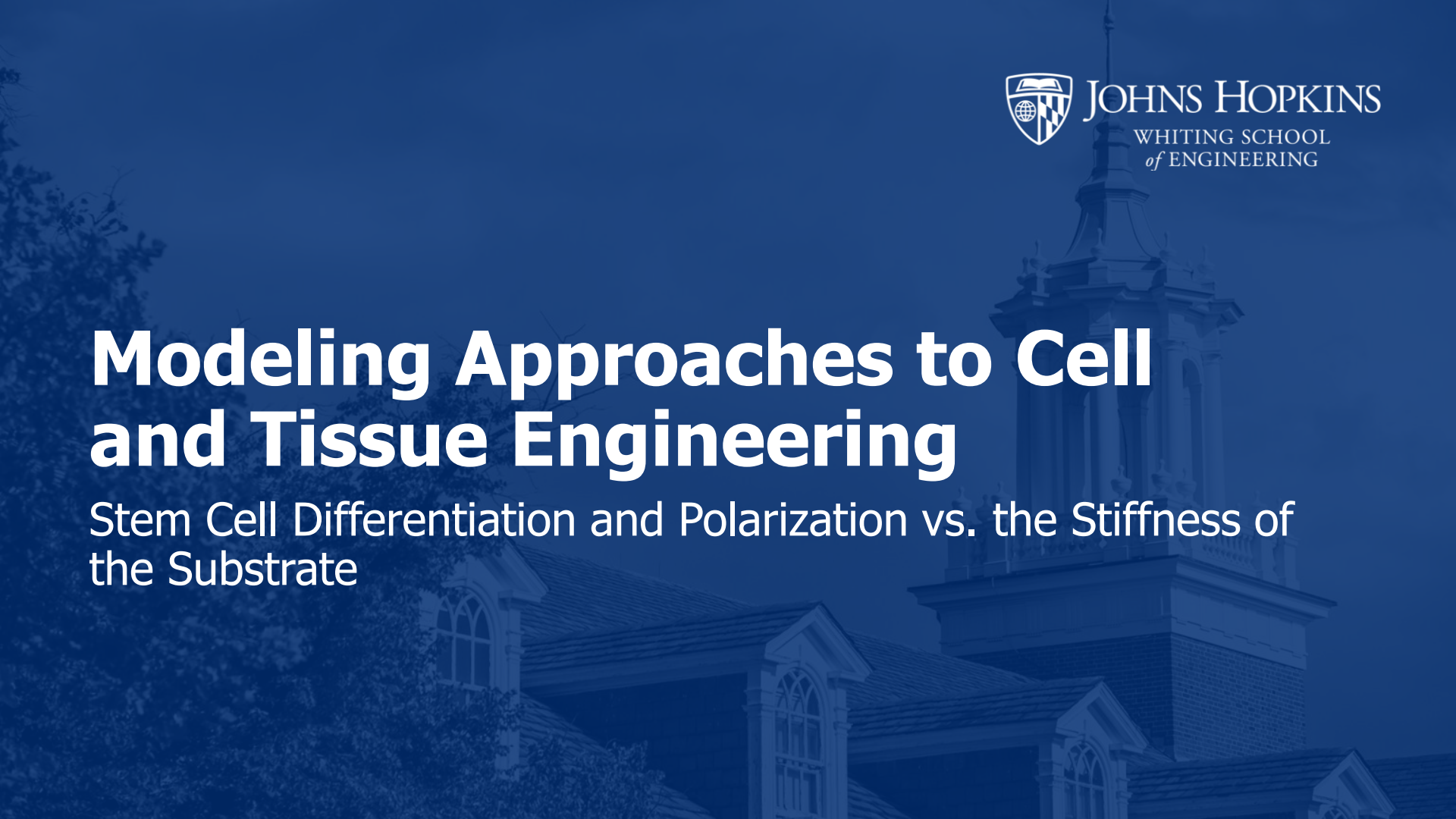


Modeling Approaches to Cell and Tissue Engineering

Stem Cell Differentiation and Polarization vs. the Stiffness of the Substrate

Notations

We denote by **Cc** and **Cm** the (fourth rank) elastic moduli tensors of the cell and the matrix respectively.

We denote by $-\mathbf{u}^0$ij the early-time, isotropic strain associated with the anchoring and spreading of the cell in an infinitely rigid matrix.

