Electronic Supplementary Information for Soft Matter manuscript:
Stress Fiber Response to Mechanics: A Free Energy Dependent Statistical Model

Li Jiang [a], Chun Yang[a], Lei Zhao[b], and Zheng QS[c]
[a]Institute of Biomechanics and Medical Engineering, Engineering Mechanics Department, School of Aerospace, Tsinghua University, Beijing100084, China
[b]School of Biological Science and Medical Engineering, Beihang University, Xueyuan Road 37, Beijing 100191, China
[c]Center for Nano/micro-Mechanics and Multidisciplinary Innovation Research, Tsinghua University, Beijing 100084, People's Republic of China

S1

The magnitude of the cell contractility in absence of the external strain

In absence of external strain, the effective free energy is

$$\overline{W}=\left[(\frac{\overline{\sigma}_c}{\overline{E}}-1)^2+(\frac{1}{\overline{E}}+\frac{1}{\overline{E}_c})\overline{\sigma}_c{}^2\right]$$.* MERGEFORMAT (A1)

The stationary point is then given by the equation $\left.\frac{\partial\overline{W}}{\partial\overline{\sigma}_c}\right|_{\overline{\sigma}_c=\overline{\sigma}_0}=0$ as

$$\overline{\sigma}_0=\frac{1}{\frac{1}{\overline{E}}+\frac{\overline{E}}{\overline{E}_c}+1}$$. * MERGEFORMAT (A2)

Since $\left.\frac{\partial^2\overline{W}}{\partial\overline{\sigma}_c{}^2}\right|_{\overline{\sigma}_c=\overline{\sigma}_0}=\frac{1}{\overline{E}}+\frac{\overline{E}}{\overline{E}_c}+1>0$, $\overline{W}$ reaches its minimum when $\overline{\sigma}_c=\overline{\sigma}_0$. Since cells moduli are observed as a linear function of substrate elasticity, $\frac{\overline{E}}{\overline{E}_c}$ is considered as a constant. Under this assumption, the cell contractility increases gradually at first, and then followed by a plateau.

S2

The contractility and alignment of the cell under static uniaxial strain

Under static uniaxial strain, the effective free energy can be written as

$$\overline{W}=(\overline{\varepsilon}_\infty\cos^2\theta+\frac{\overline{\sigma}_c}{\overline{E}}-1)^2+(\frac{1}{\overline{E}}+\frac{1}{\overline{E}_{cx}})\overline{\sigma}_c{}^2-2\overline{\sigma}_c\overline{\varepsilon}_\infty\cos^2\theta$$.*

MERGEFORMAT (A3)

Cells can adjust both the magnitude, $\overline{\sigma}_c$, and the orientation, θ, of the contractility, to minimize the free energy $\overline{W}$.

We denote the stationary point by solving the equation $\partial\overline{W}/\partial\bar{\sigma}_c = 0$ as

$$\bar{\sigma}_c = \bar{\sigma}_{static} = \frac{\bar{\varepsilon}_\infty(\bar{E}-1)cos^2\theta + 1}{1+\frac{\bar{E}}{\bar{E}_c}+\frac{1}{\bar{E}}}$$.* MERGEFORMAT (A4)

Further derivation shows that $\frac{\partial^2\overline{W}}{\partial\bar{\sigma}_c^{\ 2}} = \frac{1}{\bar{E}^2}+\frac{1}{\bar{E}}+\frac{1}{\bar{E}_c} > 0$, indicating $\overline{W}(\bar{\sigma}_c,\theta)$ reaches its minimum when $\bar{\sigma}_c = \bar{\sigma}_{static}$. Since the young's modulus E is smaller than ξ (~361kPa, see Results) in general, thus $\bar{E} < 1$, $\bar{\sigma}_{static}$ will decrease while $\bar{\varepsilon}_\infty$ increases, being self-consistent with the phenomenon of "tensional homeostasis" .

