Nonlinear Biomechanics of FibroblastMechanosensing in Fibrous ECM: Modelling, Analysis and Computation

Phoebus Rosakis

Department of Mathematics and Applied Mathematics
University of Crete
&
Institute for Computational Mathematics, F.O.R.T.H.
Cells Use Stress to Find Each Other in Fibrous Darkness

with (IACM Team)

Georgios Grekas
Charalambos Makridakis

and

Guruswami Ravichandran
Jacob Notbohm
Ayelet Lesman
David Tirrell
Continuum Mechanics
Continuum Mechanics

mathematical modeling of forces/deformations in deformable solids
Fibroblasts cause wrinkling of 2D silicone substrate
Harris, Stopak, Wild 1981
Recent development in silicone substrata involves the preparation of sheets of solid elastomers using a curing agent ([15]; Fig. 1c). This generates non-wrinkling substrata with improved mechanical characteristics. In addition, deformation of the surface is determined on the basis of micropatterns of dots or lines, generated by lithography on silicon (Si) or gallium arsenide (GaAs) molds and imprinted onto the surface of the substratum. The regular micropattern has a density of up to 1 dot per 4 mm², and allows the direct visualization of strains. But this approach is currently limited by the availability of micropatterned molds. Moreover, the micropattern creates a physically or chemically textured surface, which might affect cell adhesion and migration through the contact guidance mechanism [16]. Like the other types of silicone substrata, a method has yet to be developed for coating the surface with extracellular matrix (ECM) proteins to create a more physiological environment.

Polyacrylamide substrata

As an alternative to silicone, the flexible substratum can be made from polyacrylamide sheets, which are easy to prepare and have superior mechanical and optical properties [17]. The flexibility of the material is easily controlled by the concentration of acrylamide and/or bis-acrylamide. Furthermore, the porous nature of the material provides a more physiological environment than do solid substrata. Because most cells show no detectable affinity for polyacrylamide, several chemical approaches have been developed to coat the surface with ECM proteins [18], and one can assume that mechanical interactions with such substrata are mediated by the coated ECM or associated proteins.

Deformation is detected by using embedded fluorescent microbeads as markers [18] (Fig. 1e). Because the beads are randomly distributed throughout the substratum and their movements are dependent on the depth from the surface, the image must be carefully focused near the surface of the substratum. In addition, although bead displacements can be observed directly as the cell migrates, for stationary or slow-migrating cells the full extent of deformation must be determined by comparing images of the stressed substratum with a null-force image, which must be recorded after removing the cell by physical or chemical means. The problem with focusing can be alleviated by the recently developed technique of stacking a thin layer of polyacrylamide containing beads on top of a bead-free substratum; this then confines the beads to the top surface of the substratum [19].

Moving Fish Epidermal Keratocytes cause wrinkling of 2D silicone substrate
Burton, Park, Taylor, 1999
Explants induce densification bands in 3D fibrin extracellular matrix
Harris, Stopak, Wild 1981

Fig. 4 Two centre effects form in collagen gels even when explants are separated by a distance spanning over 1.5 cm. Collagen fibres become aligned into long axially oriented tracts interconnecting two centres of traction. Heart explants from 8-day chick embryos after 96 h in culture. Scale bar, 1 mm.
Fibroblasts exert “huge” forces onto their surroundings
Fibroblasts exert “huge” forces onto their surroundings

WHY?
mechanosensing

cells sense forces/stresses/deformations
mechanosensing

cells sense forces/stresses/deformations

tensotaxis

cells protrude/migrate toward regions of higher tension
mechanosensing
cells sense forces/stresses/deformations

tensotaxis
cells protrude/migrate toward regions of higher tension

durotaxis
cells protrude/migrate toward regions of higher stiffness
What does the ECM look like?

Network of collagen/fibrin fibers
The Role of Focal Adhesions

Adhesions allow the cell to exert traction on the ECM.
Adhesions act as force/stress/deformation detectors. Mechanical sensing is active!!
The Role of Focal Adhesions

**Adhesions** allow the cell to exert traction on the ECM.
The Role of Focal Adhesions

Adhesions allow the cell to exert traction on the ECM

Adhesions act as force/ stress/ deformation detectors.

