

Mechanical Quantification of Fibrosis in 2D and 3D models using the Pavone Nanoindenter

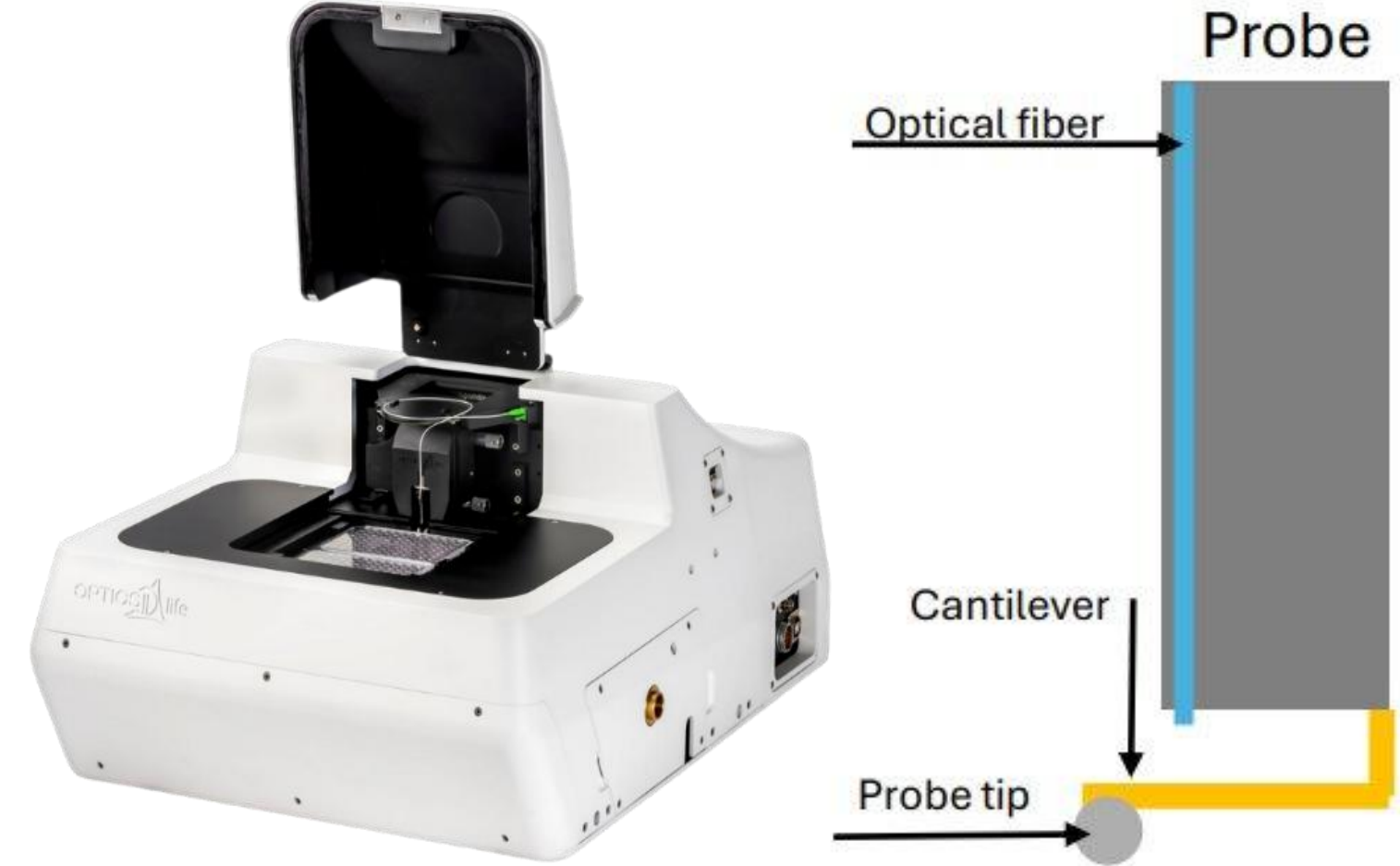


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Introduction

In IPF, increased ECM stiffness promotes scarring, fibroblast activation, and collagen deposition, reducing lung function. Target Enzyme (TE), upregulated by inflammation and fibrotic signals, worsens fibrosis by crosslinking ECM proteins, further stiffening tissue and creating a feedback loop that drives disease. Measuring ECM stiffness and TE activity is critical for monitoring IPF progression.



