ECM remodelling and tension development in tendon wound healing is mechanically regulated

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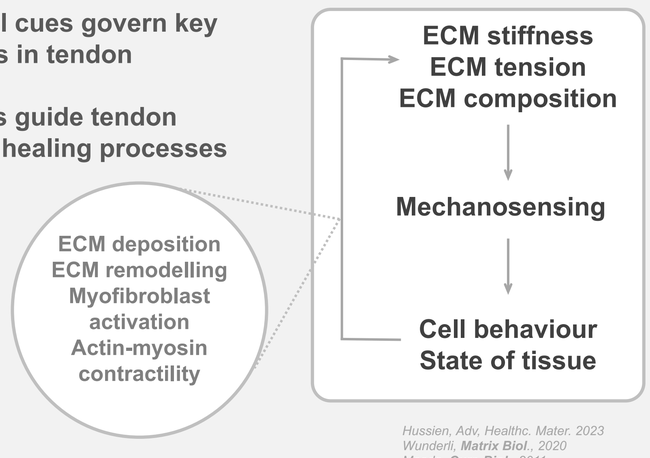
The fibrotic nature of tendon healing: Vicious cycle of ECM regulation

- Matrix mechanical cues govern key cellular processes in tendon
- Mechanical forces guide tendon tissue health and healing processes

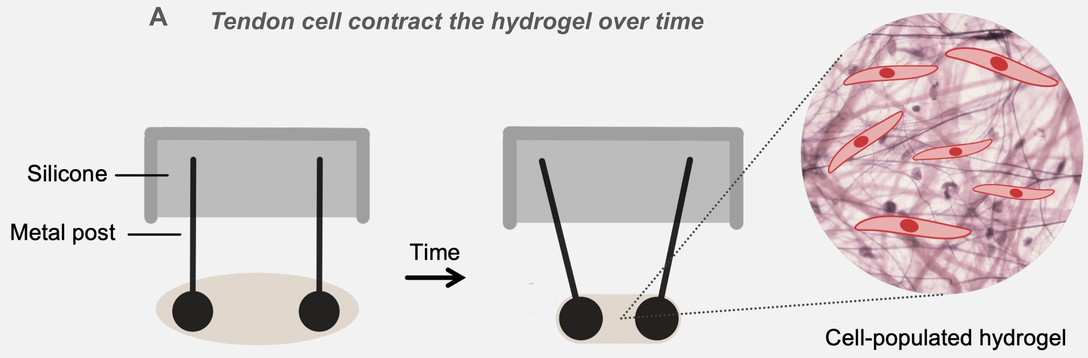


What leads the cells to exit that cycle and reach resolution and homeostasis in a sustainable way?

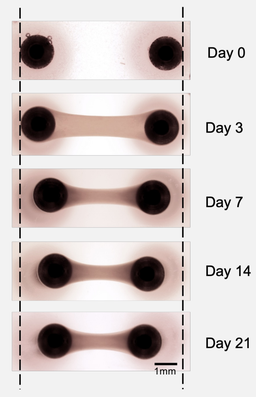
Hussien, Adv. Healthc. Mater. 2023
*Wunderli, **Matrix Biol.**, 2020*
*Maeda, **Curr. Biol.**, 2011*
*Stamenovic, **Soft Matter**, 2020*
*Humphrey, **Nat. Rev. Mol. Cell Biol.**, 2014*
*Martino, **Fron. Physiol.**, 2018*
*Nichols, **Transl. Res.**, 2019*

Engineered tendon constructs as a mechano-variant 3D *in vitro* model for tendon wound healing

A *Tendon cell contract the hydrogel over time*



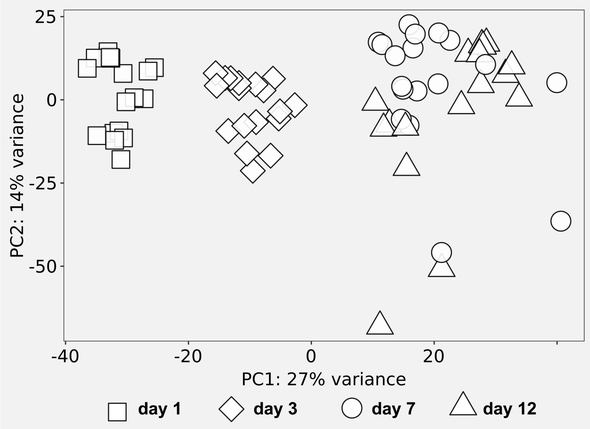
B *Development of tendon constructs over time*