Then we get

$$\begin{aligned}
&\frac{\partial\overline{W}}{\partial\theta} = 2\bar{\varepsilon}_\infty \sin 2\theta\left(\bar{\sigma}_c - \bar{\varepsilon}_\infty \cos^2\theta - \frac{\bar{\sigma}_c}{\bar{E}} + 1\right),\\
&\frac{\partial^2\overline{W}}{\partial\theta\partial\bar{\sigma}_c} = 4\bar{\varepsilon}_\infty \cos\theta\sin\theta\left(1-\frac{1}{\bar{E}}\right),\\
&\frac{\partial^2\overline{W}}{\partial\theta^2} = 2\left\{2\bar{\varepsilon}_\infty \cos 2\theta\left[\bar{\sigma}_c - \left(\bar{\varepsilon}_\infty\cos^2\theta + \frac{\bar{\sigma}_c}{\bar{E}} - 1\right)\right] + \bar{\varepsilon}_\infty^{\ 2}\sin^2 2\theta\right\}.
\end{aligned}$$ * MERGEFORMAT (A5)

For convenience, we set that

$$\frac{\partial^2\overline{W}}{\partial\sigma_c^{\ 2}} = A>0,\quad \frac{\partial^2\overline{W}}{\partial\theta\partial\sigma_c} = B,\quad \frac{\partial^2\overline{W}}{\partial\theta^2} = C$$.* MERGEFORMAT (A6)

The steady-state solutions can be given by solving the coupled equations

$$\begin{cases}\frac{\partial\overline{W}}{\partial\theta} = 0,\\ \partial\overline{W}/\partial\bar{\sigma}_c = 0.\end{cases}$$ * MERGEFORMAT (A7)

This predicts three possible orientations, $\theta_{static} = 0$, $\frac{\pi}{2}$, $\arccos\sqrt{\frac{(2\bar{E}_c+\bar{E})}{\left(\bar{E}+3\bar{E}_c-\bar{E}\bar{E}_c\right)\bar{\varepsilon}_\infty}}$.

Among those solutions, a local minimum meets the equations

$$\begin{cases}A>0,\\ AC-B^2>0.\end{cases}$$ * MERGEFORMAT (A8)

Case 1 $\theta_{static} = 0,\ \bar{\sigma}_{static} = \bar{\varepsilon}_\infty(\bar{E}-1)+1\Big/\left(1+\frac{\bar{E}}{\bar{E}_c}+\frac{1}{\bar{E}}\right)$

By substituting θ_{static} and $\bar{\sigma}_{static}$ in Eq. A6, we obtain that

$A>0$, $B=0$, $C=\dfrac{4\bar{\varepsilon}_\infty \bar{E}\left(\left(\bar{E}+2\bar{E}_c\right)\left(1-\bar{\varepsilon}_\infty\right)+\bar{\varepsilon}_\infty \bar{E}_c\left(\bar{E}-1\right)\right)}{\bar{E}^2+\bar{E}_c\bar{E}+\bar{E}_c}$.

Thus, $AC-B^2>0$ only if $\Leftrightarrow C=\dfrac{4\bar{\varepsilon}_\infty \bar{E}\left(\left(\bar{E}+2\bar{E}_c\right)\left(1-\bar{\varepsilon}_\infty\right)+\bar{\varepsilon}_\infty \bar{E}_c\left(\bar{E}-1\right)\right)}{\bar{E}_c+\bar{E}\bar{E}_c+\bar{E}^2}>0$.

This implies that parallel alignment may be the stable orientation if $\bar{E}>1$ and $\bar{\varepsilon}_\infty<1$.

Case 2 $\theta_{static}=\dfrac{\pi}{2},\ \bar{\sigma}_{static}=1\Big/\left(1+\dfrac{\bar{E}}{\bar{E}_c}+\dfrac{1}{\bar{E}}\right)$

In this case, $A>0$, $B=0$, $C=-\dfrac{4\bar{\varepsilon}_\infty \bar{E}(2\bar{E}_c+\bar{E})}{\bar{E}_c+\bar{E}\bar{E}_c+\bar{E}^2}<0$, thus $AC-B^2<0$. This indicates that perpendicular alignment is not a stable orientation.

