

ECM Stiffness Regulates Fibroblast Activation in Idiopathic Pulmonary Fibrosis

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Background

Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal lung disease characterized by excessive extracellular matrix (ECM) deposition and irreversible destruction of alveolar architecture. While ECM deposition and tissue stiffening have traditionally been considered consequences of fibrosis, increasing evidence suggests that they also actively contribute to disease progression by promoting fibroblast-to-myofibroblast activation and enhancing transforming growth factor-beta 1 (TGF- β 1) signaling. **In this study, GelMA hydrogels with different stiffnesses were used to mimic healthy and fibrotic lung ECM and investigate the effect of substrate stiffness on fibroblast activation.**

Methodology

Soft and stiff GelMA (Tessella Biosciences) disks (14 mm diameter, 200 μ m thickness) were fabricated using a spacer-assisted casting method. Hydrogel morphology was characterized by scanning electron microscopy (SEM), and mechanical properties were measured using an HR30 rheometer. Human lung fibroblasts (MRC-5) were cultured on soft GelMA, stiff GelMA, or glass substrates in the presence or absence of TGF- β 1 (10 ng/mL) for 72 hours. Fibroblast activation was evaluated by assessing α -smooth muscle actin (α -SMA) expression using confocal microscopy and Western blot analysis.

Key Findings

- **Soft and hard GelMA** exhibited storage moduli (G') of 402.6 ± 7.6 Pa and 4292.0 ± 97.0 Pa, respectively, **representing the mechanical properties of healthy and early-stage fibrotic lung tissue.**
- Confocal microscopy revealed **minimal α -SMA stress fiber** formation in MRC-5 fibroblasts cultured **on soft GelMA**, even after TGF- β 1 stimulation.
- In contrast, TGF- β 1 induced prominent **α -SMA stress fiber formation on hard GelMA and glass substrates, indicating enhanced fibroblast-to-myofibroblast activation.** These findings were further confirmed by Western blot analysis.

Conclusion

ECM stiffening is both a consequence and a driver of fibrosis, promoting fibroblast activation and disease progression.