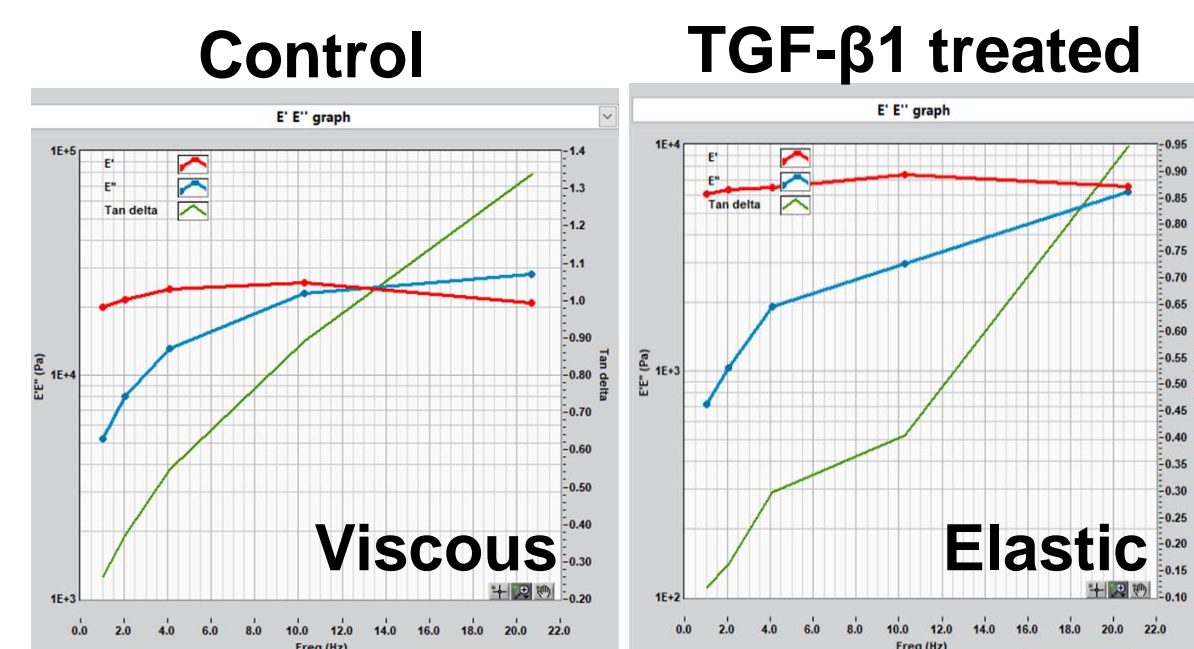
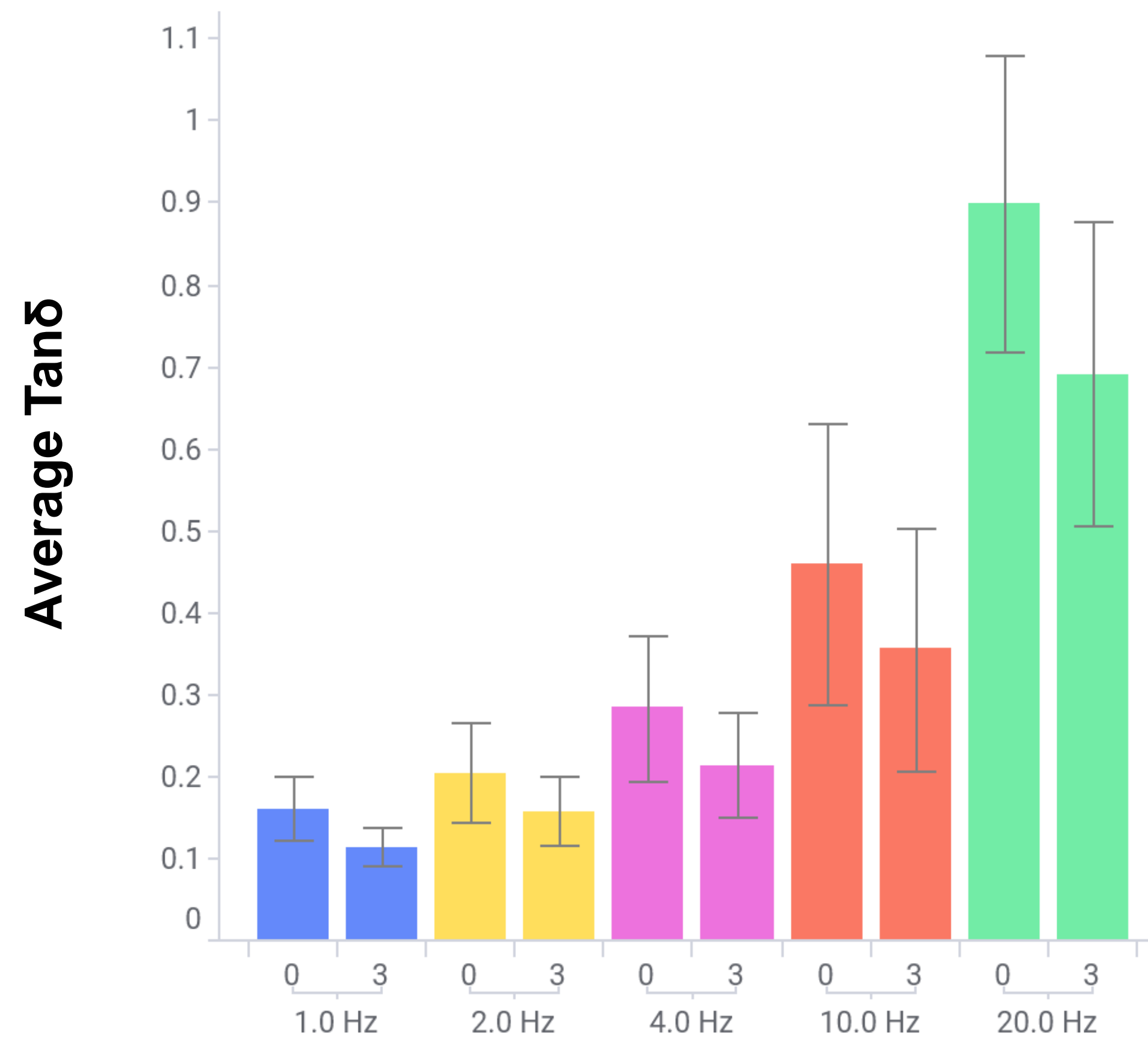
We use the **Pavone Nanoindenter** by **Optics11Life**, an automated Atomic Force Microscope that integrates indentation, imaging, and environmental control to precisely measure stiffness and viscoelasticity via vibrating cantilever probes detecting phase shifts.

