

Solutions 7: Mechanobiology

Questions on Figure 1

Consider signaling kinase activity as “biological output” (e.g. MAPKs or mitogen activated protein kinases).

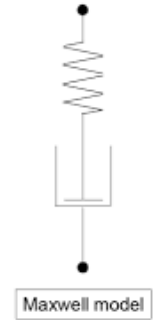
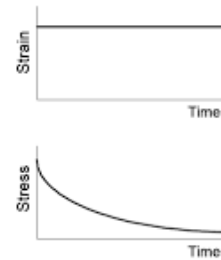
Assume that all variable factors are currently being regulated by the cell to remain steady aside from the following single factors – what is the effect on relative kinase activity if:

- **Talin density increases (e.g. by additional expression)?**
 - *Solution:* The signaling activity is proportional to the amount of unfolded talin (total number of exposed binding sites). This depends both on the total number of talin, and also on whether the force per individual talin molecule is above the threshold required for unfolding.
 - If the total force is constant, the force per talin decreases, leading to less average stress on each talin molecule
 - If the density increases to a very large degree, the stress may fall below a threshold sufficient to unfold the talins, leading to a decrease in signaling activity
 - If the average stress is above a threshold required for unfolding, more talins will be present, with more exposed binding sites.
- **Matrix density increases (e.g. by cellular secretion)?**
 - *Solution:* When the matrix density increases, the number of bound integrins will increase accordingly. This increase in bound integrins leads to more effective coupling between the cell and the matrix. Moreover, this leads to more talin being unfolded and thus, signaling activity will also increase.
- **Integrin density decreases (e.g. by reduced expression)?**
 - *Solution:* When the integrin density decreases, we will have less effective coupling between the cell and the matrix. This leads to there being less unfolded talin available and thus the signaling activity will decrease.
- **Substrate stiffness decreases (e.g. through pl. activity)?**
 - *Solution:* If we consider a generic traction force that acts on our substrate, the matrix will have to deform accordingly. Now, when we decrease the stiffness of the substrate, the matrix will move more in response to our (still constant) traction force. As the matrix moves, less deformation must be taken up by the talin molecules and therefore less talin will be unfolded. The signaling activity will therefore decrease.

Questions on Figure 2:

- Explain the observed behavior of the “stress relaxation test” shown in the strain-time and stress-time curves above. (strain is displacement normalized to length of the system; stress is force normalized to cross-section of the system);

Stress-relaxation test



- *Solution:* At time zero ($t_0 = 0$), a constant strain is applied with $\varepsilon = \text{const.}$

Since strain is given by

$$\varepsilon = \frac{\ell - \ell_0}{\ell_0} = \frac{\Delta\ell}{\ell_0}.$$

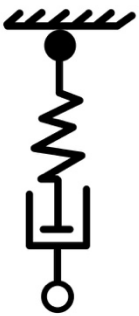
We therefore are analogously applying a constant displacement. The spring immediately deforms, with a stress response at $\sigma(t_0) = \sigma_0$.

As time evolves, the dashpot will slowly move through the viscous fluid (itself becoming longer), reducing the length of the spring gradually. As the spring shortens (Δx becomes smaller), the spring force is also reduced, since

$$F = k \cdot \Delta x$$

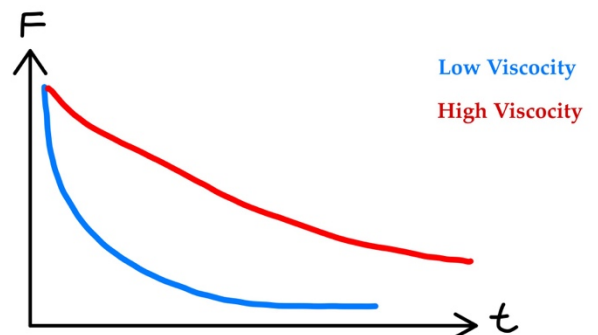
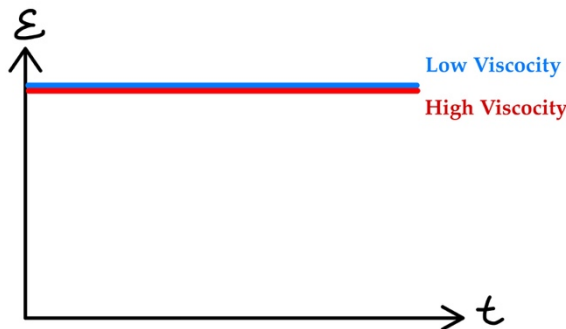
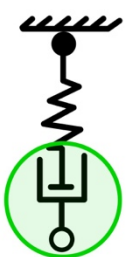
according to Hooke's Law.

