivo disc puncture viewed through a multiphoton microscope revealed immediate changes to fiber morphology indicating compromised residual strains. (Left) This ex vivo puncture acutely compromises whole disc mechanics as evidence by changes in the toe-region mechanics (n ≥ 3 discs per group).

Contact Guidance and Fibrosis

- **Release of residual strains in vivo leads to disorganized fiber networks and the emergence of fibrotic phenotypes.**



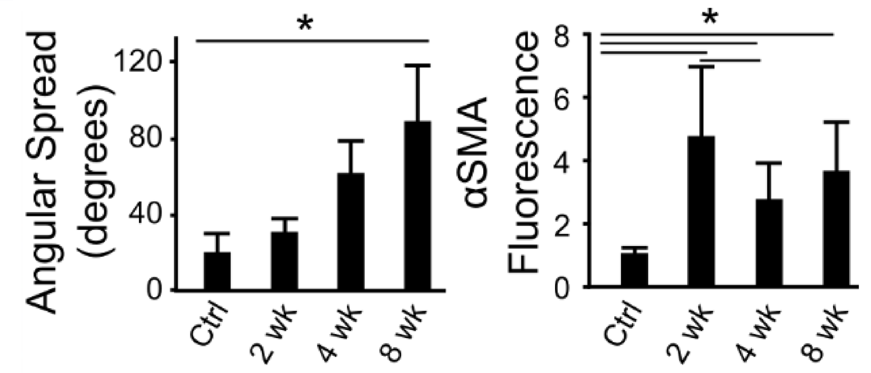


Fig 3. Survival following puncture injury led to a progressively more disorganized fiber environment (increased angular spread) of the AF and the emergence of fibrotic (i.e., αSMA+) phenotypes (n = 3 animals/group).

- **Strain-mediated fiber organization regulates mechano-sensing in engineered fiber environments**