Results

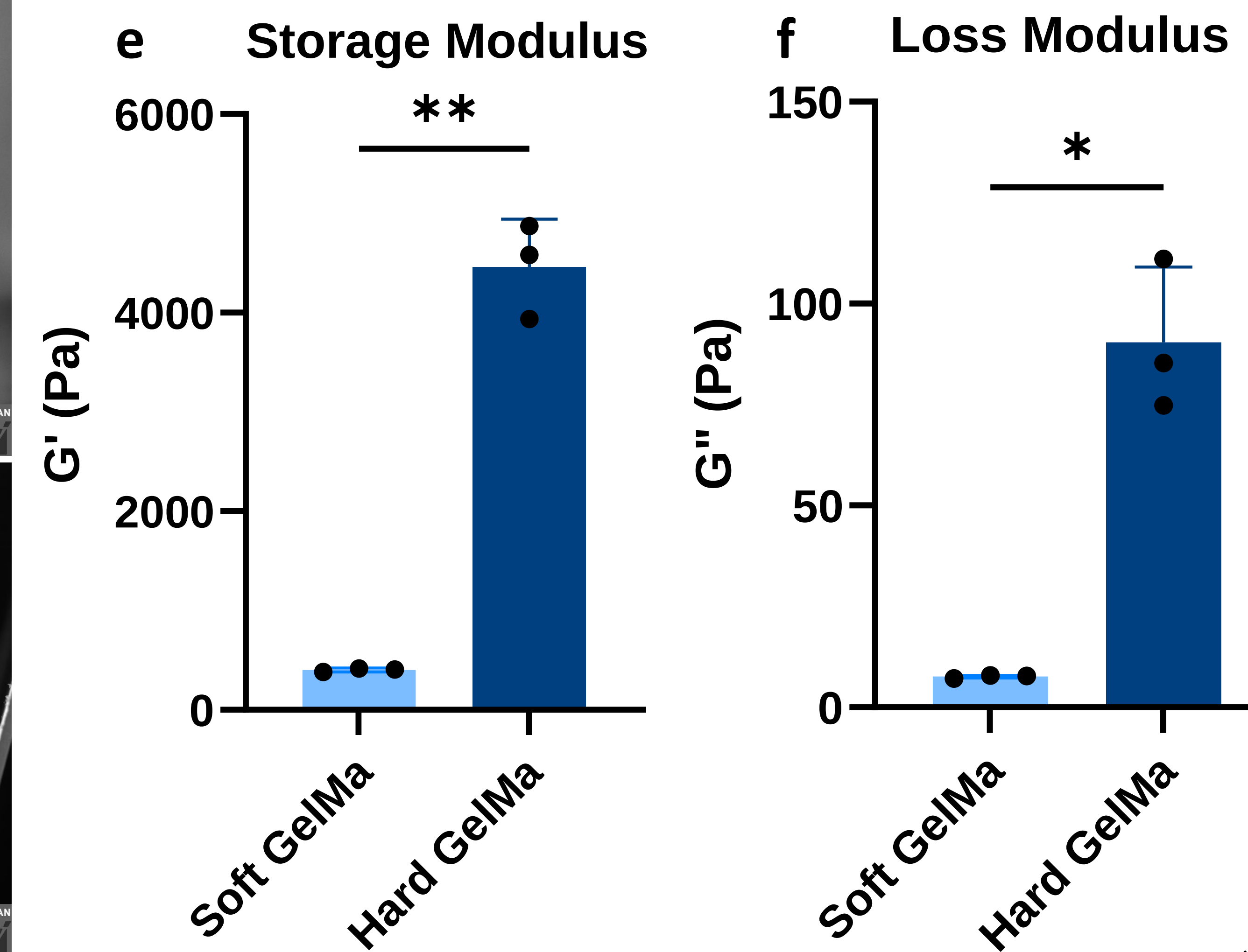
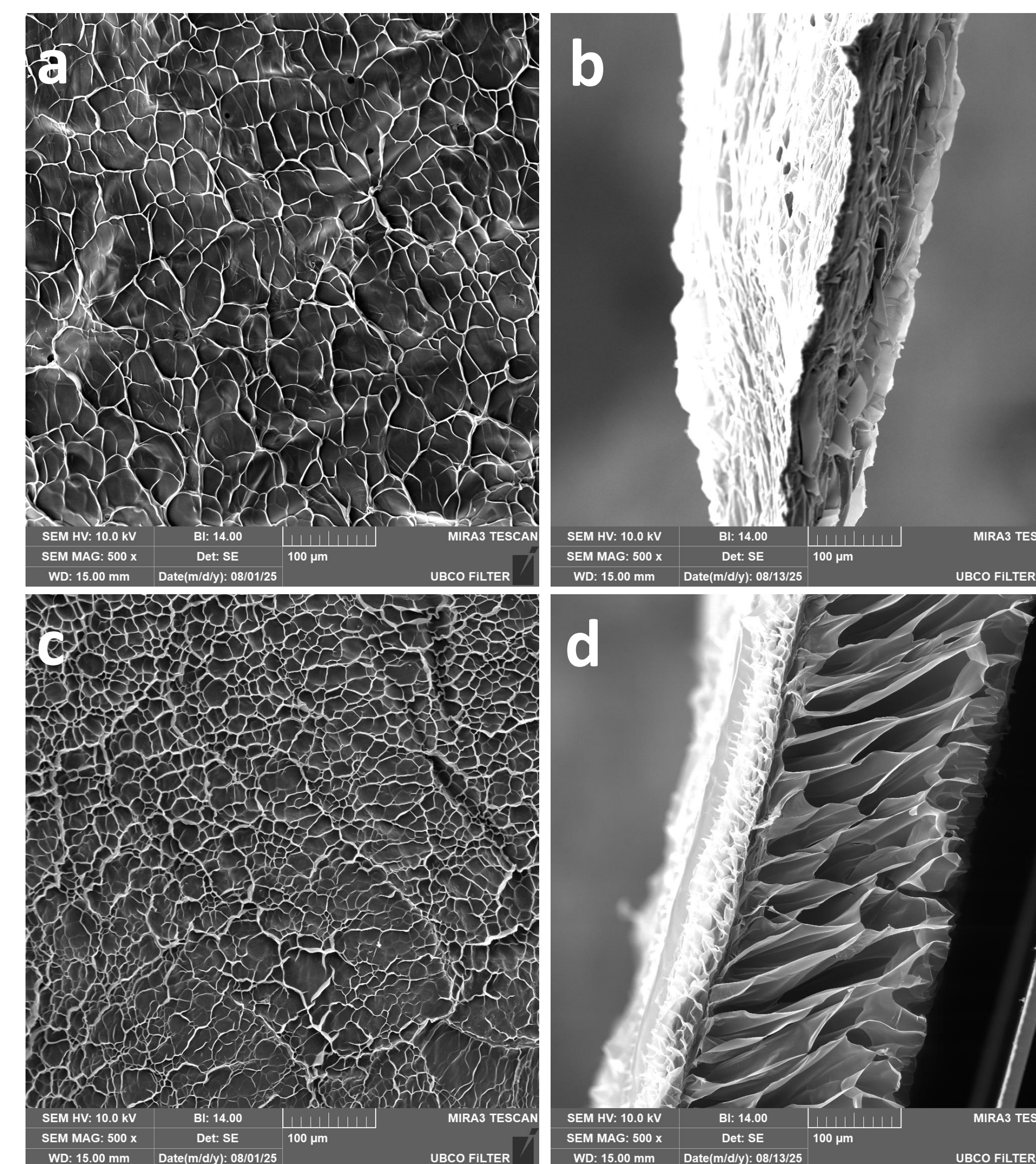


Figure 1. Material characterization. Soft GelMa (a) and hard GelMa (b) surface morphology; their cross section views (c) and (d) under SEM; Soft GelMa storage modulus G' mimic healthy lung tissue and hard GelMa G' mimic fibrotic lung tissue (e); Loss modulus G'' of soft GelMa and hard GelMa (f).

