Published in final edited form as:

Nat Biomed Eng. 2019 December ; 3(12): 951–952. doi:10.1038/s41551-019-0491-3.

Homeostasis disrupted by strain mechanosensing

Tyler J. DiStefano¹, Svenja Illien-Jünger², James C. Iatridis^{1,*}

¹Leni & Peter W. May Department of Orthopaedics, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

²Emory University School of Medicine, Atlanta, GA, USA.

Abstract

In injured intervertebral discs, disruptions in fibre organization and in cellular contractility result in a fibrotic phenotype and progressive tissue degeneration.

As essential components of every joint in the musculoskeletal system, fibrous connective tissues enable load-bearing with controlled mobility. Because these soft tissues interact with surrounding bone surfaces (which act as fixed interfaces), tissue growth and osmotic swelling cause the tissues to develop basal levels of mechanical strain (also known as ‘residual strains’) within their constituent fibres^{1,2}. Residual strains have a marked effect on the topography of the fibrillar network and on its organization within the cellular microenvironment³. Along with joint loading, these strains present biophysical cues to resident cells, ultimately influencing cellular phenotype and function through mechanotransductive mechanisms^{4,5}.

The intervertebral disc (IVD) — a fibrocartilage situated between vertebral bodies along the spinal column — consists of a highly organized angle-ply laminated annulus fibrosus (AF) that connects adjacent vertebral bodies and contains the centrally located nucleus pulposus (Fig. 1a). IVDs are susceptible to degeneration, which is strongly associated with back pain, a leading cause of global disability⁶. In healthy IVDs, the densely packed collagenous fibres of the AF develop residual strains in tension because the AF fibres restrain the high swelling pressures of the nucleus pulposus, even when under minimal muscle loads and while the body is lying down⁷. In degenerated IVDs, local tissue defects disrupt the biomechanical integrity of AF fibres and lead to an aberrant cell phenotype that manifests in organ-level dysfunction, suggesting that there is a mechanical set point that maintains overall tissue health. This is exemplified in studies that show that overloading, as well as underloading, can result in tissue trauma or in matrix remodelling that cause or accelerate IVD degeneration⁸. The accumulation of such structural disruptions in the AF are a feature of IVD degeneration that can result in pain. AF-puncture models are commonly used to mimic these disruptions and to induce IVD degeneration⁹. Yet there is limited understanding of how the loss of fibre tension from AF puncture, or of how underloading from structural disruptions more generally, affects AF cells (annulocytes). Reporting in *Nature Biomedical Engineering*, Robert Mauck and colleagues now provide evidence of a mechanobiological

* james.iatridis@mssm.edu.

interplay between mechanical cues from the microenvironment and the cellular responses leading to soft-tissue degeneration post-injury¹⁰.

Mauck and co-authors used a rabbit model of AF puncture, ex vivo puncture experiments and electrospun nanofibrous poly(ϵ -caprolactone) scaffolds recapitulating evolving boundary constraints. The authors found that an acute and prolonged reduction in residual strain elicits cellular responses, including a change in phenotype, increase in apoptosis and nascent matrix deposition within the pericellular space, which are biological hallmarks reflective of fibrotic changes in progressive IVD degeneration¹¹. Via the rabbit model, the authors demonstrate that disruption in AF boundary constraints provokes the sustained expression of the fibrotic marker α SMA starting as early as 2 weeks post-injury. By using the ex vivo puncture model, they found the emergence of active caspase-3 expression post-injury, indicating that apoptosis concomitantly occurs with aberrant degenerative matrix remodelling. To isolate the effects of fibre organization and residual strain from potential confounders in the wound-healing environment, the authors used the nanofibrous scaffolds to examine biological responses on the single-cell level. By externally controlling strain and modulating fibre alignment to simulate acute and chronic changes in residual strain in this in vitro system, they engineered culture environments that recapitulated the normal physiological state and injured conditions. They found that a disorganized and slacked fibrous environment yields a pro-fibrotic phenotype, thus recapitulating the findings from the animal and ex vivo puncture models. The use of the system also revealed that pre-strain results in an increase in cellular and nuclear aspect ratios, with a corresponding decrease in nuclear-to-cytosolic YAP-TAZ localization, indicating that annulocyte morphology and nucleocytoplasmic shuttling is dependent on fibre organization and strain. Specifically, annulocytes cultured on aligned prestrained scaffolds adopted a spindle-like morphology and retained YAP-TAZ in the cytoplasm; in contrast, annulocytes cultured on free-swollen or non-aligned fibrous matrices adopted a stellate morphology with a higher number of cellular processes and focal adhesions per cell, and triggered the nuclear translocation of YAP-TAZ. These results suggest that cells use mechanosensing processes to survey the local fibrous environment and that they actively respond to biophysical cues involving changes in fibre tension and organization.