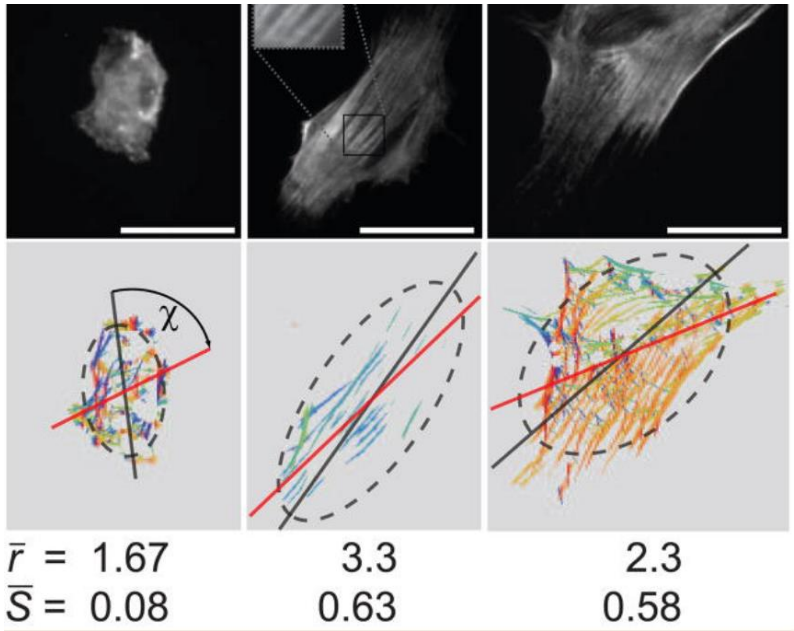
We define as $\mathbf{u^c ij} - \mathbf{u^0 ij}$ the strain in the cell.

We define as σ^cij $= \mathbf{Cc}\left(\mathbf{u^c ij} - \mathbf{u^0 ij}\right)$ the stress in the cytoskeleton.

We define the average dipole per unit volume, , $< p_{ij} >$, (equivalent to a force per unit area) exerted by the acto-myosin elastic, dipolar forces in any volume element within the cell. We assume that these force-dipoles polarize in response to the local stress in the cell, changing their magnitude and orientation from their average, isotropic initial value $< p^0 ij >$.

We assume a feedback response in the form as $p^a\mathbf{ij} = -\alpha\mathbf{Cc}\left(\mathbf{u^c ij} - \mathbf{u0ij}\right)$ where $p^{\mathbf{a}}{}_{ij} = p_{ij} - p^0\mathbf{ij}$, is the polarization tensor. The tensor $\boldsymbol{\alpha}$ determines the response of the cell to the mean cytoskeleton stress, given by: $\boldsymbol{\sigma}^{\mathbf{c}}\mathbf{ij} = \mathbf{Cc}\left(\mathbf{u^c ij} - \mathbf{u^0 ij}\right)$.

