Mechanobiological Dysregulation in Hypermobile Ehlers-Danlos Syndrome: Traction, Contractility and Morphology

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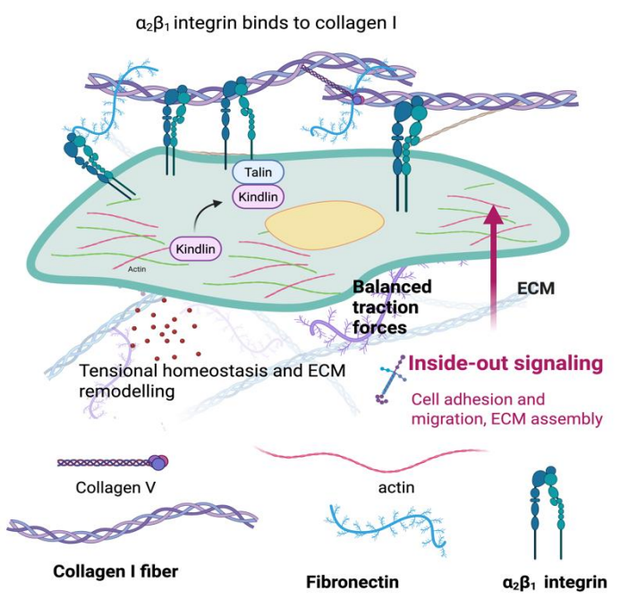
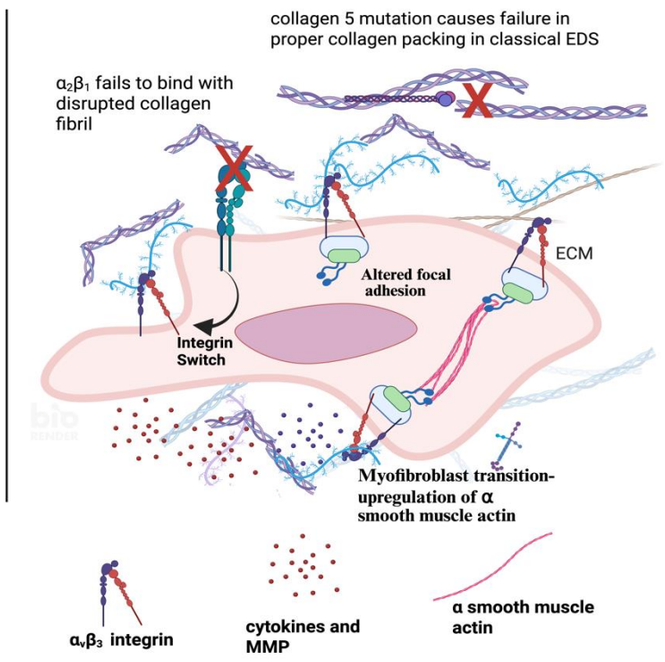
BJ Fibroblast/Healthy



Hypermobile Ehlers-Danlos Syndrome (hEDS)



BACKGROUND

hEDS & the Mechanobiological Gap

- hEDS is the most prevalent EDS subtype, affecting ~1 in 500.
- Lacks a causal gene, unlike classical EDS (cEDS), which arises from type V collagen biosynthetic defects.
- Chronic pain, joint hypermobility, and connective tissue fragility implicate aberrant fibroblast mechanosensing as a central but poorly characterised disease mechanism

The Integrin Switch Hypothesis

- Disrupted collagen fibril architecture drives an αvβ3 integrin switch, activating ILK–Snail1 signalling and promoting myofibroblast transition through upregulation of alpha smooth muscle actin (αSMA) .

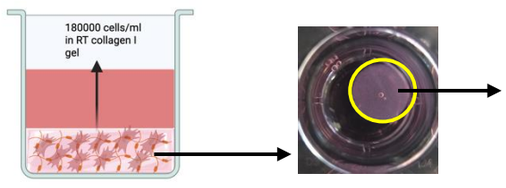
Research Aim

- We use dermal fibroblasts from healthy donors (BJF) serve as a non-pathological baseline, while classical EDS (cEDS) fibroblasts act as a disease control with a known genetic cause, allowing mechanobiological differences attributable specifically to the hEDS phenotype to be distinguished from those arising from structural collagen disruption alone.

- To determine whether hEDS fibroblasts exhibit a hypercontractile mechanical phenotype and identify the morphological correlates that distinguish them from cEDS and healthy BJF controls.

