Mechanics and Geometry of Adherent Cells and Cell Layers

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Active Matter: Cytoskeleton, Cells, Tissues and Flocks - KITP 2014
Adherent cells exert **traction forces** on elastic substrates

Silicon gel wrinkling => Cell is prestressed

What controls traction force generation in isolated cells?

- Mechano-chemical factors: ECM stiffness, Cytoskeletal Tension, Focal Adhesions
- Geometric factors: Cell spread area, adhesion geometry

Interplay between cell-cell and cell-ECM adhesions in multicellular colonies

Are there *universal* force-geometry relations across tissue length scales?
What factors control force generation during cell-matrix adhesion?


Focal Adhesions

Cell Spread Area

Substrate Stiffness

Cellular Forces/Stresses

Cell Shape

Cell Mechanical Properties


Need to isolate roles of mechanical and geometrical constraints
Cells adhere to substrates via **focal adhesions** and pull on the substrate through contractile forces generated in the **actomyosin network** → traction stresses regulated by complex signaling.

**Minimal Model for force generation**

Collagen/fibronectin

- **Actin cytoskeleton** ➔ stress fibers & cortex
- **focal adhesions**
- **substrate**
- **Traction Forces**
Traction Force Microscopy (TFM)

Traction stresses inferred using linear elasticity from the displacements of embedded beads.

Substrate displacement field

Traction stress

Strain energy

\[ u_s \]

\[ T = G_{\text{elastic}}^{-1} \cdot u_s \]

\[ W = \frac{1}{2} \int dA \ T(r) \cdot u_s(r) \]
Traction stresses are localized at the edges of cell/cell colony

F-actin

Single cell

Cell colony

Traction Magnitude

0

Max

Stationary Keratinocytes
Mertz, Banerjee et al, PRL, 2012

Smooth muscle cells

What factors determine the localization length scale?

Migrating fibroblasts
Adherent Cell as a Contractile gel

Assuming Mechanical Equilibrium

Cell

\[ \partial_\beta \sigma_{\alpha\beta} = 0 \quad (\alpha, \beta \in x, y, z) \]

Thin Film

\[ \partial_z \sigma_{iz} + \partial_j \sigma_{ij} = 0 \quad (i, j \in x, y) \]

Plane Stress Approx.

\[ h \partial_j \bar{\sigma}_{ij} = \sigma_{iz} \big|_{z=0} \equiv T_i \quad \text{(Thickness Averaged)} \]

Traction Stress

\[ \vec{T} = Y_a (\vec{u} - \vec{u}^s) \]

Focal adhesions are harmonic springs

Deforming in-plane

\[ \vec{u} \quad : \text{Cell’s in-plane displacement field} \]

\[ \vec{u}^s \quad : \text{Substrate’s in-plane displacement field} \]

\[ \vec{u}^s (\vec{r}) = \int_{\vec{r}'} G_{ij}^s (\vec{r} - \vec{r}') T_j (\vec{r}') \]

SB and MCM

EPL 2011

PRL 2012

NJP 2013
Contractile gel model...

For a thin substrate $h_s \ll L$:

$$G_{ij}^s(r - r') = \frac{1}{\mu_s/h_s} \delta_{ij} \delta(r - r')$$

Effective Force Balance:

$$h \partial_j \sigma_{ij} = Y u_i$$

Substrate Rigidity:

$$Y = \left( \frac{1}{Y_a} + \frac{h_s}{\mu_s} \right)^{-1}$$

Cellular Constitutive Relation:

$$\sigma_{ij} = B \nabla \cdot \vec{u} \ \delta_{ij} + \mu \left( \partial_i u_j + \partial_j u_i - \nabla \cdot \vec{u} \ \delta_{ij} \right) + \sigma_a \ \delta_{ij} \ (\sigma_a > 0)$$

Compression \quad \text{Shear} \quad \text{Acto-myosin Contractility}

Stress–free Boundary Condition:

$$\sigma_{ij} n_j = 0$$

$\vec{n}$: unit normal to cell boundary
One-dimension: Elongated cells

\[ \sigma = \mathcal{L}_p^2 \frac{d^2 \sigma}{dx^2} + \sigma_a \]

\[ \ell_p = \sqrt{\frac{Bh}{Y}} \text{ Penetration length} \]

\[ \sigma \big|_{x=0, L} = 0 \]

Force Balance \( \rightarrow \)

Cellular Stress \( \sigma(x) = B \partial_x u + \sigma_a \)

Traction localization and tensile stress buildup
Collective Mechanics of Epithelial Cell Layers

Aaron Mertz*       Eric Dufresne*       Valerie Horsley*       Cristina Marchetti**

*Yale University
**Syracuse University
How do collective mechanical properties of tissues emerge from cell-cell and cell-ECM interactions?
Intercellular adhesions form after calcium elevation

Calcium alters morphology and cohesiveness of colonies

Low calcium

Phalloidin (F-actin)
E-cadherin
Zyxin
Scale bars 50 µm

High calcium
Cohesive Cell Colonies – strong intercellular adhesion

High Calcium Medium

Similar distribution of strain energy regardless of cell number for highly cohesive colonies!
Strongly cohesive colonies – Contractile Gel Model