Case 3 $\theta_{static}=\arccos\sqrt{\dfrac{(2\bar{E}_c+\bar{E})}{\left(\bar{E}+3\bar{E}_c-\bar{E}\bar{E}_c\right)\bar{\varepsilon}_\infty}}$ (only if $\dfrac{(2\bar{E}_c+\bar{E})}{\left(\bar{E}+3\bar{E}_c-\bar{E}\bar{E}_c\right)\bar{\varepsilon}_\infty}\in(0,1)$),

$$\bar{\sigma}_{static}=\frac{1}{\dfrac{1}{\bar{E}_c}+\dfrac{3}{\bar{E}}-1}$$

In this case, $A>0$, $C=2\bar{\varepsilon}_\infty^{\,2}\sin^2 2\theta_{static}>0$, $B=2\bar{\varepsilon}_\infty\sin 2\theta_{static}\left(1-\dfrac{1}{\bar{E}}\right)$.

By solving the equations $AC-B^2>0$ and $0\le\dfrac{(2\bar{E}_c+\bar{E})}{\left(\bar{E}+3\bar{E}_c-\bar{E}\bar{E}_c\right)\bar{\varepsilon}_\infty}\le 1$, we then get that

$$\frac{4\bar{\varepsilon}_\infty \bar{E}\left(\left(\bar{E}+2\bar{E}_c\right)\left(1-\bar{\varepsilon}_\infty\right)+\bar{\varepsilon}_\infty \bar{E}_c\left(\bar{E}-1\right)\right)}{\bar{E}_c+\bar{E}\bar{E}_c+\bar{E}^2}<0$$

.* MERGEFORMAT (A8)

This implies that most cells may align in the direction of $\theta_{static}=\arccos\sqrt{\dfrac{(2\bar{E}_c+\bar{E})}{\left(\bar{E}+3\bar{E}_c-\bar{E}\bar{E}_c\right)\bar{\varepsilon}_\infty}}$ if $\bar{E}<1$ and $\bar{\varepsilon}_\infty>1$.

Above all, parallel orientation or $\theta_{static}=\arccos\sqrt{\dfrac{(2\bar{E}_c+\bar{E})}{\left(\bar{E}+3\bar{E}_c-\bar{E}\bar{E}_c\right)\bar{\varepsilon}_\infty}}$ is the stable orientation under static uniaxial strain, dependent on the magnitude of $\bar{E}$ and $\bar{\varepsilon}_\infty$.

Notably, $\overline{E}$ and $\overline{\varepsilon}_\infty$ are very close to 1, which makes $\theta_{static} = \arccos\frac{(2\overline{E}_c + \overline{E})}{\left(\overline{E} + 3\overline{E}_c - \overline{E}\overline{E}_c\right)\overline{\varepsilon}_\infty}$ is very close to zero.

S3

Cells alignment is guided by the magnitude and direction of cyclic uniaxial strain

Under cyclic uniaxial strain, the effective free energy averaged in one period is

$$\overline{W} = \left[(\overline{\varepsilon}_0\cos^2\theta + \frac{\overline{\sigma}_c}{\overline{E}} - 1)^2 + (\frac{1}{\overline{E}} + \frac{1}{\overline{E}_c})\overline{\sigma}_c^{\ 2} - 2\overline{\sigma}_c\overline{\varepsilon}_0\cos^2\theta\right] + \frac{1}{2}\left(\overline{\varepsilon}_0\cos^2\theta\right)^2$$ * MERGEFORMAT (A8)

The stationary point is given by solving $\partial\overline{W}/\partial\overline{\sigma}_c = 0$ as

$$\overline{\sigma}_{cyclic} = \frac{\overline{\varepsilon}_\infty(\overline{E}-1)cos^2\theta + 1}{1 + \frac{\overline{E}}{\overline{E}_c} + \frac{1}{\overline{E}}}$$ * MERGEFORMAT (A8)

Further derivation shows that $\frac{\partial^2\overline{W}}{\partial\overline{\sigma}_c^{\ 2}} = \frac{1}{\overline{E}^2} + \frac{1}{\overline{E}} + \frac{1}{\overline{E}_c} > 0$, indicating $\overline{W}(\overline{\sigma}_c, \theta)$ reaches its minimum when $\overline{\sigma}_c = \overline{\sigma}_{cyclic}$.