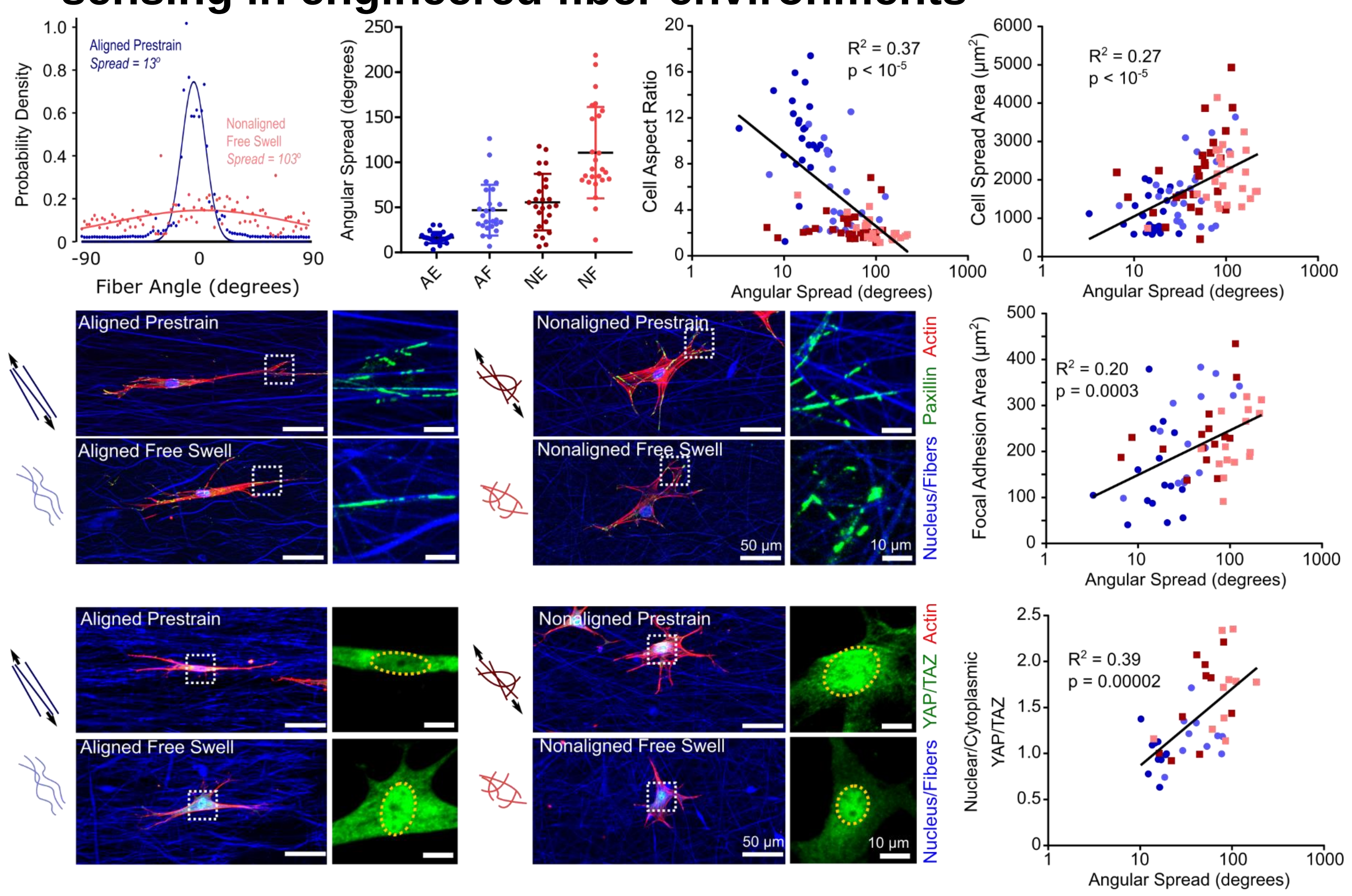


Fig 6: Prestrain governed fiber organization in both aligned and nonaligned scaffolds to dictate cell spreading (i.e., cell aspect ratio and area). Additionally, local fiber organization predicted mechanobiologic outcomes in focal adhesion formation and YAP/TAZ localization. In short, more aligned and organized fiber environments promote 1D cell elongation with low nuclear YAP/TAZ.

- **Contact guidance in highly organized environments suppresses emergence of fibrotic phenotypes**