"Pavone Nanoindenter: High-Throughput Mechanical Characterization." *Optics11 Life DEV*, 24 Sept. 2024, www.optics11life.com/products/pavone-nanoindenter/

TGF-β1 and TE treatment alters viscoelastic properties of ECM across multiple donors

(A) TGF-β1 Stimulation Increases ECM Elasticity

● 1.0 Hz ● 2.0 Hz ● 4.0 Hz ● 10.0 Hz ● 20.0 Hz



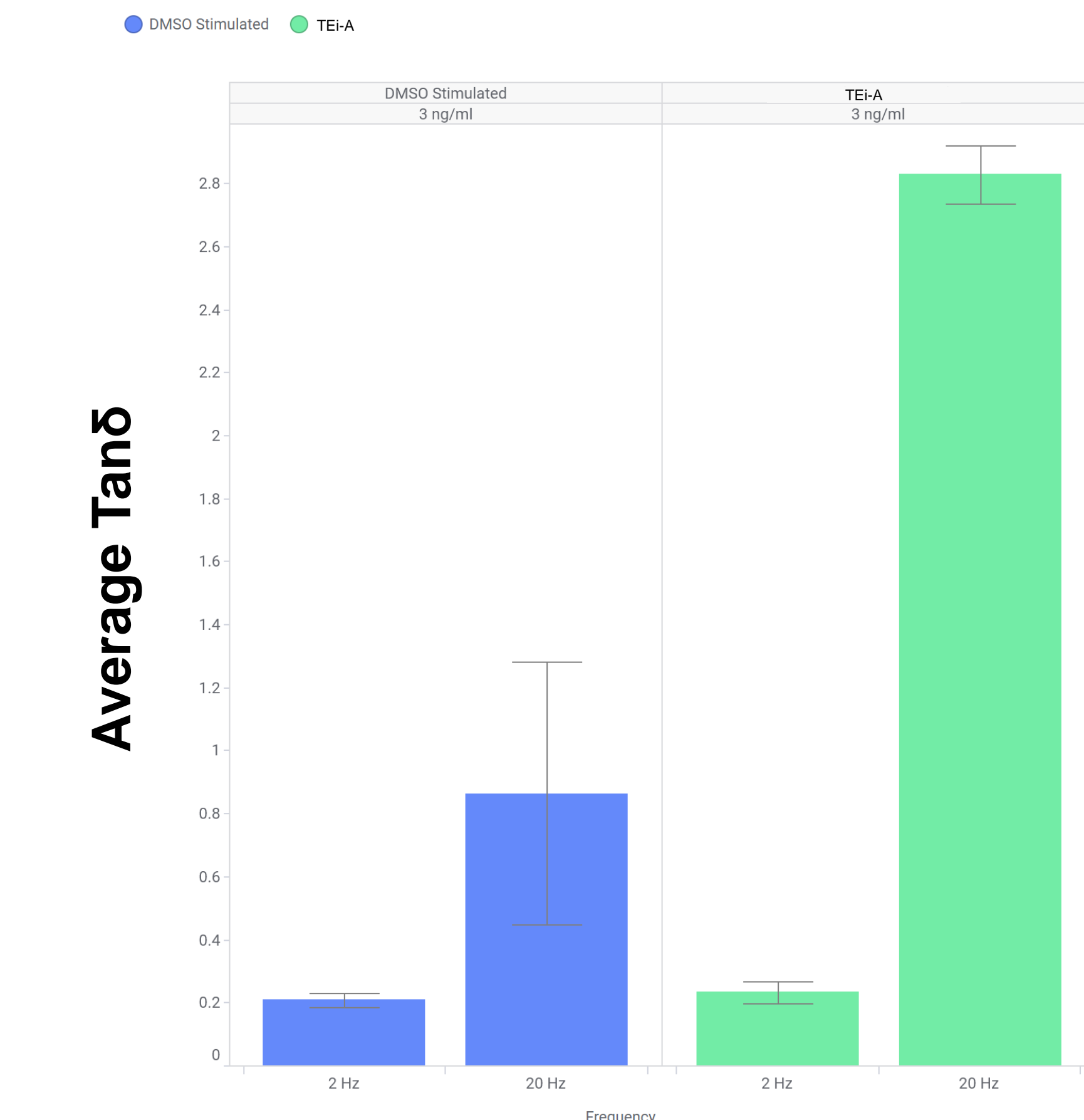
Lower $\tan \delta$ = fibrotic
Higher $\tan \delta$ = healthy

$$\text{TAN } \delta \text{ (Ratio)} = E'' / E'$$

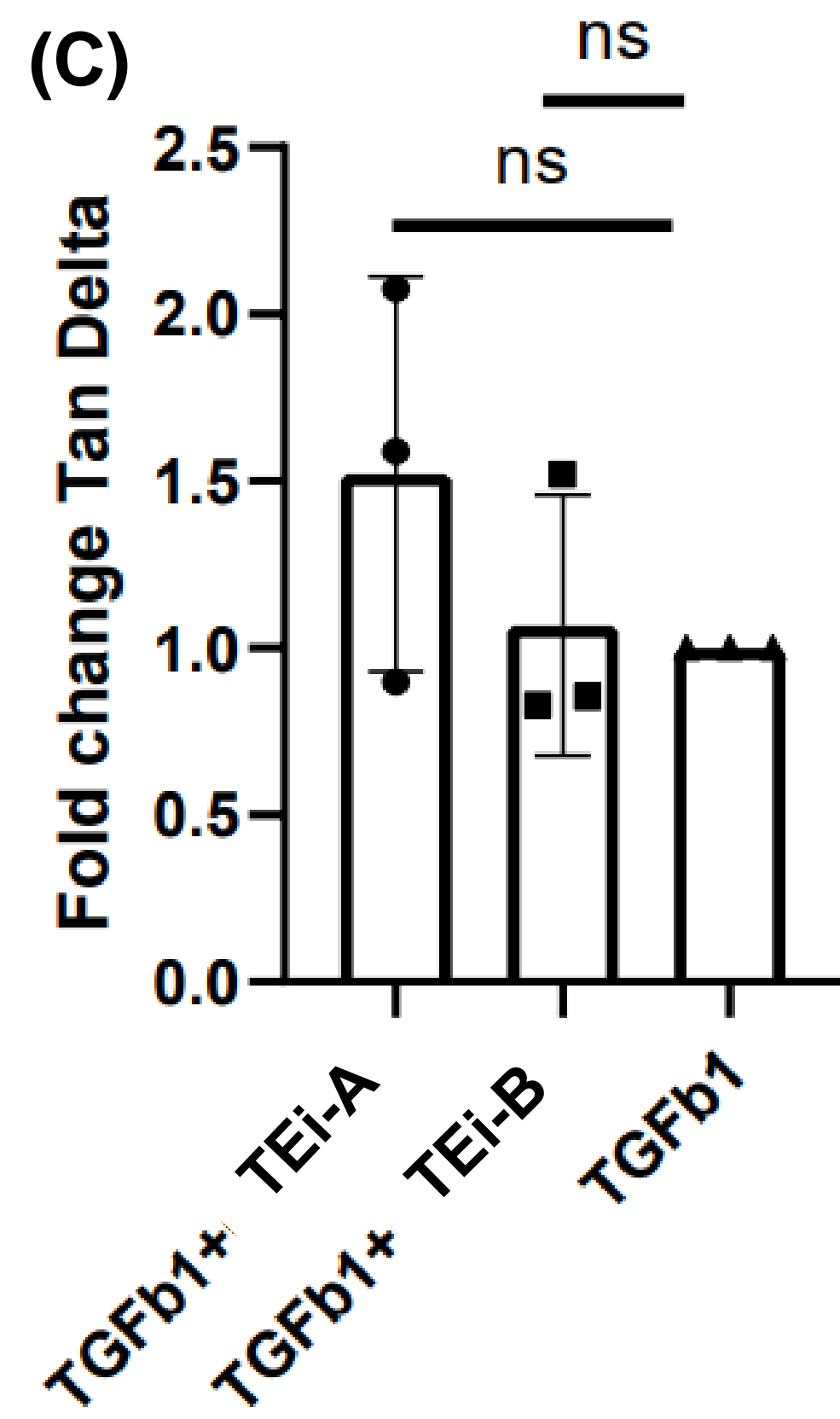
E'' = Loss Modulus
Viscosity, Fluid-like behaviour

