On-demand Secondary Crosslinking of an Extracellular Matrix Hydrogel for Modeling Myelofibrosis

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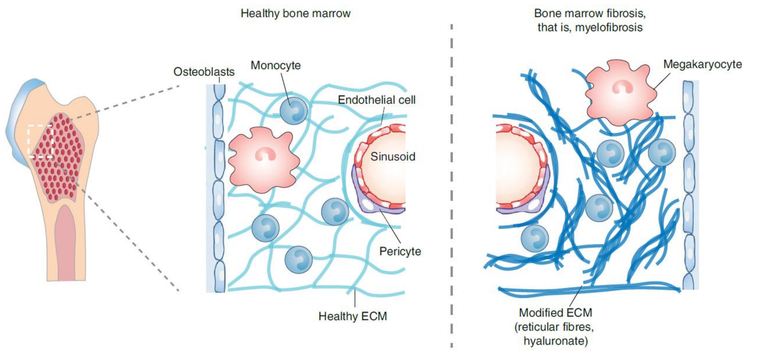
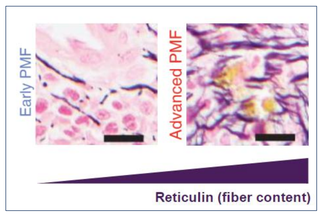
Primary myelofibrosis (PMF) is a chronic progressive myeloid malignancy

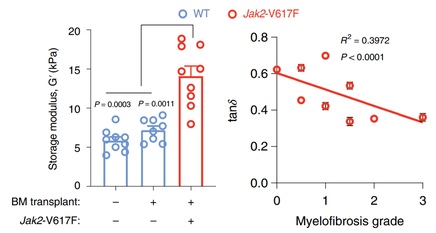
Primary myelofibrosis (PMF) features deposition of ECM in the bone marrow (BM) with a median survival of around 5-6 years.



K. Vining et al., *Nature Materials*, **2022**.

BM tissue exhibits increased stiffness and elasticity in PMF





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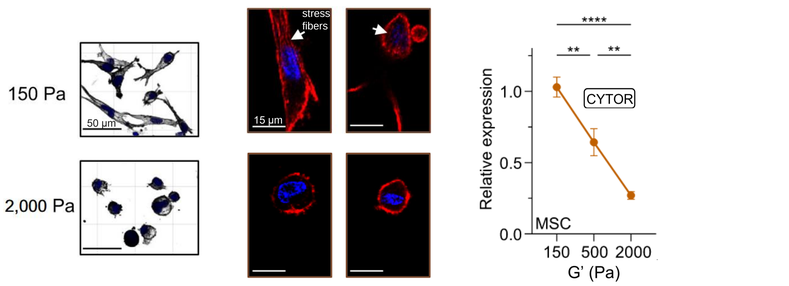
Cell morphology and gene expression of MSCs are significantly affected by ECM hydrogel stiffness

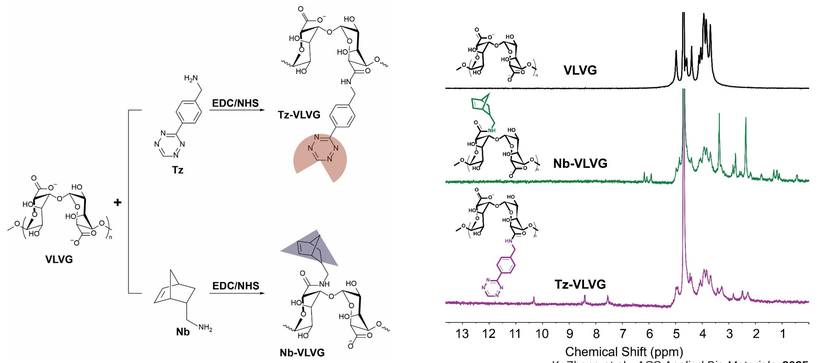


J. Lim et al., *Proc. Natl. Acad. Sci.*, **2024**.

Mesenchymal stromal cells (MSCs) in soft ECM (150Pa) exhibit a more spread morphology and different gene expression level compared to stiff ECM (2000Pa)