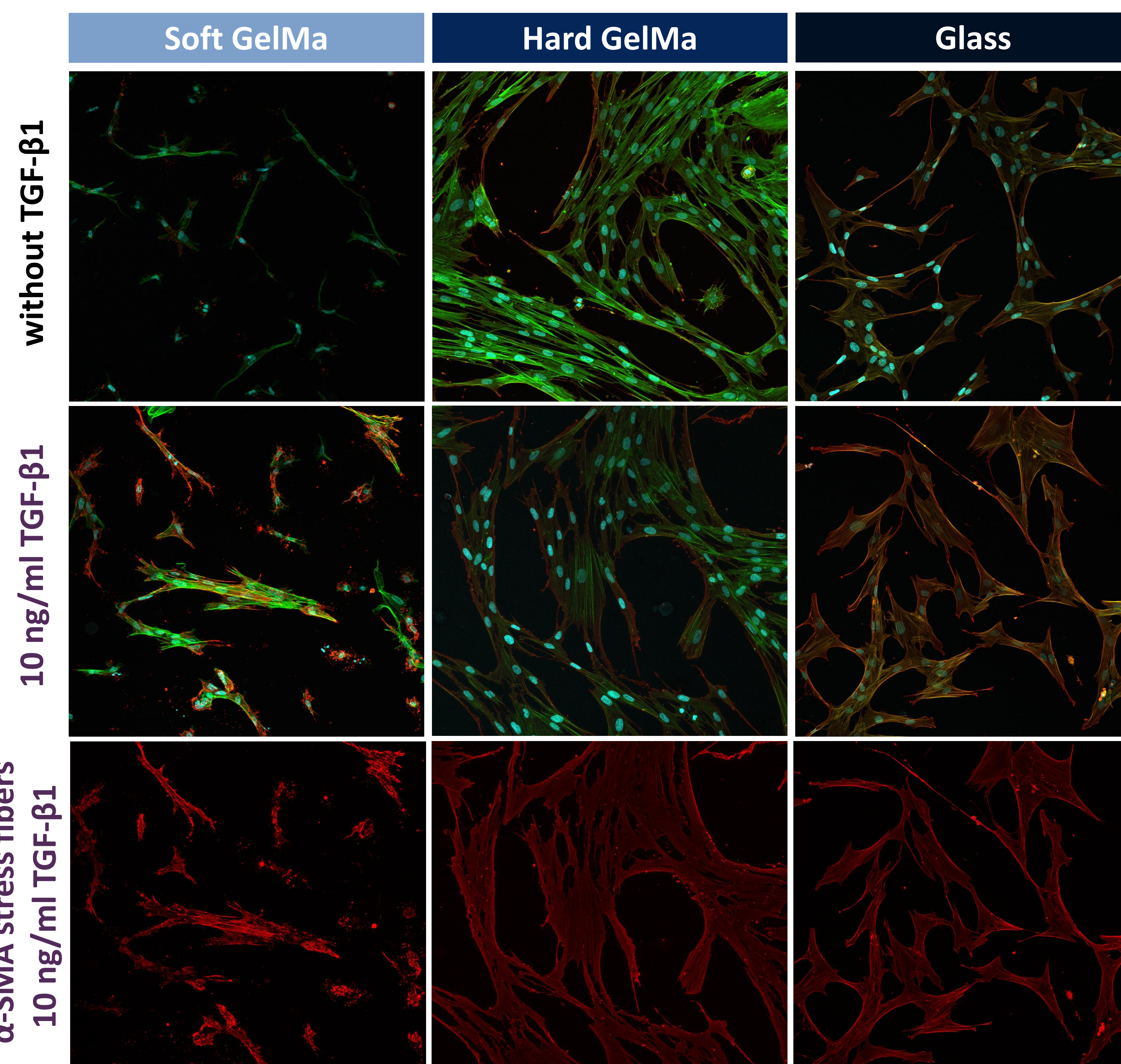


Figure 2. α -SMA expression on different stiffness ECM.

