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 both 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.

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**.**

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; 50% relative expression levels of MyoD1 after 1 week in MSCs cultured on myogenic matrices. In contrast, MyOD1 is almost absent on softer or stiffer matrices.

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

Strain in a cell is defined as the change in the cell's 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, which relates the elasticity of the cell and matrix respectively (k_c, k_m) and the initial change in cell length Δl^0_c:

$$\frac{\Delta l_c}{l_c^0} = \frac{\widetilde{k}_c}{(\widetilde{k}_c + k_m)} \frac{\Delta l_c^0}{l_c^0}$$

Where:

$\widetilde{k}_c = (1+\alpha)k_c$ is the effective rigidity of the cell;

α: ‘polarizability’ factor > 0

5. What is the definition of isotropic strain?

Isotropic strain is the initial stress caused by the cell's inward pulling forces, which are exerted in all directions without any preferred direction. It corresponds to the strain associated with the cell's anchoring and spreading within an infinitely rigid matrix.

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: $\langle p^a_{ij} \rangle$.

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 that mimic brain tissue enhance neurogenesis, intermediate stiffness promotes myogenesis, and stiff matrices that mimic bone encourage osteogenesis. The lineage specification depends on cell-generated contractility via myosin II.

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

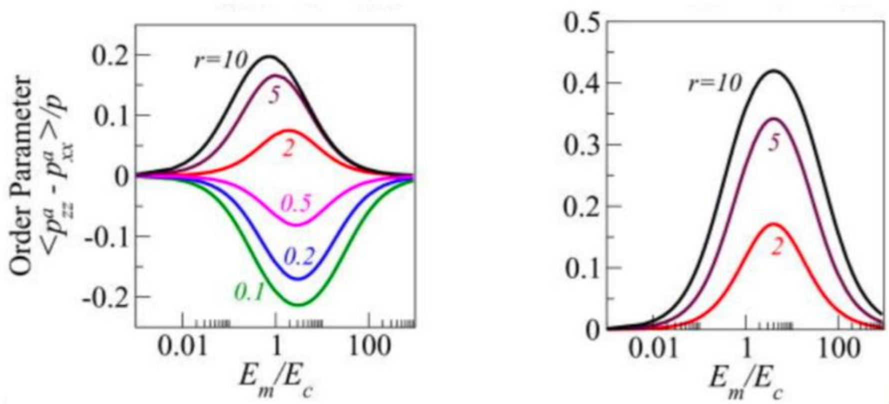
The orientation order parameter S is defined as:

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

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 α depends on the cellular ratio r.



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.

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.