Stem Cell (hMSC) Alignment on Substrates of Different Stiffness

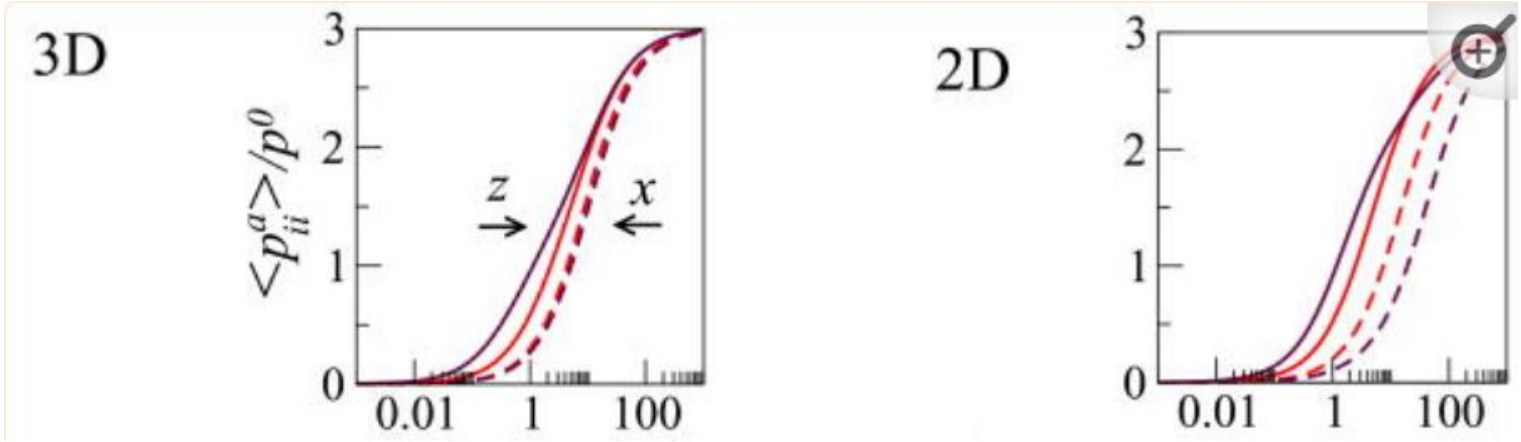


Young's moduli are 1kPa, 10 kPa, and 30 kPa

Stem Cell (hMSC) Alignment on Substrates of Different Stiffness (Young's Moduli Are 1kPa,10kPa, and 30 kPa continued)

Acto-myosin stress-fiber alignment in hMSCs sparsely plated on 2D substrates of different elasticity. The top row shows hMSCs immuno-stained for non-muscle myosin IIa (NMMIIa) 24 hours after plating on elastic substrates with a Young's modulus E_m of 1 kPa, 11 kPa, and 34 kPa that are the most representative cells of the mean values obtained for cell area *A*, aspect ratio of long to short axis *r*, and stress-fiber order parameter $S = \langle \cos 2\theta \rangle$; where θ is the angle between each stress-fiber in the cell and the long axis of the fitted ellipse. The bottom row shows the respective orientational plots, where the different orientations of myosin filaments are depicted with different colours. The dark gray dashed ellipses are best fits to the cell edge and the red line indicates the mean orientation of the stress-fibers as determined by the automated algorithm.

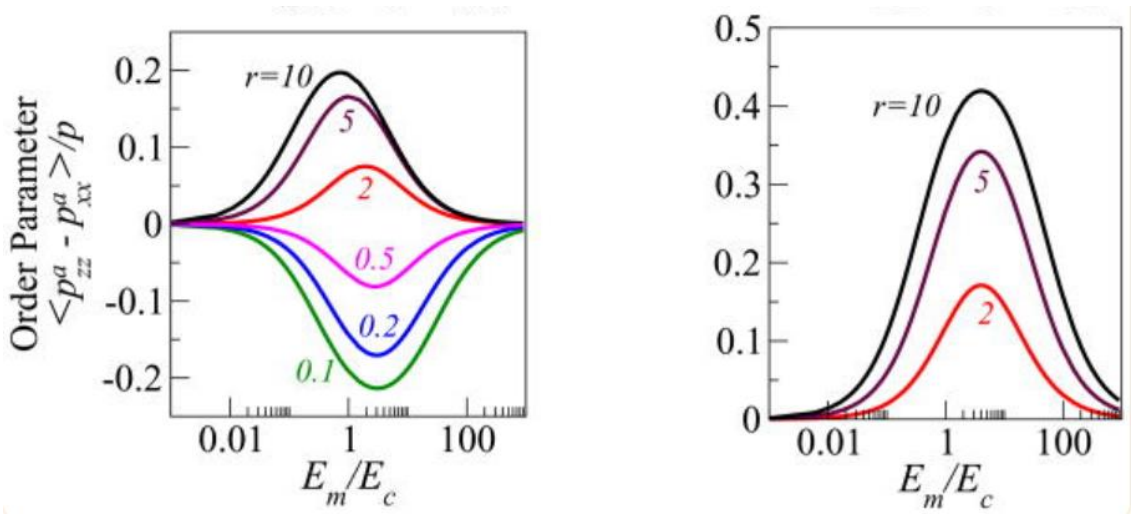
ZZ and XX Components of the Order Parameter vs. Matrix/Cell Young's Moduli Ratio



ZZ and XX Components of the Order Parameter vs. Matrix/Cell Young's Moduli Ratio (continued)

Cell polarization as a function of the ratio of the Young's modulus of the matrix, **Em**, and the cell, **Ec**, in both our two- and three-dimensional models; the plots are shown for different values of the cellular aspect ratio, r. The upper panels show (magenta: **r** = 5, red: **r** = 2) the normalized average dipole elements ⟨pazz⟩ (solid lines) and ⟨paxx⟩ (dashed lines) corresponding to the forces in the directions that are respectively parallel ($\hat{z}$) and perpendicular ($\hat{x}$) to the long axis of the cell.

