1. What is the definition of stress in the cytoskeleton?

Stress in the cytoskeleton results from shape and volume deformations during cell adhesion and spreading on the extracellular matrix (ECM). These deformations create elastic stresses in the cytoskeleton and the surrounding matrix, influenced by matrix rigidity and cell shape. The resulting stress can initiate feedback mechanisms that guide the cytoskeletal organization, including the polarization of actomyosin stress fibers.

**Ref**: Zemel, A., Rehfeldt, F., Brown, A.E.X., Discher, D.E.,& Safran, S.A. (2010). Optimal matrix rigidity for stress fiber polarization in stem cells. Nature Physics 6, 468-473. https://doi.org/10.1038/nphys1613Links to an external site..

2. What is the evidence for the neurological differentiation of stem cells?

- Immunofluorescence of MSCs on soft matrices (0.1–1 kPa), which mimic brain-like elasticity, shows expression of neuronal commitment (nestin), immature neurons (β3 tubulin), mid/late neurons (MAP2), and mature neurons (NFL, NFH, and P-NF).
- Microarray profiling of mesenchymal stem cells (MSCs) cultured on soft matrices (0.1–1 kPa), shows upregulation of neurogenic markers such as nestin**,** TUBB1**,** TUBB4**,** NCAM1**,** and MAPT. These markers are expressed significantly higher than cells cultured on stiffer matrices (11 or 34 kPa), showing that soft substrate stiffness promotes neurological differentiation**.**

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

3. What is the evidence for the myogenic differentiation of stem cells?

- Immunostaining of MSCs cultured on moderately stiff, muscle-like matrices (~ 10 kPa) shows nuclear localization and upregulation of the myogenic transcription factor MyOD1, but not on softer or stiffer matrices.
- Fluorescence intensity analysis reveals that, compared to C2C12 myoblasts, the difference in expression levels of MyoD1 is 50% after one week in MSCs cultured on myogenic matrices. In contrast, MyoD1 is almost absent on softer or stiffer matrices.

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

4. What is the definition of full strain in the cell?

Strain in a cell is defined as the change in length or shape relative to its relaxed state. In the 1D Spring model, the full strain is defined as the change in cell length relative to its original length; it is the elastic deformation that would arise if the cellular springs were maximally stretched when spreading on a matrix of infinite rigidity:

$$-\Delta \dot{l}_c^0 = l_c^0 - l_c^R > 0$$

- $l_c^R$: relaxed length of the cellular spring
- $l_c^0$: fully stretched cellular spring in infinitely rigid matrix

**Ref**: Zemel, A., Rehfeldt, F., Brown, A.E.X., Discher, D.E.,& Safran, S.A. (2010). Optimal matrix rigidity for stress fiber polarization in stem cells. Nature Physics 6, 468-473. https://doi.org/10.1038/nphys1613Links to an external site..

5. What is the definition of isotropic strain?

Isotropic strain means that the relative change in length (or volume) **is** the same in every direction**.**
Isotropic strain is the same relative change in length (or volume) in every direction.
**Ref**: Zemel, A., Rehfeldt, F., Brown, A.E.X., Discher, D.E.,& Safran, S.A. (2010). Optimal matrix rigidity for stress fiber polarization in stem cells. Nature Physics 6, 468-473. https://doi.org/10.1038/nphys1613Links to an external site..

6. What is the definition of polarization tensor?

In response to the local stress in the cell, force-dipoles polarize, changing their magnitude and orientation from their average, isotropic initial tensor $\langle p^0_{ij} \rangle$ to the average dipole per unit of volume $\langle p_{ij} \rangle$, the difference between $\langle p_{ij} \rangle$ and $\langle p^0_{ij} \rangle$ is the anisotropic polarization tensor: $\langle p^a_{ij} \rangle = \langle p_{ij} \rangle - \langle p^0_{ij} \rangle$.
**Ref**: Zemel, A., Rehfeldt, F., Brown, A.E.X., Discher, D.E.,& Safran, S.A. (2010). Optimal matrix rigidity for stress fiber polarization in stem cells. Nature Physics 6, 468-473. https://doi.org/10.1038/nphys1613Links to an external site..