E' = Storage Modulus
Elasticity, solid-like behaviour

(B) TE inhibitor Increases ECM Viscosity



(C) Fold Change $\tan \delta$ n=3 pooled (Fixed ECM)



(A) Tan delta across 1–20 Hz comparing control and TGF-β1-stimulated ECM. TGF-β1 samples show consistently lower values, indicating increased stiffness and elasticity.

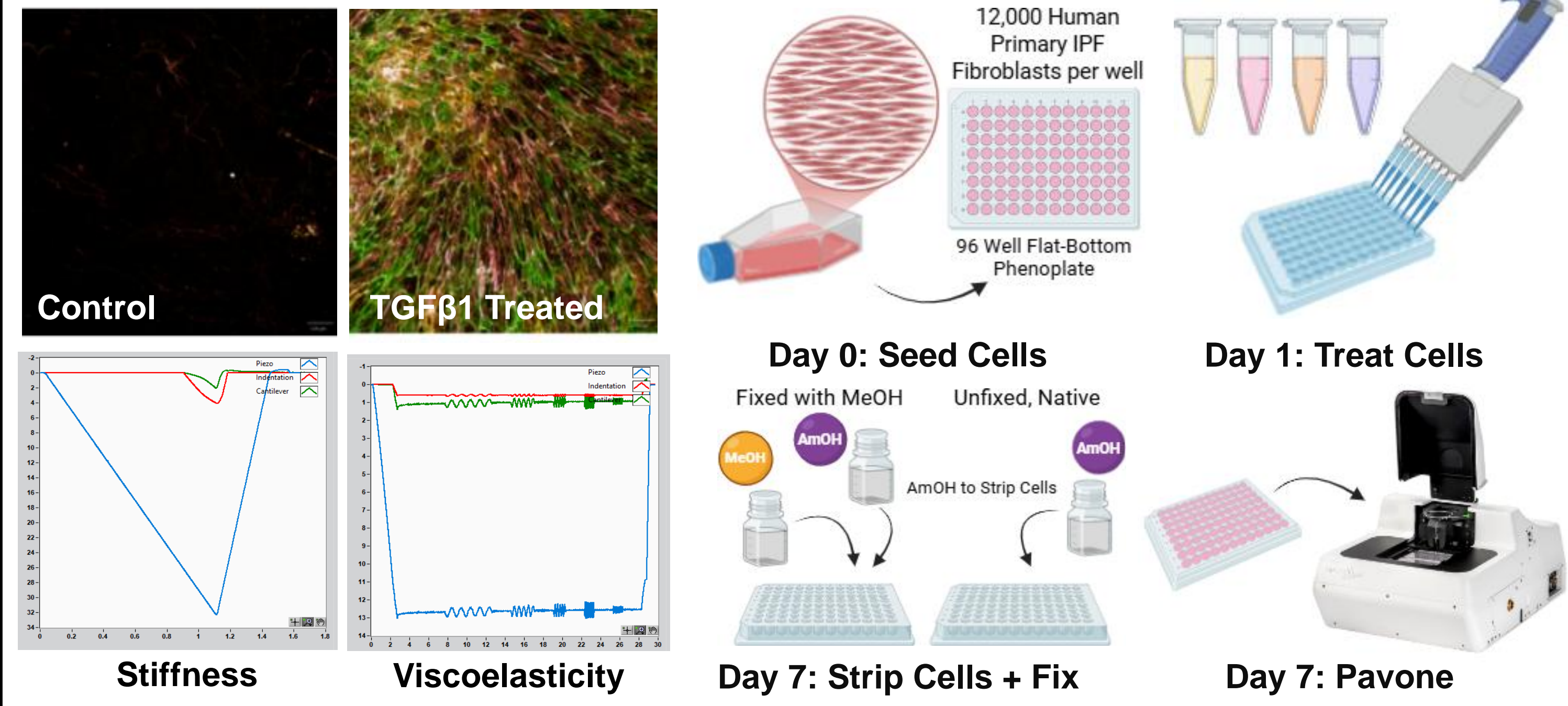
(B) Tan delta at 2 Hz and 20 Hz comparing TGF-β1 vs. TE inhibitor + TGF-β1. Inhibitor-treated samples show higher values, suggesting increased viscosity and reduced elasticity. Control samples excluded due to insufficient ECM thickness.