We then get

$$\frac{\partial\overline{W}}{\partial\theta} = \overline{\varepsilon}_\infty\sin 2\theta\left[2\overline{\sigma}_c\left(1 - \frac{1}{\overline{E}}\right) + 2 - 3\overline{\varepsilon}_\infty\cos^2\theta\right],$$
$$\frac{\partial^2\overline{W}}{\partial\theta\partial\overline{\sigma}_c} = 4\overline{\varepsilon}_\infty\cos\theta\sin\theta\left(1 - \frac{1}{\overline{E}}\right),$$
$$\frac{\partial^2\overline{W}}{\partial\theta^2} = 2\overline{\varepsilon}_\infty\left\{\cos 2\theta\left[2\left(\overline{\sigma}_c - \frac{\overline{\sigma}_c}{\overline{E}} + 1\right) - 3\overline{\varepsilon}_\infty\cos^2\theta\right] + \frac{3}{2}\overline{\varepsilon}_\infty\sin^2 2\theta\right\}.$$ * MERGEFORMAT (A9)

Similar as described above, three stationary points can be derived from Eq. A7:

Case 1 $\theta_{cyclic} = 0$, $\overline{\sigma}_{cyclic} = \overline{\varepsilon}_\infty(\overline{E}-1) + 1\Big/\left(1 + \frac{\overline{E}}{\overline{E}_c} + \frac{1}{\overline{E}}\right)$

By substituting θ_{static} and $\overline{\sigma}_{static}$ in Eq. A6, we obtain that $A>0$, $B = 0$,

$$C = \frac{2\overline{\varepsilon}_\infty\left[2\overline{E}\left(2\overline{E}_c + \overline{E}\right)\left(2 - 3\overline{\varepsilon}_\infty\right) + \overline{\varepsilon}_\infty\overline{E}_c\left(2\overline{E}+1\right)\left(\overline{E}-1\right)\right]}{\overline{E}_c + \overline{E}\overline{E}_c + \overline{E}^2}.$$ $AC - B^2 > 0$ requires

$$C=\frac{2\bar{\varepsilon}_\infty\left[2\bar{E}\left(2\bar{E}_c+\bar{E}\right)\left(2-3\bar{\varepsilon}_\infty\right)+\bar{\varepsilon}_\infty\bar{E}_c\left(2\bar{E}+1\right)\left(\bar{E}-1\right)\right]}{\bar{E}_c+\bar{E}\bar{E}_c+\bar{E}^2}>0$$, denoting that $\bar{E}>1$ and $\bar{\varepsilon}_\infty<\frac{2}{3}$ is one of the solutions. This implies that parallel alignment may dominate when the substrate is stiffer than the cell and the magnitude of the external cyclic strain is small.

Case 2 $\theta_{cyclic}=\frac{\pi}{2}$, $\bar{\sigma}_{cyclic}=\frac{1}{1+\frac{\bar{E}}{\bar{E}_c}+\frac{1}{\bar{E}}}$

Then $A>0$, $B=0$, $C=-\frac{4\bar{\varepsilon}_\infty\bar{E}(2\bar{E}_c+\bar{E})}{\bar{E}_c+\bar{E}\bar{E}_c+\bar{E}}<0$, which means that $AC-B^2<0$. This indicates that perpendicular alignment is not a stable orientation.

Case 3 $\theta_{cyclic}=\arccos\sqrt{\frac{2\bar{E}\left(\bar{E}+2\bar{E}_c\right)}{\bar{\varepsilon}_\infty\left(7\bar{E}\bar{E}_c+3\bar{E}^2+\bar{E}_c-2\bar{E}^2\bar{E}_c\right)}}$ (only if $\frac{2\bar{E}\left(\bar{E}+2\bar{E}_c\right)}{\bar{\varepsilon}_\infty\left(7\bar{E}\bar{E}_c+3\bar{E}^2+\bar{E}_c-2\bar{E}^2\bar{E}_c\right)}\in(0,1)$), $\bar{\sigma}_{cyclic}=\frac{\bar{E}_c\bar{E}\left(1+2\bar{E}\right)}{7\bar{E}\bar{E}_c+3\bar{E}^2+\bar{E}_c-2\bar{E}^2\bar{E}_c}$

Then we obtain $A>0$, $B=2\bar{\varepsilon}_\infty\sin2\theta\left(1-\frac{1}{\bar{E}}\right)$, $C=3\bar{\varepsilon}_\infty^2\sin^2 2\theta$.

