The Mechanical Environment Modulates Myofibroblast Contractile Activity

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AIM OF THE STUDY

To investigate how mechanical challenges affect myofibroblast contractile activity by analyzing intracellular calcium dynamics.

BACKGROUND

- Myofibroblast contraction of the extracellular matrix promotes normal tissue healing but also fosters fibrosis.
- The mechanical environment varies during wound repair. In general, tissue stiffness increases with remodeling over time.
- The impact of mechanical factors on the contractile activity of myofibroblasts remains elusive.
- Periodic spontaneous calcium oscillations in myofibroblasts are instrumentally linked to cell contraction.

SUMMARY

Modulation of extracellular stress

- Calcium oscillations in myofibroblasts accelerate gradually with increasing stiffness of silicone soft substrates **(Panel 2)** and collagen gels **(Panel 3)**.

Modulation of intracellular stress

- The calcium oscillation frequency is high in myofibroblasts attaching to adhesive fibronectin-coated coverslips and decreases with increasing concentration of non-adhesive poly-L-lysine **(Panel 4)**.
- Acute blocking of actin polymerization by addition of Cytochalasin D reduces the calcium oscillation frequency in myofibroblasts **(Panel 5)**.

CONCLUSIONS

Our results suggest that increasing extracellular and intracellular mechanical stresses enhance myofibroblast contractile activities.

These results provide new insights about myofibroblast behaviour and can be useful to understand normal and pathological wound healing.

ACKNOWLEDGEMENTS

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Panel 1: Methods

Cells

- We cultured primary rat subcutaneous fibroblasts in the presence of transforming growth factor β1 for 4-6 days to induce differentiation into myofibroblasts.
- We then trypsinized and seeded cells according to the experimental conditions (Panels 2-5).

Monitoring of calcium dynamics

- After 2 additional days, we incubated cells with calcium indicators (Fura-2 AM or Fluo-4 AM).
- We recorded intracellular calcium dynamics using fluorescence microscopy.

Analysis of calcium oscillations

- We determined the mean period of spontaneously occurring regular calcium oscillations in myofibroblasts (**Figure 1a**).
- We compiled oscillation periods in a histogram and fitted a curve (**Figure 1b**).

Figure 1a

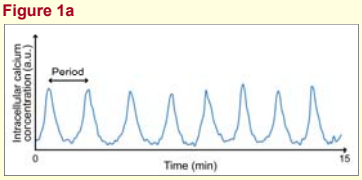
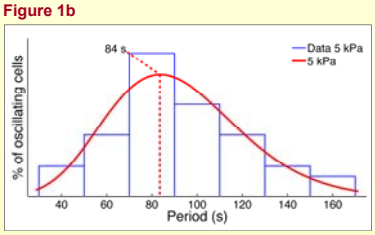


Figure 1b



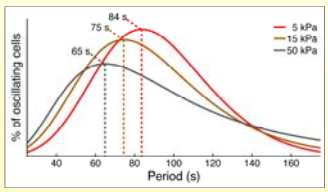
Panel 2: Modulation of extracellular stress – silicone substrates with tunable stiffness (2D model)

Method

Myofibroblasts were seeded onto silicone substrates of various stiffness.

Results

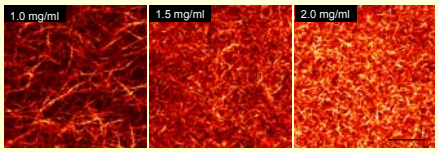
Increasing substrate stiffness decreases calcium oscillation periods (n≥44 cells).



Panel 3: Modulation of extracellular stress – type I collagen gels of different concentration (3D model)

Method

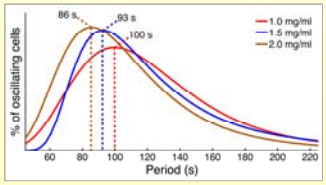
- Myofibroblasts were seeded into collagen gels of various concentrations (n≥60 cells) to modulate extracellular matrix stiffness.
- Confocal reflection microscopy shows gel density differences (below).



Scale bar = 50 µm

Results

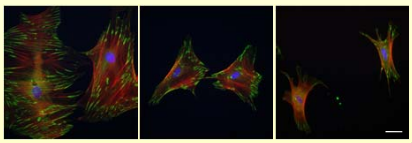
Increasing collagen gel concentration decreases calcium oscillation periods (=increases frequency).



Panel 4: Modulation of intracellular stress – adhesion strength

Method

Myofibroblasts were seeded onto coverslips coated with adhesive fibronectin or non-adhesive poly-L-lysine at different concentrations (n≥ 68 cells). Focal adhesions (vinculin), organization of stress fibers (actin) are reduced by increasing concentration of poly-L-lysine (below), indicating loss intracellular stress



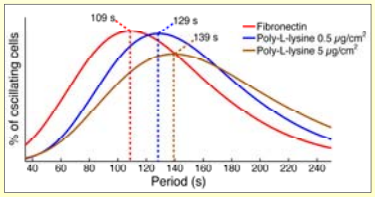
Fibronectin | Poly-L-lysine 0.5 µg/cm² | Poly-L-lysine 5 µg/cm²

Scale bar = 50 µm

Vinculin Actin Nucleus

Results

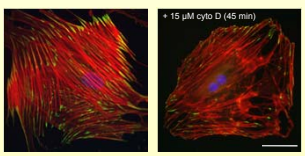
Calcium oscillation periods are short in myofibroblasts cultured on fibronectin and become progressively longer in cells grown on increasing densities of poly-L-lysine.



Panel 5: Modulation of intracellular stress – depolymerizing actin fibers

Method

- Calcium oscillations were recorded in myofibroblasts seeded on fibronectin-coated coverslips before and 15 minutes after addition of Cytochalasin D (n=25 cells).
- Cytochalasin D decreases intracellular stress by blocking actin polymerization and thus disrupting stress fiber network (below).

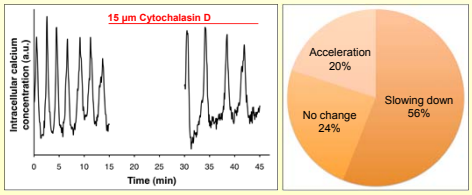


Vinculin Actin Nucleus

Scale bar = 50 µm

Results

After addition of Cytochalasin D, calcium oscillation frequency is reduced (period increase) in the majority of cells (~55-60%).



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