(C) Fold change plot of pooled fixed ECM from 3 IPF donors shows a slight, non-significant trend toward higher tan delta with TE inhibitor, suggesting more fluid-like ECM. Trend was not reproducible across donors. Control samples excluded due to insufficient ECM thickness.

Summary

- **TE promotes fibrosis**, activates TGF-β1 and crosslinks ECM, increasing tissue stiffness.
- **ECM stiffening accelerates fibrosis** and fibroblast activation in IPF.
- **Pavone measurements** show TGF-β1 increases stiffness and TE inhibition reduces it—**only in fixed samples**.
- **2D ECM is thin and fragile**, limiting reliable indentation and causing data exclusions.
- **Hard plastic substrates** in 2D models artificially influence cell stiffness and structure.
- These limitations **support a shift to 3D models** for more accurate, physiologically relevant assessments.
- **Ongoing work** focuses on optimizing spheroid models for realistic fibrosis testing.

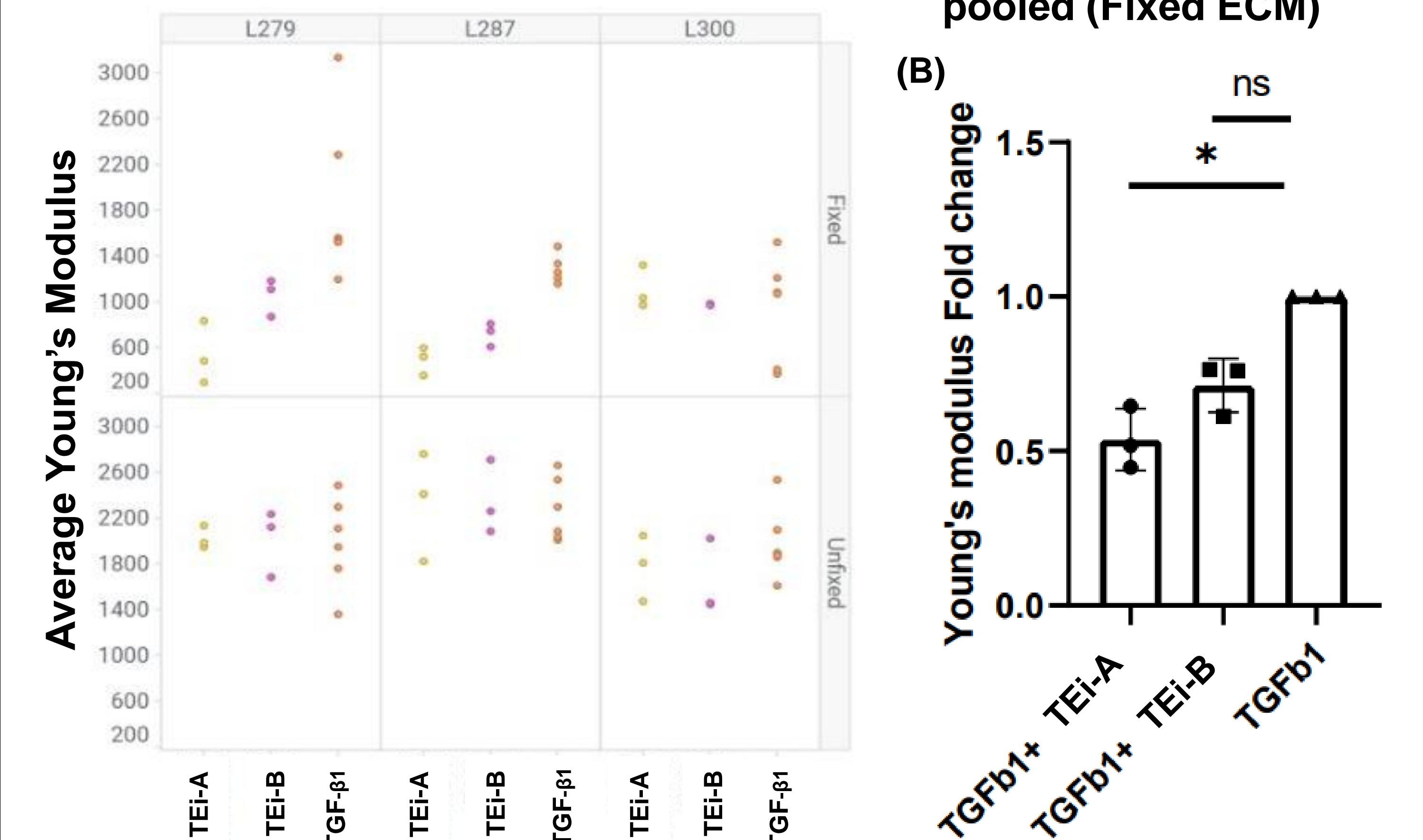