Order Parameter (Dimensionless) vs. the Matrix/Cell Young's Moduli Ratio (for Different Cell Aspect Ratios)



The bottom panels show the calculated orientational order parameter of the stress-fibers that is given by the normalized difference $(\langle p_{azz}) - \langle p_{axx}\rangle)/p$ The color coding indicates the aspect ratio.

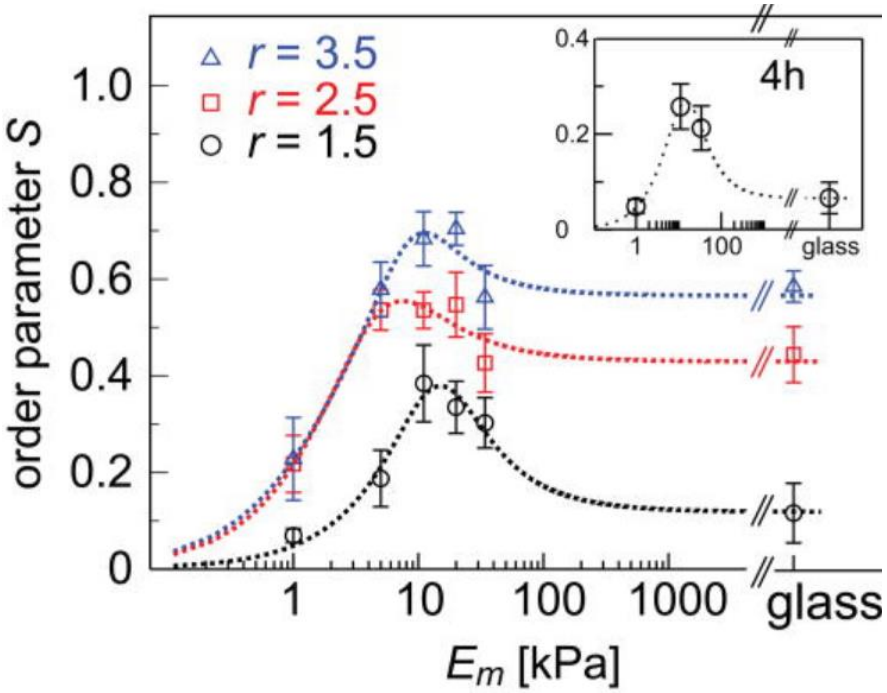
In this plot the Poisson ratio of the matrix and the cellular domain are taken to be $v_m = 0.45, v_c = 0.3$ and the magnitude of the polarizability is $\alpha = 3$

Order Parameter (Dimensionless) vs. the Matrix/Cell Young's Moduli Ratio (for Different Cell Aspect Ratios, continued)

Cell polarization as a function of the ratio of the Young's modulus of the matrix, E_m, and the cell, E_c, in both our two- and three-dimensional models; the plots are shown for different values of the cellular aspect ratio, *r*. The bottom panels show the calculated orientational order parameter of the stress-fibers that is given by the normalized difference $(\langle p^a_{zz}\rangle - \langle p^a_{xx}\rangle)/\mathrm{p}$.

The color coding indicates the aspect ratio. In this plot the Poisson ratio of the matrix and the cellular domain are taken to be, $\nu_m = 0.45, \nu c = 0.3$ and the magnitude of the polarizability is $\alpha = 3$.