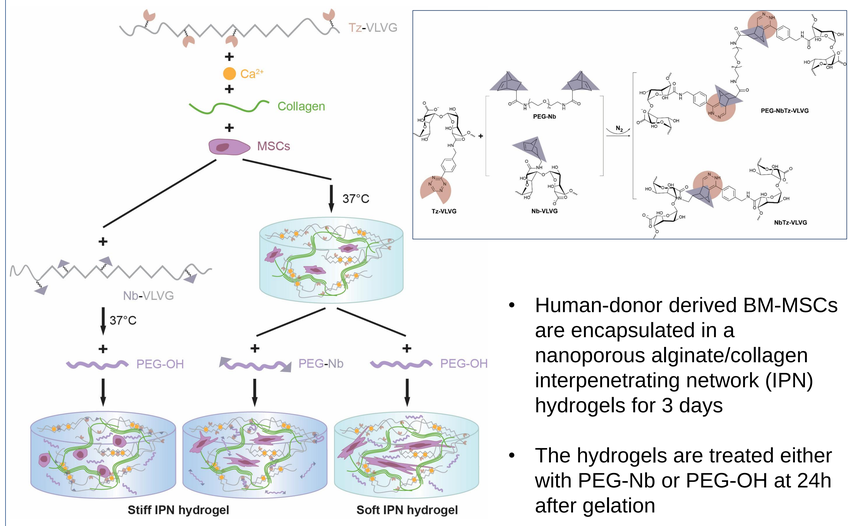
- **Here we propose a novel biopolymer-based hydrogel platform that can be stiffened on-demand, enabling initial cell spreading prior to matrix stiffening.**
- **This approach overcomes a key limitation of existing models in which cells are directly encapsulated in stiff matrices, restricting cell spreading and thereby impairing cellular function.**

BM-MSCs are encapsulated in click-alginate/collagen IPN hydrogel



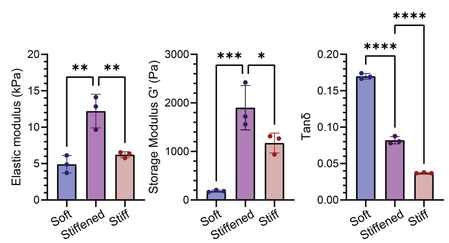
K. Zhang et al., *ACS Applied Bio Materials*, **2025**.

Sodium alginate (VLVG) is functionalized with norbornene (Nb) and tetrazine (Tz) based amines



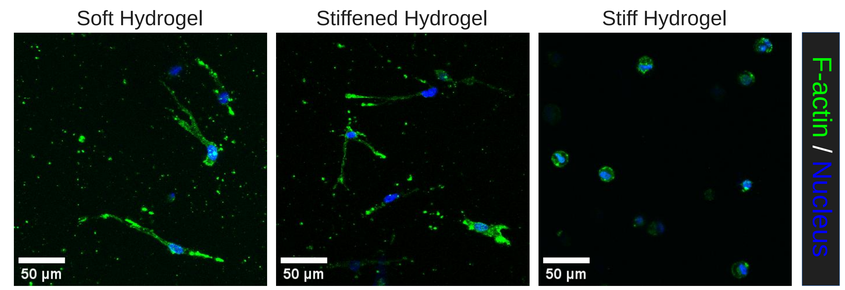
- Human-donor derived BM-MSCs are encapsulated in a nanoporous alginate/collagen interpenetrating network (IPN) hydrogels for 3 days
- The hydrogels are treated either with PEG-Nb or PEG-OH at 24h after gelation

PEG-Nb tuned stiffness and viscoelasticity of IPN hydrogel



PEG-Nb treated Tz-VLVG hydrogel showed significantly increased compressive elastic modulus, G', and elasticity

BM-MSCs retain morphology in stiffened ECM hydrogels



- Cells spread in the soft hydrogel before PEG treatment while remain spherical shape in stiff hydrogel
- Following PEG treatment, cells that were initially spread maintained their spread morphology, while the matrix was stiffened through crosslinking between Tz-VLVG and PEG-Nb

Cells in soft hydrogel show significantly increased expression of F-actin, suggesting stiffness-dependent cell response to matrix mechanics

Conclusions and future direction

- A biopolymer-based IPN hydrogel platform was fabricated with ionically crosslinked Tz-VLVG and self-assembled collagen type I, which replicates the fibrillar architecture of the BM ECM.
- Pre-gelled samples were stiffened by treating with PEG-Nb crosslinkers, mimicking the progressive stiffening of the BM niche during PMF.
- BM-MSCs were encapsulated in soft-stiffened and stiff hydrogels and showed distinct changes in cell morphology and F-actin expression.
- Future work will involve investigating the secretome of MSCs to ECM stiffness and viscoelasticity change, as well as the myeloid-stromal crosstalk by co-culturing MSCs and immune cells in the construct.

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