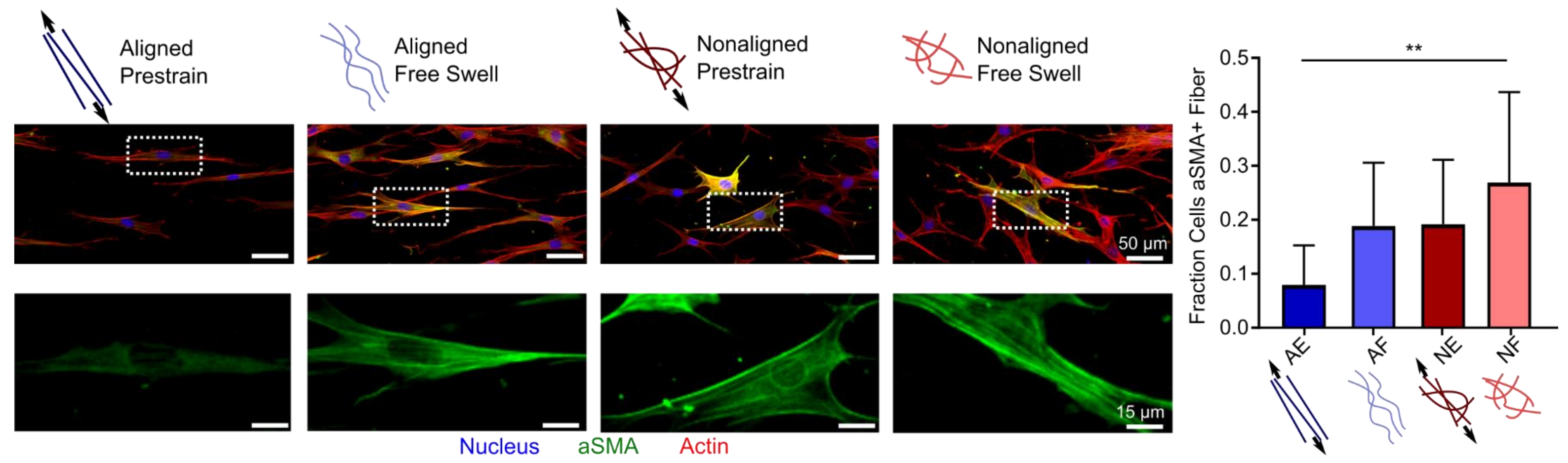
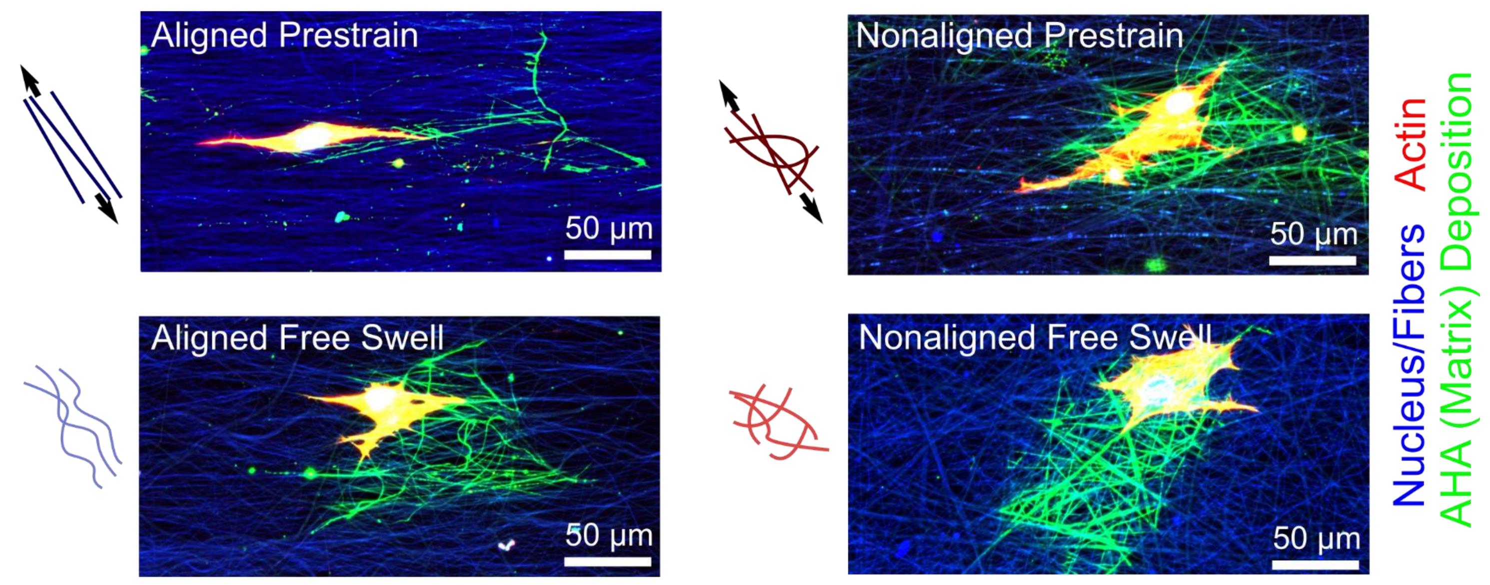


Fig 7. In agreement with the focal adhesion and YAP/TAZ outcomes, prestrain and baseline organization modulated the emergence of αSMA+ phenotypes, where prestrain in aligned environments suppressed this fibrotic phenotype (n = 6 scaffolds/group).