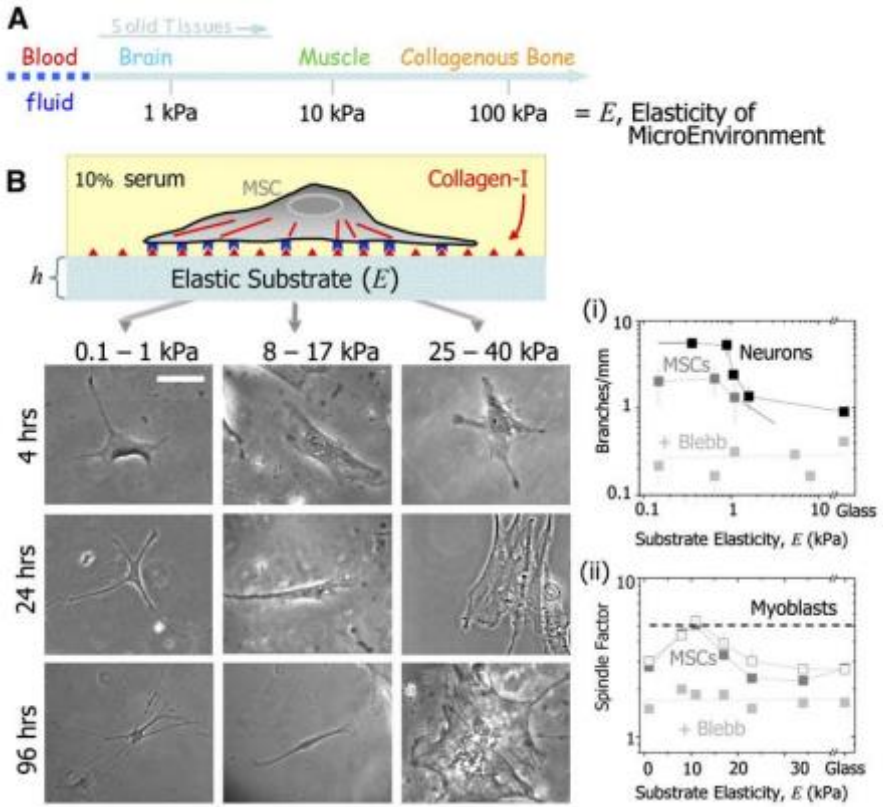
Order Parameter vs. Young's Modulus of ECM for Three Different Aspect Ratios of the Cell (Optimal Value for ECM Stiffness)



Order Parameter vs. Young's Modulus of ECM for Three Different Aspect Ratios of the Cell (Optimal Value for ECM Stiffness, continued)

The effect of axial cell elongation on stress-fiber polarization and experimental values of the order parameter S for different elastic substrates. Upper panel shows a calculation of the 2D order parameter as a function of the matrix rigidity. The bottom panel shows the experimental values of the stress-fiber order parameter, $S = \langle \cos 2\theta \rangle$, 24 hours after plating the cells, for the three groups of cells (of aspect ratios r = 1.5, 2.5, 3.5) as a function of the Young's modulus of the matrix, Em; θ is the angle between each stress-fiber in the cell and the long axis of the fitted ellipse. Within each of the different groups, S is maximal for Em = 11 kPa and generally increases with aspect ratio r, in agreement with our theoretical predictions.

ECM Stiffness Directs Stem Cell Lineage Specification



(Engler et al., 2006))

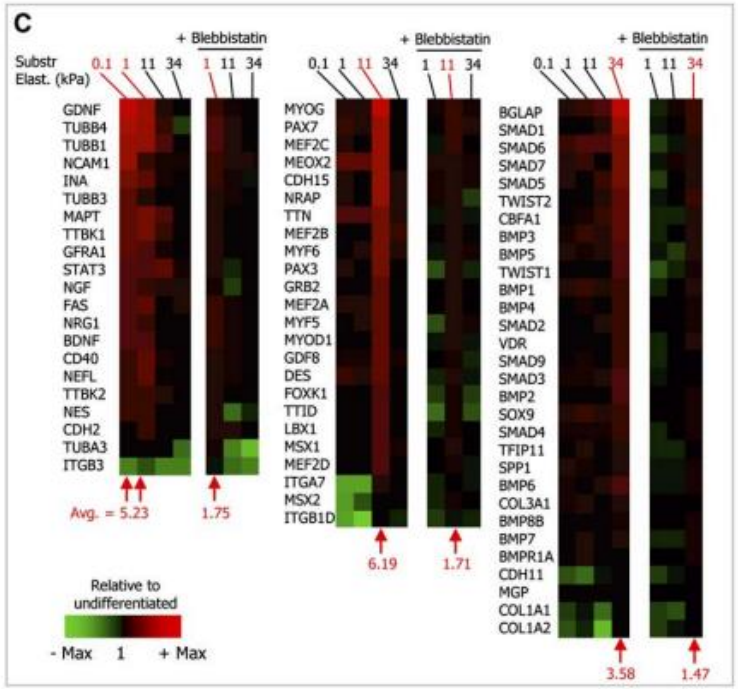
ECM Stiffness Directs Stem Cell Lineage Specification (continued)

A. Solid tissues exhibit a range of stiffness, as measured by the elastic modulus, *E*.

B. The in vitro gel system allows for control of E through crosslinking, control of cell adhesion by covalent attachment of collagen-I, and control of thickness, *h*. Naive MSCs of a standard expression phenotype (Table S1) are initially small and round but develop increasingly branched, spindle, or polygonal shapes when grown on matrices respectively in the range typical of $\sim E_{\text{brain}}\,(0.1-1\text{kPa}), \sim E_{\text{muscle}}(8-17\,\text{kPa})$, or stiff crosslinked-collagen matrices (25-40 kPa). Scale bar is $20\ \mu m$.