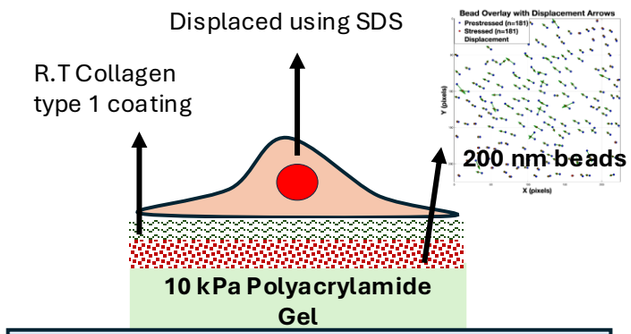
METHODOLOGY

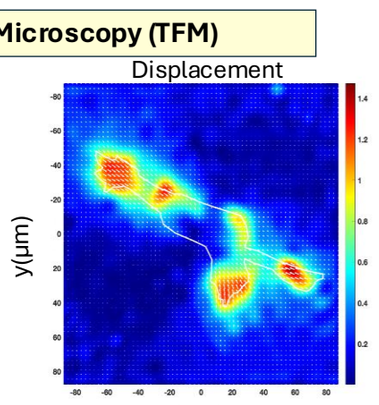
Bulk population: **Gel Contraction assay**



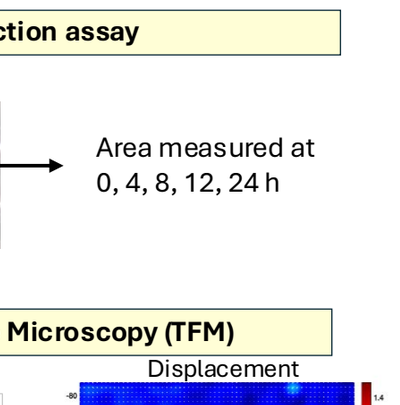
Area measured at 0, 4, 8, 12, 24 h

Single Cell population: **Traction Force Microscopy (TFM)**





Regularised Constrained Traction

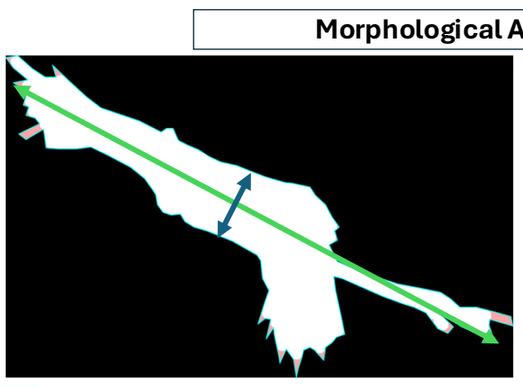


Traction, T from displacement u using the green function followed by regularisation:

Strain energy: Elastic energy stored in material from stress-strain relationship

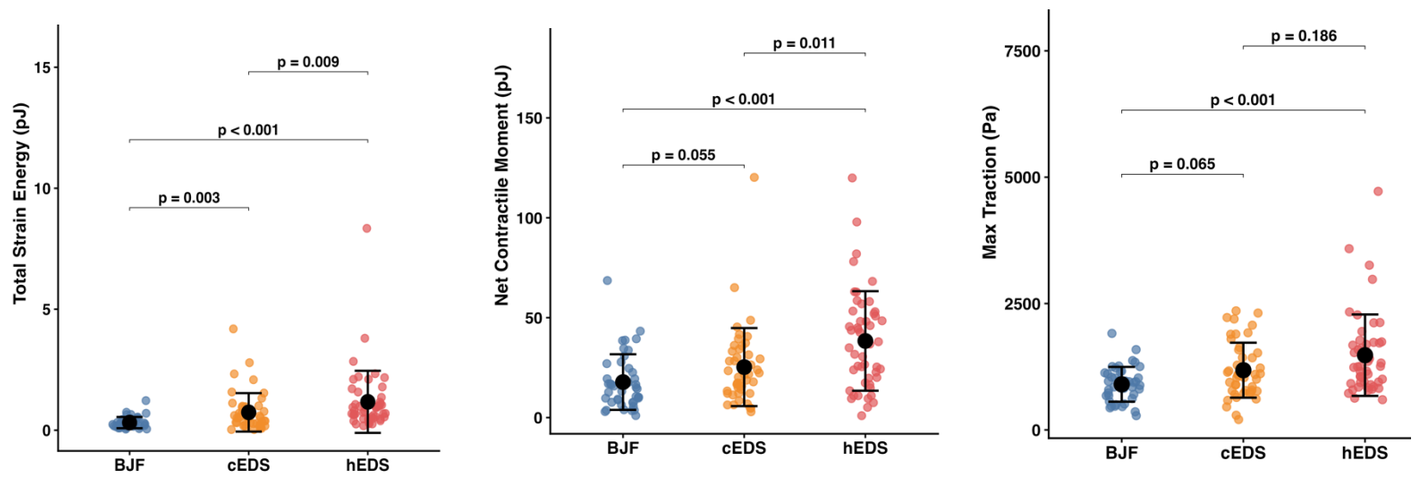
Net Contractile Moment: traction forces time displacement field integrated over area gives a view about cellular contractile strength