7. What is the property of guidance of the stem cell differentiation by the substrate stiffness?

Stem cells detect the stiffness of matrices and convert this information into mechanical signals that guide lineage specification. Soft matrices ($E_{brain}$0.1–1 kPa), that mimic brain tissue enhance neurogenesis, intermediate stiffness promotes myogenesis ($E_{muscle}$ ~ 8-17 kPa), and stiff matrices that mimic bone encourage osteogenesis ($E_{osteo}$ ~ 25 – 40 kPa). The lineage specification depends on cell-generated contractility via myosin II.
**Ref:** Engler, A.J., Sen S., Lee Sweeney, H.,& Discher, D.E. (2006). Matrix elasticity directs stem cell lineage specification. Cell, 126(4):677-689.

8. Formulate the extremal property of the order parameter as a function of the ECM stiffness.

The orientation order parameter S is defined as:

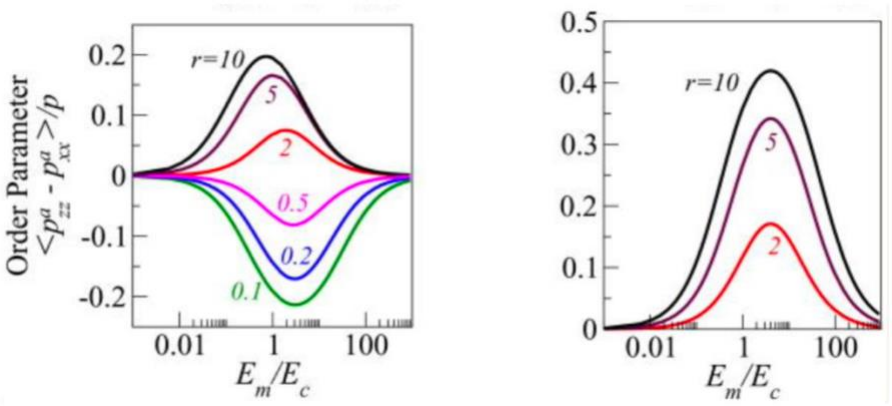
$$S(E_m/Ec) = a\,(E_m/E_c)\,/[b\,(Em - E_0)\,/\,Ec)^2 + 1]$$

Where

- $E_m$: matrix stiffness
- Ec: cell stiffness

- a, b, E0 are functions of the aspect ratio, cell polarizability, Poisson ratio of the cell, and matrix and dimensionality of the system.

The order parameter reaches a maximum at an optimal ratio, which occurs near Em/Ec ~ 1, but the exact value for a given magnitude polarizability $\alpha$ depends on the cellular ratio r.




**Ref**: Zemel, A., Rehfeldt, F., Brown, A.E.X., Discher, D.E.,& Safran, S.A. (2010). Optimal matrix rigidity for stress fiber polarization in stem cells. Nature Physics 6, 468-473. https://doi.org/10.1038/nphys1613Links to an external site..

9. What is the definition of isotropic dipole per unit volume?

Initially, the force-dipole within the cell cytoskeleton is not polarized and has no preferred direction. The average of the cell's initial force-dipole per unit volume is the isotropic dipole per unit volume.

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10. Describe the introduction of dipole per unit volume.

The active forces of actomyosin in the cytoskeleton are represented as a local distribution of “force-dipole.” These dipolar forces result from equal and opposite forces applied by myosin motors at two close points along the actin filaments within the cell. The dipole per unit volume represents the average dipolar forces per unit volume within the cell.

**Ref**: Zemel, A., Rehfeldt, F., Brown, A.E.X., Discher, D.E.,& Safran, S.A. (2010). Optimal matrix rigidity for stress fiber polarization in stem cells. Nature Physics 6, 468-473. https://doi.org/10.1038/nphys1613Links to an external site..

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