To mechanistically probe the deleterious changes seen in their animal and ex vivo models, Mauck and co-authors used the nanofibrous scaffolds to perform dynamic-loading experiments that emulated both annular injury and overloading events. They found that the cells responded to changes in fibre strain via calcium signalling, independent of loading direction. Notably, scaffolds that underwent acute unloading (9–4% strain) 24 hours after cell seeding exhibited an apoptotic response, whereas static and acute overloading conditions led to minimal levels of apoptosis. To assess whether these responses were mediated by cellular contractility and mechanosensing processes, the authors performed these dynamic loading experiments with two Rho-associated protein kinase (ROCK) inhibitors (Y27632 and Fasudil). They found, via traction force microscopy, that treatment with both ROCK inhibitors led to a reduction in cellular contractility. Moreover, treatment with both ROCK inhibitors precluded a cellular fate towards programmed cell death, as indicated by a large reduction in active caspase-3 in the unloading condition (all other static and overloading conditions led to no such changes).

Taken together, Mauck and colleagues' findings posit a working conceptual model of the onset of soft-tissue degeneration and of its advancement into a progressive state. In healthy IVDs, intradiscal loads are transferred amongst annular fibres in tension, and as a result establish a taut pre-strained fibrous environment (Fig. 1b, left) that maintains a normal phenotype for resident annulocytes¹². The anatomical form and biomechanical function of the healthy AF thus provides a tensional homeostatic environment for annulocytes. Following injury or degeneration, a loss in matrix integrity and physical strain (Fig. 1b, right) leads to the disruption of tensional homeostasis, ultimately causing a pro-fibrotic phenotype, matrix remodelling and apoptosis, all of which are biological responses seen in progressive degeneration. Small molecules that target mechanosensitive cell-contractility proteins can inhibit the progression of these cellular behaviours by altering the cell's ability to sense aberrant fibrous topographies.

Mauck and co-authors' findings indicate that matrix organization and fibre tension are critical factors in the maintenance of a healthy physiological state, and highlight that in the progression of IVD degeneration the loss of residual strains is at least as important as overloading. Although the use of ROCK inhibitors was effective in attenuating cellular contractility and apoptotic responses in the nanofibrous scaffolds, whether these inhibitors would be effective in the in vivo context of IVD degeneration remains to be studied. In fact, given the orchestrated immune responses that follow puncture injury or de novo degeneration¹³, a scaffold that incorporates the pro-inflammatory cytokines present in the degenerative environment might better recapitulate the in vivo microenvironment. Inflammatory cytokines are known to affect intracellular signal transduction and can change the sensitivity of the cell's response to mechanical cues¹⁴.

By highlighting the importance of an organized fibrous network with residual strains in maintaining a healthy phenotype, Mauck and colleagues' study suggests that cell-laden constructs for treating IVD degeneration will be most effective when recapitulating the native tissue architecture. The study also suggests that key proteins involved in cellular mechanosensitization (such as proteins in the ROCK AGC-kinase subfamily involved with cellular contractility and cytoskeletal dynamics¹⁵) could become targets of interest for small-molecule therapeutics for delaying progressive IVD degeneration.

References

1. Narmoneva DA et al. *J. Biomech* 32, 401–408 (1999). [PubMed: 10213030]
2. Lanir YJ *Biomech. Eng* 131, 044506 (2009).
3. Muiznieks LD et al. *Biochim. Biophys. Acta* 1832, 866–875 (2013). [PubMed: 23220448]
4. Gasiorowski JZ et al. *Annu. Rev. Biomed. Eng* 15, 155–176 (2013). [PubMed: 23862676]
5. Humphrey JD et al. *Nat. Rev. Mol. Cell. Biol* 15, 802–812 (2014). [PubMed: 25355505]
6. Hartvigsen J et al. *Lancet* 391, 2356–2367 (2018). [PubMed: 29573870]
7. Michalek AJ et al. *J. Biomech* 45, 1227–1231 (2012). [PubMed: 22342138]
8. Stokes IA et al. *Spine J* 29, 2724–2732 (2004).
9. Iatridis JC et al. *Spine J* 13, 243–262 (2013). [PubMed: 23369494]
10. Bonnevie ED et al. *Nat. Biomed. Eng* 10.1038/s41551-019-0458-4 (2019).
11. Yee A et al. *Osteoarthritis Cartilage*. 24, 503–513 (2016). [PubMed: 26463451]
12. Torre OM et al. *Ann. NY Acad. Sci* 1442, 61–78 (2019). [PubMed: 30604562]

13. De Geer CM J. *Chiropr. Med* 17, 54–62 (2018). [PubMed: 29628809]
14. Fearing BV et al. *JOR Spine*. 1, e1026 (2018). [PubMed: 30569032]
15. Arencibia JM et al. *Biochim. Biophys. Acta* 1834, 1302–1321 (2013). [PubMed: 23524293]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 1 |. Fibre pre-strain regulates homeostasis and maintenance of the viability, phenotype and morphology of cells in the intervertebral disc's annulus fibrosus.

a, The structure of the human intervertebral disc (situated between superior and inferior vertebrae), showing the laminated annulus fibrosus encircling the nucleus pulposus. **b**, Relationships between cellular responses and the fibrous microenvironment when residual strains are present amongst taut fibres (left), and when injury or modified boundary conditions release residual strains within extracellular fibres (right). Panel **a** reproduced with permission from Cortes D.H. & Elliott D.M. in *The Intervertebral Disc* (eds Shapiro, I. & Risbud, M.) 17–31 (Springer, 2014). Panel **b**, courtesy of Mount Sinai Health System.