- **Functional non-canonical amino acid tagging reveals association between local fiber topography and cellular activity.**



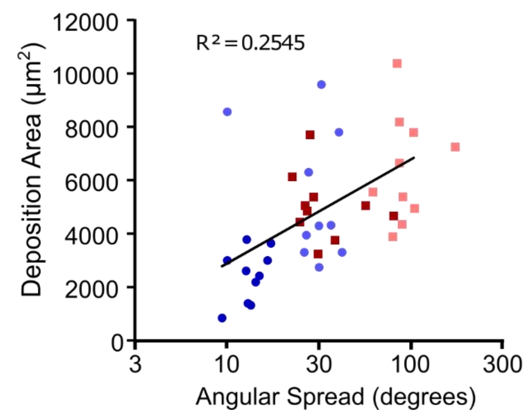
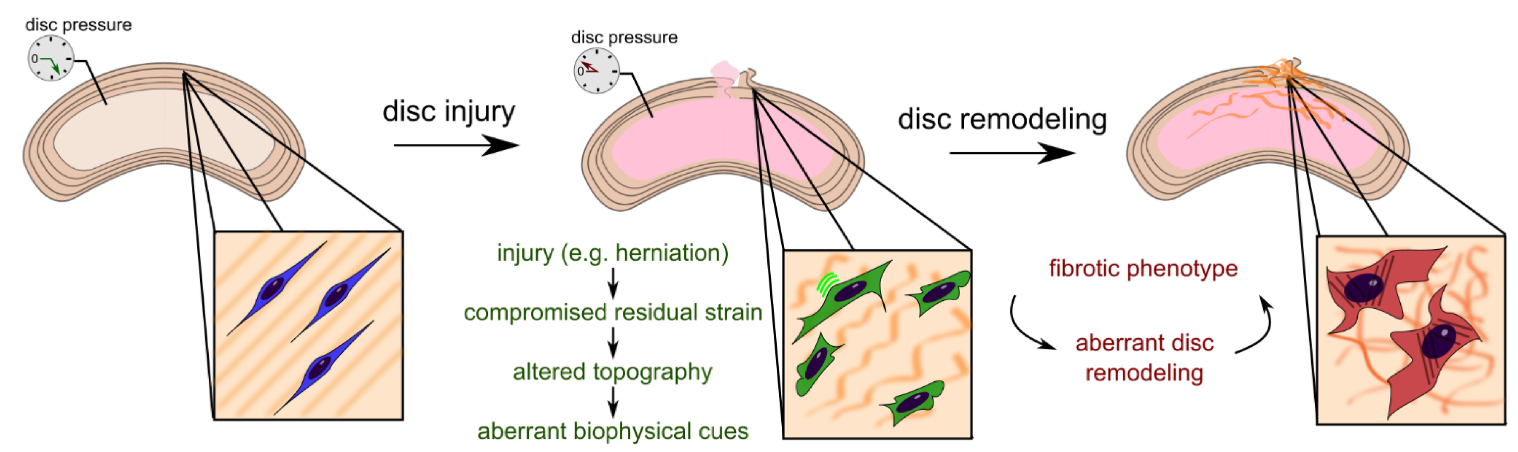


Fig 8. Methionine was replaced by the methionine analog, L-Azidohomoalanine (AHA), in cell culture media and imaged through click chemistry (green). (Above) AHA staining revealed the extent of the fiber environment that cells have engaged with and deposited matrix on. (Left) the area of deposition was predicted well by the local fiber organization, where cells in more disorganized environments deposited more matrix.

Discussion

- Here, we presented evidence that release of residual strains triggers mechanobiologic responses of annulus fibrosus cells both in vivo and in vitro.
- Following release of residual strains, the emergence of fibrotic phenotypes in the AF accompanies progressive disorganization of the local fiber environment.
- In vitro analyses indicate that local fiber organization provides contact-guidance cues that alter cellular attachment and spreading to modulate the emergence of fibrotic phenotypes.

Altered mechanosensing in soft tissue degeneration



References & Acknowledgments

[1] Michalek *et al.* 2012, [2] Gardner-Morse and Stokes 2003, [3] Engler *et al* 2006, [4] Masuda *et al* 2005

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