Mechanosensing is active!!
They induce ECM stress/deformation by contracting (as much as 50%) while adhering to the ECM. They exert much larger traction than other cell types. Harris-Stopak-Wild, 1981. These tractions can be high enough to create distinct spatial patterns (tissue morphogenesis). Stopak-Harris, 1982.
Fibroblasts Use Stress, But WHY?

To see and be seen and to change things around them i.e.
To detect and approach each other by spreading/protruding
To remodel the matrix around them (possibly for tissue morphogenesis) Stopak-Harris, 1982
Fibroblasts Use Stress, But WHY?

To see
Fibroblasts Use Stress, But WHY?

To see

and be seen
Fibroblasts Use Stress, But WHY?

To see

and be seen

and to change things around them
Fibroblasts Use Stress, But WHY?

To see

and be seen

and to change things around them

i.e.
Fibroblasts Use Stress, But **WHY?**

To see

and be seen

and to change things around them

i.e.

To detect and approach each other by spreading/protruding
Fibroblasts Use Stress, But WHY?

To see

and be seen

and to change things around them

i.e.

To detect and approach each other by spreading/protruding

To remodel the matrix around them (possibly for tissue morphogenesis Stopak-Harris, 1982)
Cells contract and tethers form in the ECM joining them. Tethers (white) are regions of high ECM density.
Cells contract and tethers form in the ECM joining them. Tethers (white) are regions of high ECM density.
Later, cells grow appendages along the tethers towards each other.

Green: cell actin.
Later, cells grow appendages along the tethers towards each other.

Green: cell actin.
Claim:
This behavior relies on a special nonlinearity of the ECM’s mechanical behavior.
Claim:
This behavior relies on a special (instability) of the ECM’s mechanical behavior

this tethering behavior does not occur in homogeneous linear elastic ECM (e.g. hydrogels)
Microbuckling

Individual fibers will buckle under compression
Microbuckling

Individual fibers will buckle under compression stiffer in tension than in compression (rubber band)
A Nonlinear Constitutive Law for Large Deformations
Fig. 4  Two centre effects form in collagen gels even when explants are separated by a distance spanning over 1.5 cm. Collagen fibres become aligned into long axially oriented tracts interconnecting two centres of traction. Heart explants from 8-day chick embryos after 96 h in culture. Scale bar, 1 mm.
A Nonlinear Constitutive Law for Large Deformations

Macroscopic tether (1.5cm) between contracting (multi-cell) explants (2-3mm) in collagen fibrous ECM

Harris-Stopak 1981

Fig. 4  Two centre effects form in collagen gels even when explants are separated by a distance spanning over 1.5 cm. Collagen fibres become aligned into long axially oriented tracts interconnecting two centres of traction. Heart explants from 8-day chick embryos after 96 h in culture.

Scale bar, 1 mm.
A Nonlinear Constitutive Law for Large Deformations

Harris-Stopak 1981

Macroscopic tether (1.5cm) between contracting (multi-cell) explants (2-3mm) in collagen fibrous ECM

**Hypothesis:** This can be explained by microbuckling.
Elastic Strain Energy Function

Start with a single fiber with force stretch relation $F(\lambda)$ that is weaker in compression than tension and energy

$$\bar{W}(\lambda) = \int_1^\lambda F(\zeta) d\zeta$$

$$F(\lambda) = \mu (\lambda^N - 1)$$
Elastic Strain Energy Function

Start with a single fiber with force stretch relation $F(\lambda)$ that is weaker in compression than tension and energy

$$\bar{W}(\lambda) = \int_{1}^{\lambda} F(\zeta) d\zeta$$
Elastic Strain Energy Function