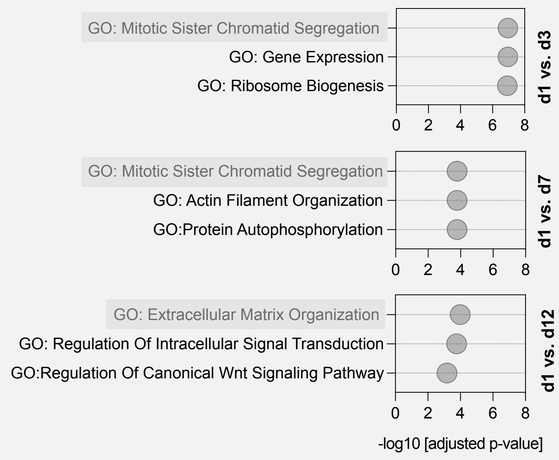
*Patient-derived tendon stromal cells in a collagen I hydrogel. Casting the hydrogel between posts anchored in silicone allows adjusting the post stiffness [**A**]. Static mechanical stimulation: cells remodel the (unorganized) collagen representing wound healing mechanisms [**B**]. Used post stiffnesses: k = 1N/m, k = 5N/m and completely rigid posts (placed into PLA top part).*

Transcriptomic comparison across time points and boundary conditions

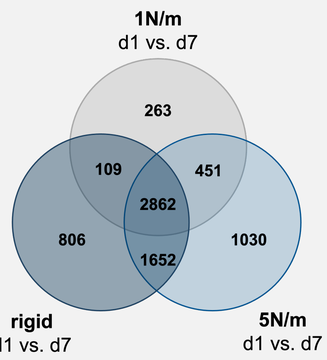
Overall PCA based on mRNA expression from RNAseq



Top 3 enriched gene ontology biological processes over time of all conditions



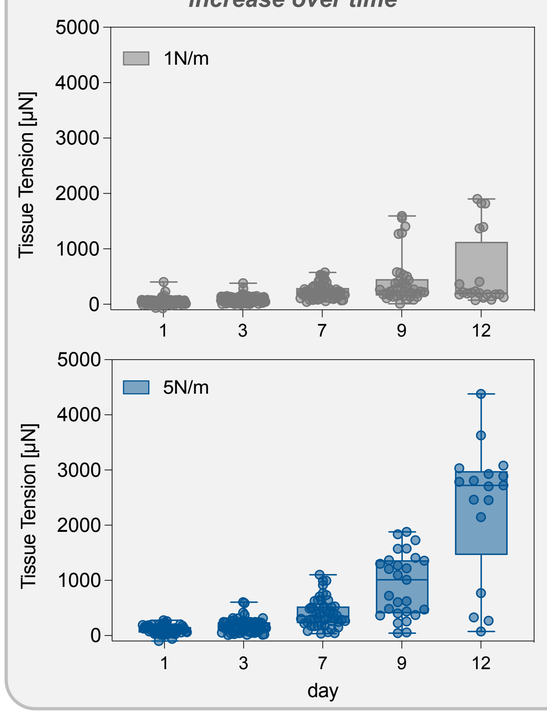
Large overlap of DEGs across stiffnesses



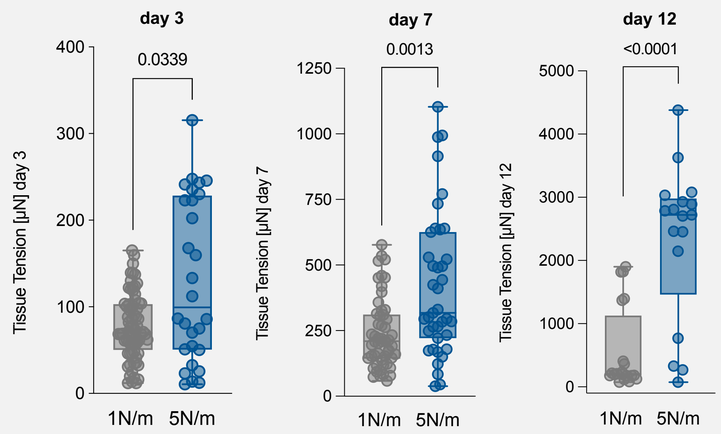
RNAseq was performed using tendon constructs cultured at 1N/m, 5N/m, rigid posts for 1, 3, 7, 12 days. N = 6 donors, 3 tendon constructs pooled each time point and condition. Differential expression was determined using DESeq2 method. Over-representation analysis was performed using hypergeometric overrepresentation test against the GO database with both up- and downregulated DEGs.