   Inset graphs quantify the morphological changes (mean ± SEM) versus stiffness, *E*: shown are (*i*) cell branching per length of primary mouse neurons (Flanagan et al., 2002), MSCs, and blebbistatin-treated MSCs and (*ii*) spindle morphology of MSCs, blebbistatin-treated MSCs, and mitomycin-C treated MSCs (open squares) compared to C2C12 myoblasts (dashed line) (Engler et al., 2004a).

Microarray Profiling of MSC Transcripts



Cells Cultured on 0.1, 1, 11, or 34 Kpa Matrices With or Without Blebbistatin Treatment

(Engler et al., 2006))

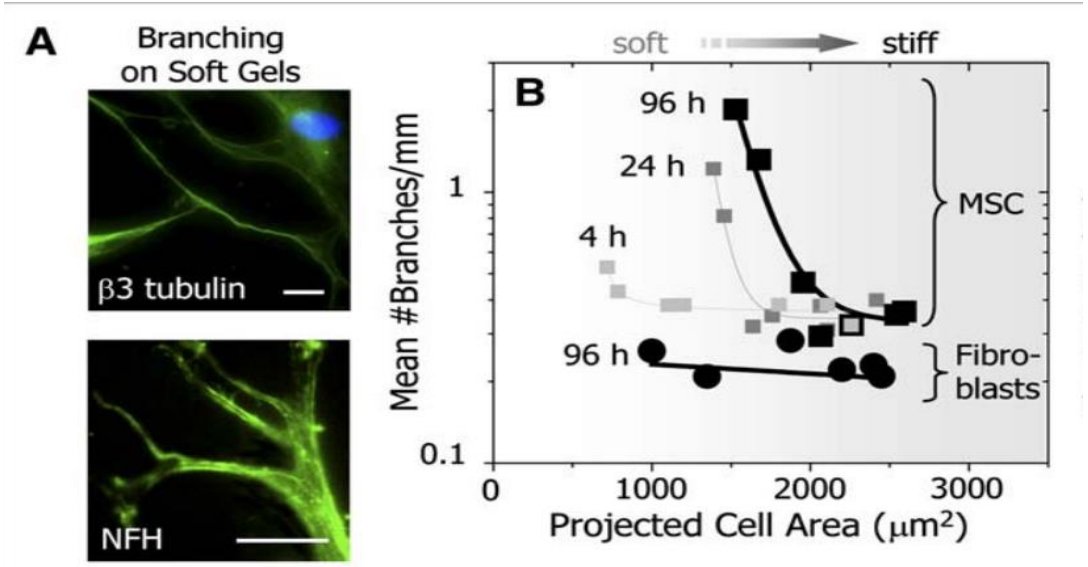
Microarray Profiling of MSC Transcripts in Cells Cultured on 0.1, 1, 11, or 34 Kpa Matrices With or Without Blebbistatin Treatment (Continued)

(C) Microarray profiling of MSC transcripts in cells cultured on 0.1, 1, 11, or 34 kPa matrices with or without blebbistatin treatment. Results are normalized to actin levels and then normalized again to expression in naive MSCs, yielding the fold increase at the bottom of each array. Neurogenic markers (left) are clearly highest on 0.1-1 kPa gels, while myogenic markers (center) are highest on 11 kPa gels and osteogenic markers (right) are highest on 34 kPa gels. Blebbistatin blocks such specification (<2-fold different from naive MSCs).