Start with a single fiber with force stretch relation $F(\lambda)$ that is weaker in compression than tension and energy

$$\tilde{W}(\lambda) = \int_{1}^{\lambda} F(\zeta) d\zeta$$

Suppose ECM (2D) has uniform angular distribution of fibers

$$\hat{W}(\lambda_1, \lambda_2) = \frac{1}{2\pi} \int_{0}^{2\pi} \tilde{W} \left( \sqrt{(\lambda_1 \cos \theta)^2 + (\lambda_2 \sin \theta)^2} \right) d\theta$$
Elastic Strain Energy Function

Explicitly determined

\[ W(F), F = \nabla u \]

Elastic Deformation Energy /unit volume, function of deformation gradient matrix

Nonlinear Stress-Strain Relation

\[ S(F) = \frac{dW(F)}{dF} \]
Elastic Strain Energy Function

Explicitly determined

\[ W(F), \quad F = \nabla u \]

Elastic Deformation Energy /unit volume, function of deformation gradient matrix \( F \) (strain)
Elastic Strain Energy Function

Explicitly determined

\[ W(F), \quad F = \nabla u \]

Elastic Deformation Energy /unit volume, function of deformation gradient matrix \( F \) (strain)
Elastic Strain Energy Function

Explicitly determined

\[ W(F), \quad F = \nabla u \]

Elastic Deformation Energy / unit volume, function of deformation gradient matrix \( F \) (strain)

Nonlinear Stress-Strain Relation

\[ S(F) = \frac{dW(F)}{dF} \]
Elastic Strain Energy Function

Explicitly determined

\[ W(F), \quad F = \nabla u \]

Elastic Deformation Energy /unit volume, function of deformation gradient matrix \( F \) (strain)

Nonlinear Stress-Strain Relation

\[ S(F) = \frac{dW(F)}{dF} \]
Interesting Properties of $W$

Uniaxial compression
Interesting Properties of $W$

Uniaxial compression
Interesting Properties of $W$

Uniaxial compression

Nonmonotone uniaxial compression:
Interesting Properties of $W$

Uniaxial compression

Nonmonotone uniaxial compression: densification (compressive) phase transition,
Rank-one convexity fails!
Knowles-Sternberg strong ellipticity conditions fail in compression.
Interesting Properties of $W$
Interesting Properties of $W$

$W$ is a multi-well isotropic strain energy
Interesting Properties of $\mathcal{W}$

$\mathcal{W}$ is a multi-well isotropic strain energy

Level curves of $\hat{\mathcal{W}}(\lambda_1, \lambda_2)$
Interesting Properties of $\mathcal{W}$

$\mathcal{W}$ is a multi-well isotropic strain energy

Level curves of $\hat{\mathcal{W}}(\lambda_1, \lambda_2)$

Two wells: two phases (stable states) because of microbuckling
Interesting Properties of $\mathcal{W}$

$\mathcal{W}$ is a multi-well isotropic strain energy

Level curves of $\hat{\mathcal{W}}(\lambda_1, \lambda_2)$

Two wells: two phases (stable states) because of microbuckling Instabilities, Discontinuities, Interfaces...
Elastic Energy of the ECM

- Model ECM as elastic body with holes (cells/explants)
- Strain Energy Function $W$.
- Elastic Energy of the ECM

$$\mathcal{E}[u] = \int_{\Omega} W(\nabla u) dV$$
Elastic Energy of the ECM

- Model ECM as elastic body with holes (cells/explants)
- Strain Energy Function $W$.
- Elastic Energy of the ECM

$$\mathcal{E}[u] = \int_{\Omega} W(\nabla u) dV$$

Minimize Energy
**Cells** are the holes. They contract: they apply centripetal forces proportional to distance from their center.