Solving the coupled equations $AC-B^2>0$ and $0<\frac{2\bar{E}\left(\bar{E}+2\bar{E}_c\right)}{\bar{\varepsilon}_\infty\left(7\bar{E}\bar{E}_c+3\bar{E}^2+\bar{E}_c-2\bar{E}^2\bar{E}_c\right)}<1$, we then get $\frac{2\bar{\varepsilon}_\infty\left[2\bar{E}\left(2\bar{E}_c+\bar{E}\right)\left(2-3\bar{\varepsilon}_\infty\right)+\bar{\varepsilon}_\infty\bar{E}_c\left(2\bar{E}+1\right)\left(\bar{E}-1\right)\right]}{\bar{E}_c+\bar{E}\bar{E}_c+\bar{E}^2}<0$. $\bar{E}<1$ and $\bar{\varepsilon}_\infty>\frac{2}{3}$ is one of the solutions. This suggests that $\theta_{cyclic}=\arccos\sqrt{\frac{2\bar{E}\left(\bar{E}+2\bar{E}_c\right)}{\bar{\varepsilon}_\infty\left(7\bar{E}\bar{E}_c+3\bar{E}^2+\bar{E}_c-2\bar{E}^2\bar{E}_c\right)}}$ will be a local minimum of the effective free energy when the substrate is softer than the cell and the magnitude of the external cyclic strain is large. Notably, if $\bar{E}=1$ and $\bar{\varepsilon}_\infty ? \frac{2}{3}$, $\arccos\sqrt{\frac{2\bar{E}\left(\bar{E}+2\bar{E}_c\right)}{\bar{\varepsilon}_\infty\left(7\bar{E}\bar{E}_c+3\bar{E}^2+\bar{E}_c-2\bar{E}^2\bar{E}_c\right)}}$ will be very close to $\frac{\pi}{2}$.

In conclusion, parallel orientation or $\theta_{cyclic} = \arccos\sqrt{\frac{2\overline{E}\left(\overline{E}+2\overline{E}_c\right)}{\overline{\varepsilon}_\infty\left(7\overline{E}\overline{E}_c+3\overline{E}^2+\overline{E}_c-2\overline{E}^2\overline{E}_c\right)}}$ will be the stable orientation under static uniaxial strain, dependent on the magnitude of $\overline{E}$ and $\overline{\varepsilon}_\infty$. Cells are more likely to align parallel to uniaxial cyclic strain when $\overline{\varepsilon}_\infty < \frac{2}{3}$, while in the direction of $\theta_{cyclic} = \arccos\sqrt{\frac{2\overline{E}\left(\overline{E}+2\overline{E}_c\right)}{\overline{\varepsilon}_\infty\left(7\overline{E}\overline{E}_c+3\overline{E}^2+\overline{E}_c-2\overline{E}^2\overline{E}_c\right)}}$ when $\overline{\varepsilon}_\infty > \frac{2}{3}$, indicating that varying the magnitude of external cyclic strain will result in different cells orientations. In addition, $\theta_{cyclic} = \arccos\sqrt{\frac{2\overline{E}\left(\overline{E}+2\overline{E}_c\right)}{\overline{\varepsilon}_\infty\left(7\overline{E}\overline{E}_c+3\overline{E}^2+\overline{E}_c-2\overline{E}^2\overline{E}_c\right)}}$ will shift towards $\frac{\pi}{2}$ along with the growth of $\overline{\varepsilon}_\infty$.