Development of tissue tension is modulated by boundary rigidity

A *Cell-mediated tensional forces increase over time*



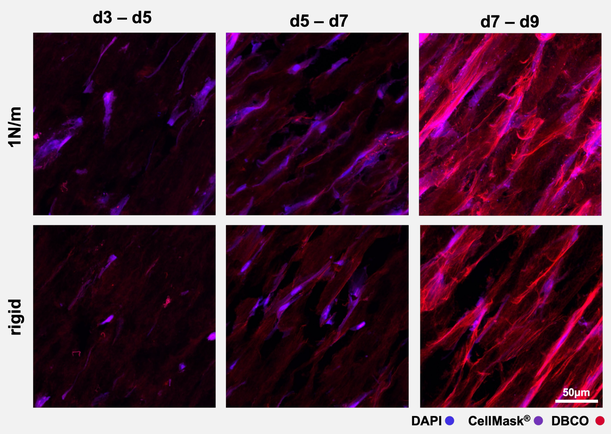
B *Higher post stiffness leads to higher forces on tissue-level*



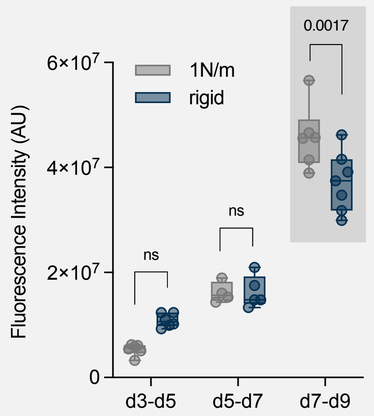
Quantification of cell-mediated tensional forces using post deflection, N = 6 independent donors, Kruskal-Wallis-Test

Post stiffness modulates deposition and structure of nascent ECM

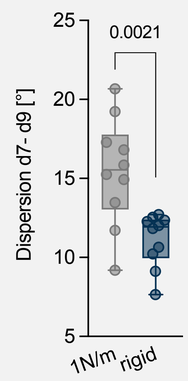
A *Matrix is produced at later stages of culture*



B *More ECM synthesized on soft posts*



C *Less matrix alignment on soft posts*

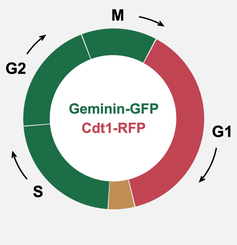


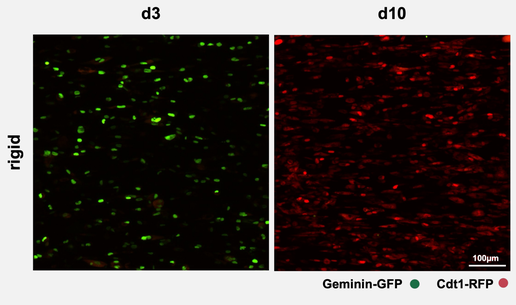
*Visualization of newly deposited ECM with metabolic marking of nascent proteins using L-azidohomoalanine as an L-methionine analogue and fluorescent labeling with DBCO click-chemistry (red). Cells labelled with DAPI (nuclei, blue) and CellMask® (membrane, purple) [**A**]. Quantification of amount and orientation of matrix fibers using ImageJ [**B**]. N = 5 -10 gels each condition and time point, unpaired t-test*

*Loebel, **Nat. Protoc.**, 2022*

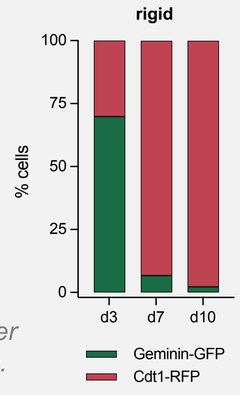
Tendon cells display an tension-dependent exit to quiescence

FUCCI reporter gene-based cell cycle analysis





Increase of cells in G1 phase over time



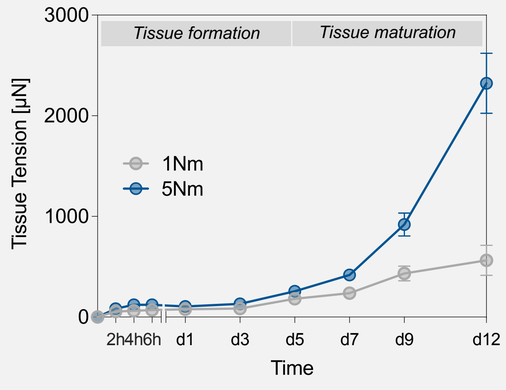
Fluorescent ubiquitination-based cell cycle indicator [FUCCI] reporter allows observation of cell cycle state in FUCCI expressing tendon cells. Confocal microscope images, N = 5 gels each time point

*Fischer, **MIMB**, 2023*

Mechanically gated switch leading to tissue-level attainment of homeostasis

Tendon cells balance cellular state, matrix remodelling and matrix synthesis during different phases of tissue repair reaching tension levels and matrix status as a function of the mechanical microenvironment.

Mechanoresponsive pathways and molecular sensors guiding tension-mediated exit from wound healing towards homeostasis will be further investigated.



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#mechanobiology #tendon #woundhealing #invitromodel #ECM #SnedekerLab

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