**Explants**: same as cells but at a much bigger scale.
Typical Numerical Results (Finite Element Computation)
Typical Numerical Results (Finite Element Computation)
Typical Numerical Results (Finite Element Computation)

Stopak-Harris 1981

Fig. 4  Two centre effects form in collagen gels even when explants are separated by a distance spanning over 1.5 cm. Collagen fibres become aligned into long axially oriented tracts interconnecting two centres of traction. Heart explants from 8-day chick embryos after 96 h in culture. Scale bar, 1 mm.
Typical Numerical Results (Finite Element Computation)

Stopak-Harris 1981
Mesh Dependence
Mesh Dependence
Mesh Dependence

Discontinuities, Oscillations
Mesh Dependence

Discontinuities, Oscillation
Stopak-Harris 1981
Higher Gradients

... added to the energy to limit gradient oscillations. Related to discreteness, bending stiffness of the fibers and “rotational springs” at network nodes.

\[ \Phi[u] = E[u] + C[u] + \frac{\varepsilon}{2} \int_{\Omega} |\nabla \nabla u|^2 \]
(a) $\epsilon = 0$.  

(b) $\epsilon_1 > 0$.  

(b) $\epsilon_1 > 0$.

(c) $\epsilon_2 > \epsilon_1$. 
Length Scale $\sqrt{\varepsilon} \ll$ explant size
Length Scale $\sqrt{\varepsilon} \approx$ cell size

Compare of simulations with experiments to calibrate $\varepsilon$ based on number of protrusions.
Conclusions

- cells use stress to see and be seen by peers
Conclusions

- cells use stress to see and be seen by peers
- mechanosensing is active (cells exert force, detect resulting stress/deformation).
Conclusions

▶ cells use stress to see and be seen by peers

▶ mechanosensing is active (cells exert force, detect resulting stress/deformation).

▶ they exploit a special phase transition (microbuckling of fibrin) to increase detection range by tether formation
Conclusions

▶ cells use stress to see and be seen by peers

▶ mechanosensing is active (cells exert force, detect resulting stress/deformation).

▶ they exploit a special phase transition (microbuckling of fibrin) to increase detection range by tether formation (stress decays rapidly in linear solids)
Conclusions

- cells use stress to see and be seen by peers

- mechanosensing is active (cells exert force, detect resulting stress/deformation).

- they exploit a special phase transition (microbuckling of fibrin) to increase detection range by tether formation (stress decays rapidly in linear solids)

- highly nonlinear problem requires specialized modelling/simulation techniques to predict/explain experimental observations by this mechanism
Conclusions

- cells use stress to see and be seen by peers
- mechanosensing is active (cells exert force, detect resulting stress/deformation).
- they exploit a special phase transition (microbuckling of fibrin) to increase detection range by tether formation (stress decays rapidly in linear solids)
- highly nonlinear problem requires specialized modelling/simulation techniques to predict/explain experimental observations by this mechanism
- yes but why?
Cell Networks
Lesman-Notbohm-Ravichnadran-Tirrell unpublished experiments

Cell-cell interactions in 3D

100 μm

Labeled fibrin: 10mg/ml,
3T3 Fibroblast: 3 K cells/10 μl
Cell Networks
Lesman-Notbohm-Ravichnadran-Tirrell unpublished experiments

Cell-cell interactions in 3D

100 μm

Labeled fibrin: 10mg/ml, 3T3 Fibroblast: 3 K cells/10 μl
Cell Networks
Lesman-Notbohm-Ravichnadran-Tirrell unpublished

Cell-cell interactions in 3D

Labeled fibrin: 10mg/ml,
3T3 Fibroblast: 3 K cells/10 μl

100 μm
Cell Networks
Lesman-Notbohm-Ravichnadran-Tirrell unpublished

**Cell-cell interactions in 3D**

Labeled fibrin: 10mg/ml,
3T3 Fibroblast: 3 K cells/10 μl
Cell Networks
Lesman-Notbohm-Ravichnadran-Tirrell unpublished

Cell-cell interactions in 3D

Labeled fibrin: 10mg/ml,
3T3 Fibroblast: 3 K cells/10 μl

100 μm
Cell Networks

Hypothesis: Matrix Remodeling
Hypothesis: Matrix Remodeling

Tether network changes ECM mechanical properties (stiffness)
Cell Networks

2D Simulation