Neurogenic Branching

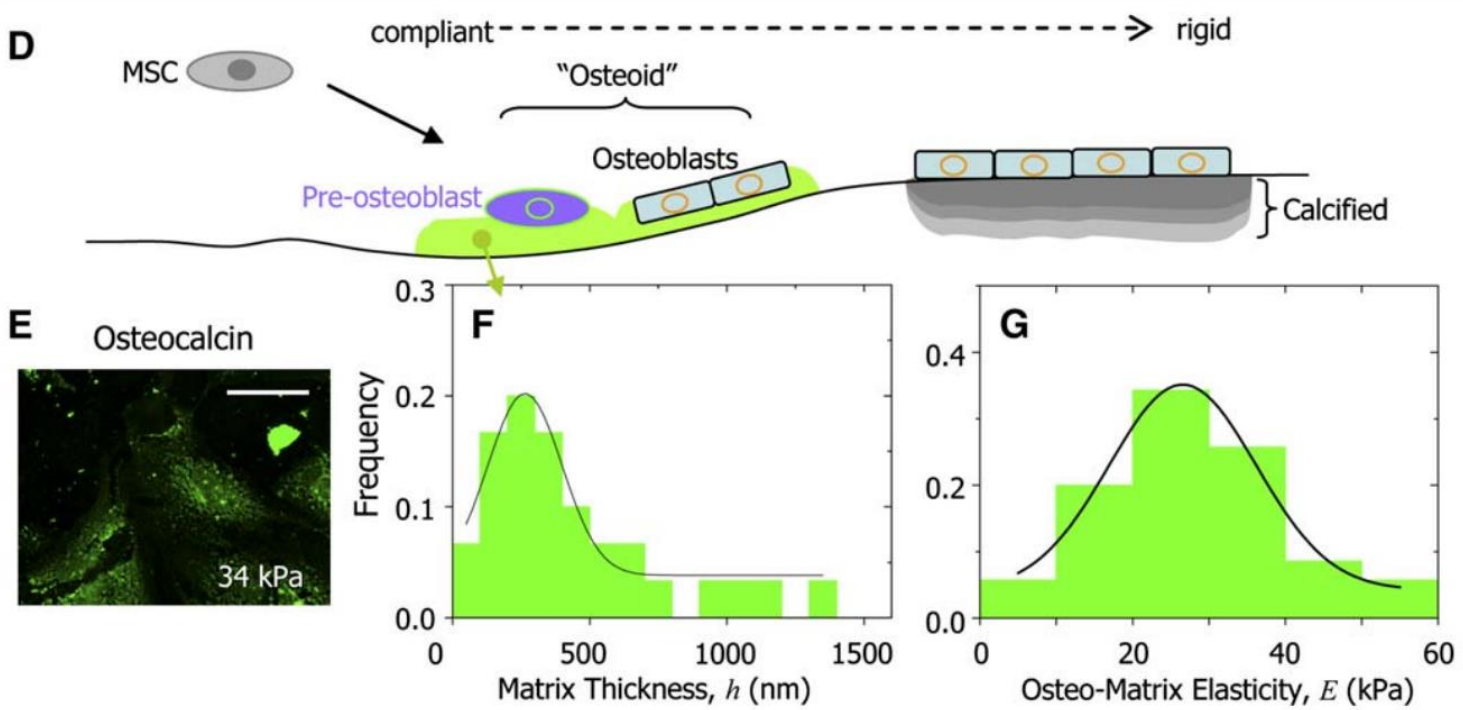
A. Immunofluorescence images of $\beta 3$ tubulin and NFH in branched extensions of MSCs on soft matrices $(E \sim 1\, kPa)$. Scale bars are $5\ \mu m$.

B. MSCs and fibroblasts on a range of elastic matrices show an increase in projected area with matrix stiffness, but only MSCs on the softest gels (with smallest areas) show an increasing number of branches per extension length with time.



(Engler et al., 2006))

Osteogenic Microenvironment



(Engler et al., 2006))

Osteogenic Microenvironment (continued)

D. Schematic of the compliant, collagenous "osteoid" microenvironment (green) that MSCs encounter in initial remodeling of bone matrix (adapted from Raisz, 1999). Committed osteoblasts remodel microenvironments by secreting matrix proteins that are slowly calcified.

E. hFOB osteoblasts secrete osteocalcin after being plated on glass. By day 7, the matrix is thick (F) and compliant with $E_{\text{osteo}} \sim 25 - 40\text{kPa(G)}$ based on measurements made by AFM

Resources

- Engler, A. J., Sen, S., Sweeney, H. L., & Discher, D. E. (2006). Matrix elasticity directs stem cell lineage specification. *Cell*, *126*(4), 677–689.