Morphological Analysis



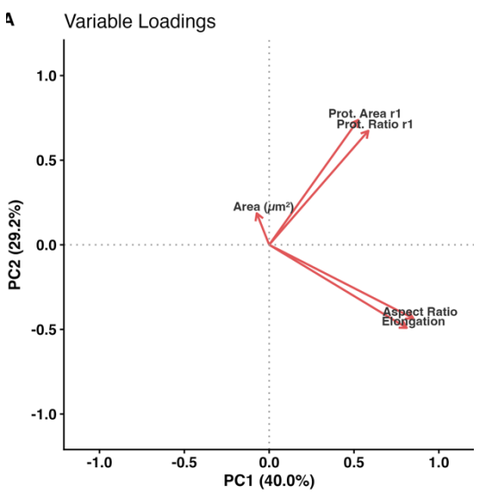
Brightfield image → Binary mask → Morphological opening. → Protusion ratio (Perimeter of original mask ÷ perimeter after morphological opening)

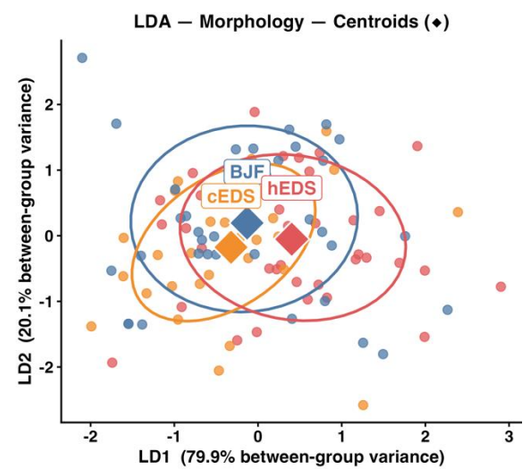
hEDS cells demonstrate higher traction and contractility in TFM



- Strain energy, maximum traction, and net contractile moment are all significantly elevated in hEDS fibroblasts, collectively indicating that the hypercontractile phenotype manifests as greater substrate deformation, higher peak focal adhesion forces, and stronger directional contractility compared to cEDS and BJF controls.

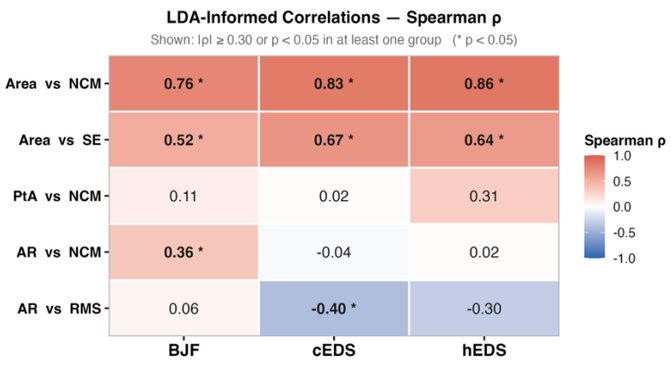
Traction shows a correlation with morphology





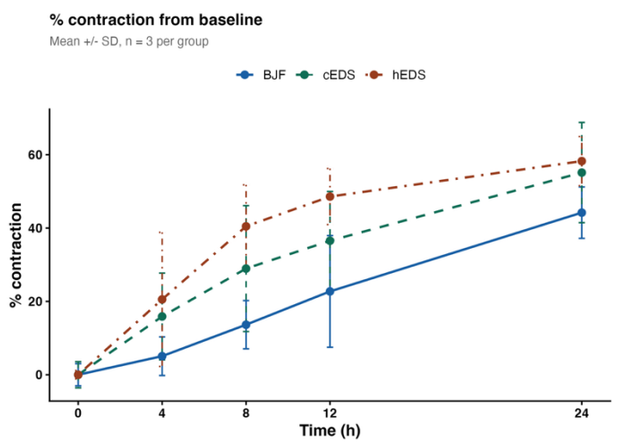
- LDA separates hEDS from BJF and cEDS along a single protrusion-size axis (LD1 = 79.9% between-group variance), driven by greater cell area and protrusion complexity rather than any single morphological parameter .