Methods



For the 2D model, human IPF fibroblasts (12,000 cells/well) were plated in 96-well plates and treated with control or TGF-β1 ± TE inhibitor for 1 week. Two plates were prepared: one methanol-fixed, one unfixed. After cell removal with AmOH, samples were tested on the Pavone to measure stiffness (indentation) and viscoelasticity (frequency oscillation).

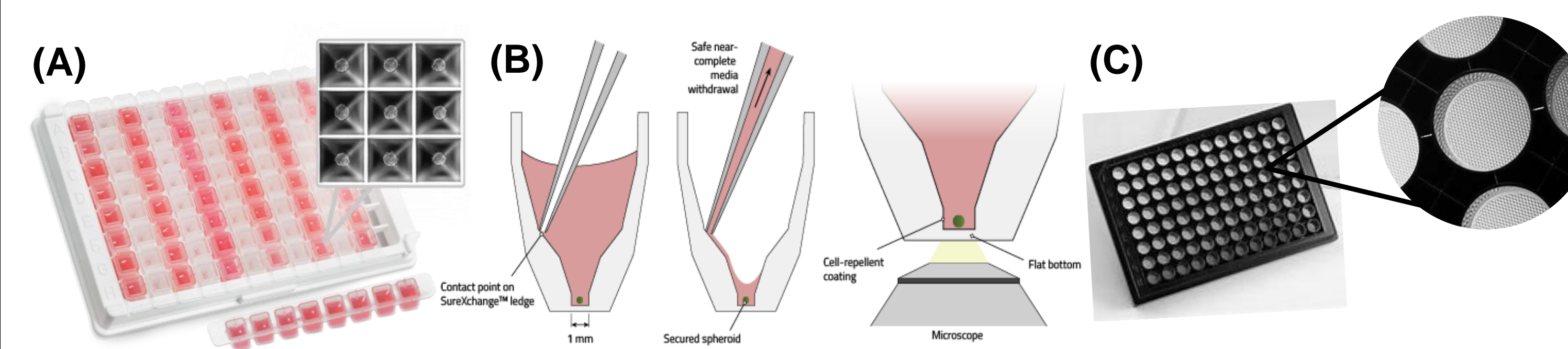
Both Fixing and TE inhibitors alter Stiffness of ECM

(A) TE Inhibitor Reduces Stiffness across donors, but only in Fixed ECM



(A) Scatter plot of average Young's modulus values across multiple donors. L279 and L287 show increased stiffness in TGF-β1-stimulated ECM compared to TE inhibitor-treated samples, while L300 shows no significant differences. Trends observed only in fixed ECM. Control samples were excluded again due to thin ECM. Kruskal Wallis (B) testing revealed significant reduction in ECM stiffness with TE inhibitor across multiple donors (fixed only).