Model Predictions

\[ R \ll \ell_p : \quad \mathcal{F}(R) \propto R^3 \]

\[ R \gg \ell_p : \quad \mathcal{F}(R) \approx 2\pi h\sigma_{\alpha} R \propto R \]

Effective surface tension?
Scaling of Traction Forces with colony radius

- **Total traction** grows monotonically with colony radius and **not** the number of cells.
- Linear scaling at large colony radius suggests emergence of an **effective surface tension** originating from contractility.

\[
\frac{F(R)}{2R} = h \alpha (8 \pm 2) \times 10^{-4} \text{N/m}
\]

**Micropillars**

- Mertz, Banerjee *et al.*, PRL 2012
- Large colonies appear to behave like liquid droplets wetting a surface!
Strain energy localizes to colony periphery as adherens junctions form

Mertz, Che, Banerjee et al., PNAS, 2013
**Low Calcium**

- Weak cell-cell coupling

**High Calcium**

- Strong cell-cell coupling

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**G**

![Image G](image1.png)

**H**

![Image H](image2.png)

**I**

![Image I](image3.png)

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**D**

![Image D](image4.png)

**E**

![Image E](image5.png)

**F**

![Image F](image6.png)

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**J**

![Image J](image7.png)

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**1 h**

![Graph 1 h](image8.png)

**7 h**

![Graph 7 h](image9.png)

**11 h**

![Graph 11 h](image10.png)
Cell-cell adhesion as an elastic bond

Cells adhere to each other via hookean springs with adjustable stiffness

Traction Forces

Weakly cohesive cell colony

Elevating Calcium Level

Strongly cohesive cell colony
2D model with shapes and springs captures experimental data

Increasing stiffness of cell-cell springs

Time after Ca²⁺ addition

Mertz, Che, Banerjee et al., PNAS, 2013
Can Geometry solely regulate traction stresses in adherent cells?

*University of Chicago
**Syracuse University

Manuscript Submitted, under review
Adherent Cells maintain a characteristic Surface Tension

\[ W = \frac{1}{2} \int dA \ T(r) \cdot u_s(r) \]

\[ W \propto A \]

\[ \gamma = W/A \simeq 10^{-5} \text{N/m} \]

Cellular Surface Tension dependence

Substrate Stiffness (weak)

Myosin-II based Contractility (strong)
Strain Energy is insensitive to Substrate Stiffness

Pattern Area=800 μm²
Uniform Three-element Model

\[ \sigma_{cell} = E_{cell} \epsilon + \sigma_a \]
\[ \sigma_f = E_f \epsilon_f \]
\[ \sigma_s = E_s \epsilon_s \]

\[ \epsilon_s = \sigma_a \frac{E_{eff}}{E_s (E_{eff} + E_{cell})} \]

\[ E_{eff} = (E_s^{-1} + E_f^{-1})^{-1} \]

\[ T = E_{eff} \sigma_a / (E_{cell} + E_{eff}) \]

\[ W = \left[ \frac{h \sigma_a^2 E_{eff}^2}{2 E_s (E_{cell} + E_{eff})^2} \right] A \]

Using, \( h \sim 1 \mu m, E_{cell} \sim 10 \text{ kPa}, E_{eff} \sim E_s \sim 10 \text{ kPa}, \sigma_a \sim 1 \text{ kPa} \)
\[ \gamma \sim 1.25 \times 10^{-5} \text{ N/m} \]
Micropatterns with Constant Curvature

Substrate shear modulus = 16 kPa

Radius of Curvature=15 μm

• Strain Energy scales with Cell Spread Area

• Traction Stresses strongly localize to curved regions
Micropatterns with Constant Area

Pattern Area=1600 μm²

Substrate shear modulus = 16 kPa

• Strain Energy invariant.

• Maximum stress increased with aspect ratio
Line Tension

\[ U = \frac{h}{2} \int dA \, \sigma_{ij} u_{ij} + \frac{Y}{2} \int dA \, u^2 + \lambda \oint ds \]

\[ \lambda > 0 \]

Edge Contractility

Line Tension Scales with Cell Perimeter

\[ \lambda = f_m P \]

Force/length exerted by molecular motors acting in parallel across the cell boundary
Micropatterns with Constant Curvature

$$E_{cell} = 5.4 \text{ kPa}$$

$$\sigma_a = 2.4 \text{ kPa}$$

$$f_m = 0.7 \text{ nN/\mu m}$$

Surface Tension $\gamma = 6.06 \times 10^{-5} \text{ N/m}$
Micropatterns with Constant Area
Unconstrained Fibroblasts

Force Distribution predictions
Concluding Remarks

Single Cells

• *Global Mechanics*: Substrate stiffness, number of focal adhesions and cell shape have little effect on total cellular strain energy. Strain Energy of an adherent cell is regulated by its spread area alone, for a particular cell type.

• *Local Mechanics*: Traction stresses are highly sensitive to substrate stiffness, cell shape or adhesion geometry.

Cell Colonies

• Cohesive cell colonies wet the substrate underneath with an effective surface tension.

• Colony surface tension emerges from having strong intercellular adhesions and acto-myosin contractility.

• Cadherin based adhesions organize cell-matrix forces to the periphery of the colony.