- Protrusion area predicts contractile moment specifically in hEDS (ρ = 0.31), while aspect ratio negatively correlates with traction in cEDS (ρ = −0.40), indicating disease-specific morphology–mechanics coupling.



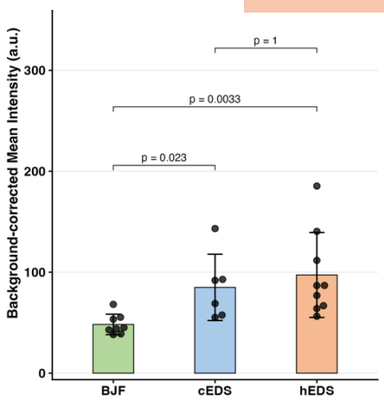
AR: Aspect Ratio, **RMS**: RMS traction, **NCM**: Net contractile Moment, **SE**: Strain Energy, **PtA**: protusion area

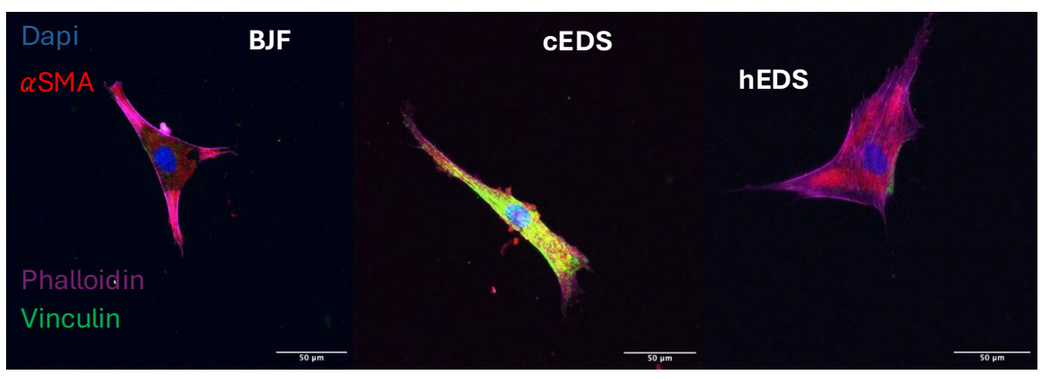
hEDS cells show elevated bulk contractility



- hEDS fibroblasts produced significantly greater gel contraction than both cEDS and BJF at all timepoints,.
- cEDS fibroblasts contracted less than hEDS, suggesting that structural collagen disruption partially elevates contractility.

hEDS cells upregulate α-SMA on collagen coated gel





αSMA intensity is significantly elevated in hEDS fibroblasts compared to both cEDS (p = 0.004) and BJF (p = 0.023), confirming progressive myofibroblast activation across the disease spectrum.

FUTURE WORK

Cell Intrinsic

- ROCK inhibitor: Test myosin-II dependence of contractility.
- MMP inhibitors : Determine if proteolytic remodelling modulates contractility.
- Integrin-blocking antibodies: Specifically block α5β1 or αvβ3 to test whether integrin-switching drives contractile changes.

ECM Dependent

- Dissect ECM-dependent vs intrinsic determinants of contractile dysfunction through reciprocal ECM swapping traction force microscopy.
- Transfect cells with paxillin-GFP to visualize focal adhesion assembly/disassembly in real-time
- Quantify focal adhesion parameters across ECM combinations: number, size, lifetime, and distribution
- Apply perturbations across all ECM combinations to determine if rescue mechanisms are ECM dependent.

DISCUSSION AND CONCLUSION

- hEDS fibroblasts exhibit a robust hypercontractile phenotype evidenced convergently by elevated traction forces, net contractile moment, and strain energy at the single-cell level, and greater collagen gel compaction at the population level.

- Cell area emerges as the strongest morphological predictor of contractility across all three cell lines (ρ = 0.76–0.86), while protrusion area correlates specifically with contractile moment in hEDS, suggesting that the hypercontractile phenotype is coupled to a more protrusive, spread morphology consistent with myofibroblast transition driven by the αvβ3 integrin switch.

- Elevated α-SMA intensity in hEDS fibroblasts further supports myofibroblast activation as the mechanistic basis of the observed contractile excess, distinguishing it from the structural ECM deficiency driving cEDS contractility.

- Methodological constraints including operator-dependent boundary tracing, limited sample size, and single-channel bead imaging introduced variability; future work should incorporate automated segmentation, larger cohorts, and live focal adhesion imaging to establish causal mechanistic links.

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