Shifting to a 3D Model for Accuracy in Measurements



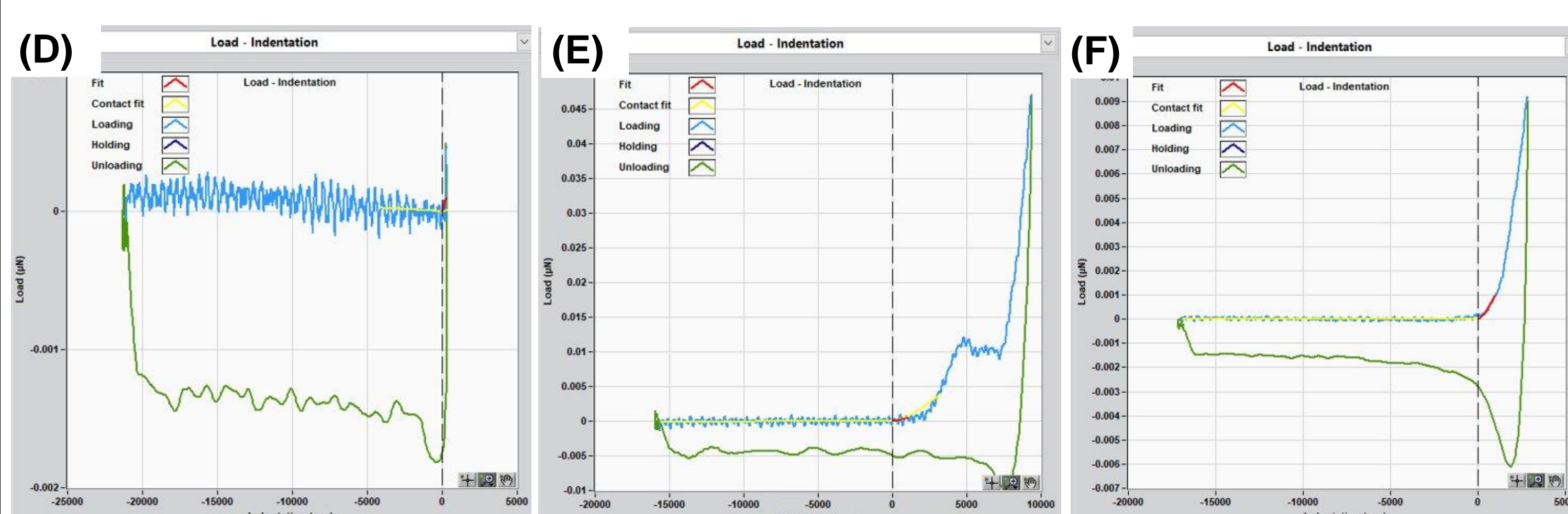
"96-Well Strip Format | Sphericalplate 5D." *Sp5d.com*, 2025, www.sp5d.com/96-well-plate/.

"Akura™ 96 Spheroid Microplate - InSphero." *InSphero*, 27 Nov. 2024, insphero.com/3d-cell-culture-tools/akura-96-spheroid-microplate/.

"Corning® Eplasia® 96-Well Black/Clear, Plasma Treated, Microcavity Microplate, with Lid | Corning." *Corning.com*, 2025, catalog.corning.com/life-sciences/Microplates.

Kugelmier (A) and Corning Eplasia plates (A) allow high-throughput culture (25+ spheroids/well) but are incompatible with Pavone due to v-bottom design. Akura plates (B) support larger spheroids but have narrow wells (1 mm), limiting probe access. Currently, spheroids (25,000 cells) are cultured in Akura plates and transferred to collagen coated flat-bottom plates for testing with Pavone.

Capability Build for 3D Model with Spheroids



(D) 2,500-cell spheroids are too small and soft, leading to indentation failure. (E) 5,000-cell spheroids are large enough but unstable, causing probe interference with the plate. (F) 25,000-cell spheroids were optimal, yielding consistent Young's Modulus and DMA data. Over time, spheroids showed increased stiffness: Week 1 lack structure, Week 3 are measurable but soft, Week 6 support complex testing.

Acknowledgements

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