S4

Drugs-tuned contractility impacts on cell alignment under cyclic uniaxial strain

The effective free energy averaged in one period is

$$\overline{W} = \left[(\overline{\varepsilon}_0\cos^2\theta + \frac{\overline{\sigma}_c}{\overline{E}} - 1)^2 + (\frac{1}{\overline{E}} + \frac{1}{\overline{E}_c})\overline{\sigma}_c^{\ 2} - 2\overline{\sigma}_c\overline{\varepsilon}_0\cos^2\theta\right] + \frac{1}{2}\left(\overline{\varepsilon}_0\cos^2\theta\right)^2$$

As $\overline{\sigma}_c$ is dominated by drugs and regarded as a constant, the minimum of $\overline{W}$ is achieved when

$$\partial\overline{W}/\partial\theta = \overline{\varepsilon}_\infty\sin 2\theta\left[2\overline{\sigma}_c\left(1-\frac{1}{\overline{E}}\right)+2-3\overline{\varepsilon}_\infty\cos^2\theta\right]=0, \quad \text{* MERGEFORMAT (A10)}$$

$$\frac{\partial^2\overline{W}}{\partial\theta^2} = 2\overline{\varepsilon}_\infty\left\{\cos 2\theta\left[2\left(\overline{\sigma}_c-\frac{\overline{\sigma}_c}{\overline{E}}+1\right)-3\overline{\varepsilon}_\infty\cos^2\theta\right]+\frac{3}{2}\overline{\varepsilon}_\infty\sin^2 2\theta\right\}>0. \quad \text{* MERGEFORMAT (A11)}$$

Three stationary points can be solved from the Eq. A10:

Case 1 $\theta_{drug} = 0$

By substituting $\theta_{drug} = 0$ in Eq. A11, we then get $\overline{E} > 1$ and $\overline{\varepsilon}_\infty < \frac{2}{3}$ is one of the solutions for the equation $\frac{\partial^2\overline{W}}{\partial\theta^2} = 2\overline{\varepsilon}_\infty\left[2\overline{\sigma}_c\left(1-\frac{1}{\overline{E}}\right)+\left(2-3\overline{\varepsilon}_\infty\right)\right]>0$. This implies that parallel alignment will locally minimize the effective free energy when the substrate is stiffer than the cells and the magnitude of the external cyclic strain is small.

Case 2 $\theta_{drug} = \frac{\pi}{2}$

In this case, $\frac{\partial^2 \overline{W}}{\partial \theta^2} = -4\bar{\varepsilon}_\infty \left[\bar{\sigma}_c \left(1 - \frac{1}{\bar{E}} \right) + 1 \right]$. Thus $\bar{E} = 1$ and $\bar{\sigma}_c > \frac{1}{\frac{1}{\bar{E}} - 1}$ is one of the solutions for $\frac{\partial^2 \overline{W}}{\partial \theta^2} > 0$. This suggests that perpendicular alignment will be a local minimum of free energy when the substrate is softer than the cell and the contractile stress is relatively large.

Case 3 $\theta_{drug} = \arccos \sqrt{\frac{2\left[\bar{\sigma}_c \left(1 - \frac{1}{\bar{E}} \right) + 1 \right]}{3\bar{\varepsilon}_\infty}}$ (only if $\frac{2\left[\bar{\sigma}_c \left(1 - \frac{1}{\bar{E}} \right) + 1 \right]}{3\bar{\varepsilon}_\infty} \in (0, 1)$)

In this case, $\frac{\partial^2 \overline{W}}{\partial \theta^2} = 3\bar{\varepsilon}_\infty^{\,2} \sin^2(2\theta_{drug}) > 0$, indicating that

$\theta_{drug} = \arccos \sqrt{\frac{2\left[\bar{\sigma}_c \left(1 - \frac{1}{\bar{E}} \right) + 1 \right]}{3\bar{\varepsilon}_\infty}}$ is a local minimum.

In summary, $\theta_{drug} = 0$, $\frac{\pi}{2}$, and $\arccos \sqrt{2\left[\bar{\sigma}_c \left(1 - \frac{1}{\bar{E}} \right) + 1 \right] / 3\bar{\varepsilon}_\infty}$ are all possible to minimize the effective free energy, dependent on the magnitudes of $\bar{E}$, $\bar{\varepsilon}_\infty$, and $\bar{\sigma}_c$. This explains why cells orientation varies when exposed to contractility-regulating reagents of different concentration.