Red: α -SMA, Green: phalloidin, Blue: DAPI. Top row: MRC-5 fibroblast. Middle row: MRC-5 with TGF- β 1. Bottom row: α -SMA stress fiber corresponding to the middle row. Minimal α -SMA stress fiber formation was observed on soft GelMA, even in the presence of TGF- β 1. In contrast, α -SMA stress fiber formation increased with increasing ECM stiffness.

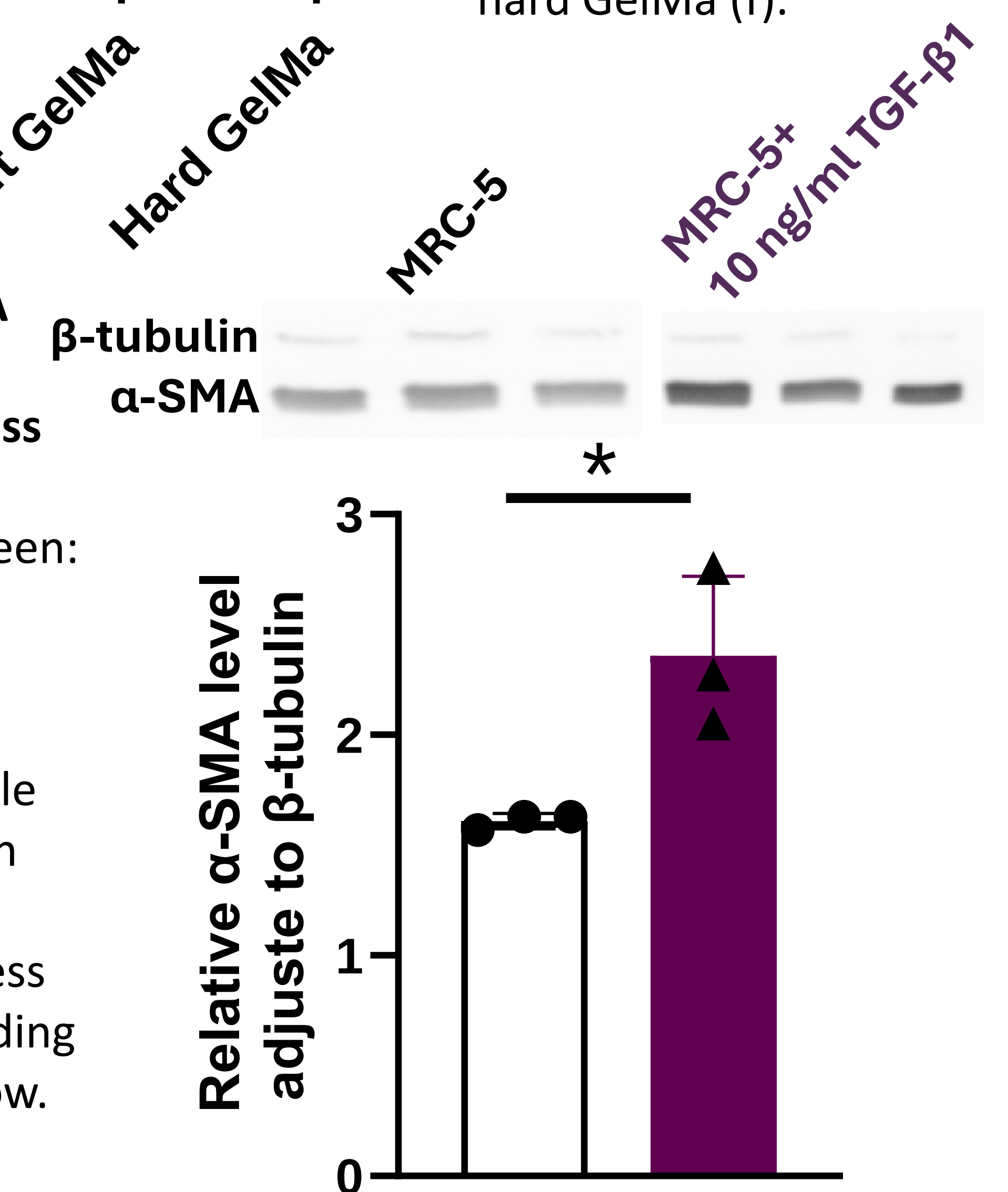


Figure 3. Western blot analysis for α -smooth muscle actin expression of MRC-5 vs. myofibroblast (MRC-5 with 10 ng/ml TGF- β 1) on glass surface.

Reference

- Lodyga, M. et al. Sci. Signal. 12, eaao3469 (2019).
- Astrab, L. R. et al. Preprint at <https://doi.org/10.1101/2024.10.13.618034> (2024).
- Astrab, L. R. et al. Biomater. Sci. 14, 1276–1291 (2026).

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