Once the spring reaches its beginning length ℓ_0 , the internal deformation energy and internal stresses are dissipated. Intuitively, we can think of this dissipation happening when the dashpot travels through the viscous fluid, transferring its energy in form of heat into it.



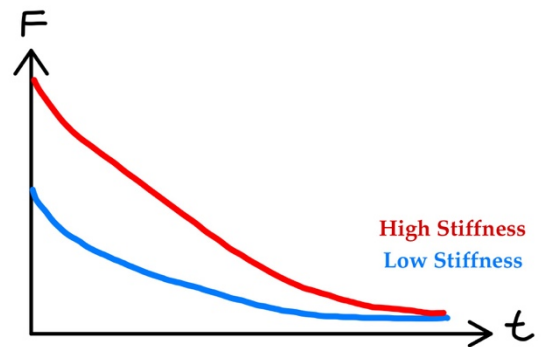
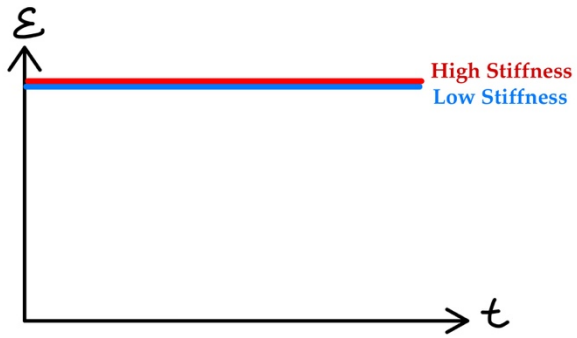
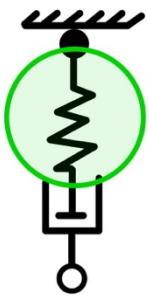
- Make a sketch of these two curves that illustrates the difference between a highly viscous fluid vs a low viscosity fluid (assume an equivalent spring)

- *Solution:*



- Make a sketch of these two curves that illustrates the difference between a high stiffness vs low stiffness spring.

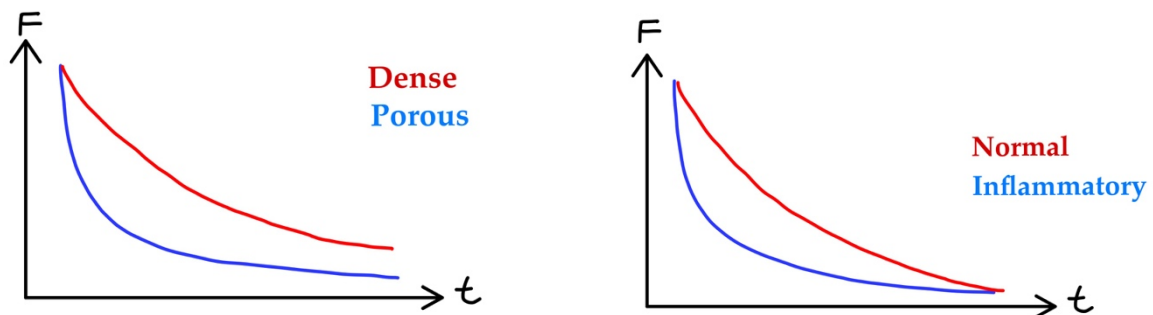
○ *Solution:*



Questions combining concepts of figure 1 (biology) & 2 (mechanical models):

- Assuming a sudden onset of cellular traction with then constant forces provided by the actin-myosin machinery, **draw the force vs. time curves for forces at a single focal adhesion for the following cases:**
 - matrix is very dense vs. very porous; in both cases assume a normal interstitial fluid (mostly water, ions, and soluble factors)
 - fluid in the matrix is of normal viscosity (healthy interstitial fluid), versus inflammatory (containing proteins that confer low viscosity properties).

Solution:



- Assuming a matrix with viscoelastic mechanical properties, and assuming a sudden force onset driven by constant myosin activity:
 - Describe the relative changes (as a function of time) of:
 - **the integrin-ECM binding site locations with respect to the cell center:**
 - *Solution:* As a sudden force is applied to the matrix, the integrin-ECM binding sites will move closer to the cell center over time.
 - **the structural “conformation” (shape) of talin:**
 - *Solution:* Talin will be less deformed over time.
 - **kinase activity:**
 - *Solution:* Kinase activity will decrease over time.
- **Assuming that the cell will attempt to maintain constant cell contractility, and that kinase activity is required to maintain myosin activity, how do you suspect a cell reacts to a time-dependent displacement of the viscoelastic substrate?**
 - *Solution:* The cell will try to “move” its focal adhesions increasingly outward. As the matrix moves toward the cell center, the cell grabs new (“fresh”) areas of the matrix in an effort to keep itself “fully spread” with fully tensioned cytoskeletal (actin) structures.