**Abstract**

Fibroblasts in connective tissues often interact with a fibrillar extra-cellular matrix (ECM) that restricts their shape along one-dimension (1D, along the fiber). At the same time, the fibroblast responds to and affects the mechanical nature of its microenvironment which consists of the inter-woven fibrillary ECM, other matrix components and cells. To simultaneously constrain a fibroblast along 1D and still let it mechanically interact with an extended microenvironment of defined stiffness, we plated NIH 3T3 fibroblasts on micropatterned 1.5 µm-wide fibronectin lines on polyacrylamide gels of stiffness 13 or 45 kPa. We used traction force microscopy to quantify the cellular traction force transmitted to and deforming the substrate and the associated strain energy. We found that even though cell length depends on substrate stiffness, the strain energy stored in the substrate and the maximum traction forces exerted were independent of substrate stiffness. We also found that the fibrillar force exerted by fibroblasts and cell length depend on the actin nucleator formin, consistent with the predominance of linear actin structures.

**Background**

Fibroblasts in fibrillar environments assume a shape that is often predominantly 1-dimensional (1D), but exert forces on (and are influenced by) the 3D microenvironment [1]. Even though fibrillar force generation shapes the extra-cellular matrix (ECM) in many physiological and pathological situations, our knowledge of cell force generation in this context is limited.

**Methods**

NIH 3T3 cells were plated on polyacrylamide gels micropatterned as depicted below. Traction force measurements were performed using regularized Fourier traction cytometry [2].

**Results**

Strain energy and maximum traction stress exerted by fibrillar fibroblasts are not correlated with the cell length.

![Traction Maps](image1)

Figure 2. (A, B) Traction maps of 3T3 cells on a fibronectin line on a 45 kPa polyacrylamide (PAA) gel. (C, D) Scatter plot of strain energy and maximum traction stress exerted by the fibroblasts, versus cell length.

Strain energy and maximum traction stress are independent of substrate stiffness, but the cell length depends on substrate stiffness.

![Cell Length vs Substrate Stiffness](image2)

Figure 3. (A, B and C) Bar plots of cell length (in µm), strain energy (in fJ) and maximum traction stress (in Pa) exerted by 3T3 cells on a fibronectin line on 13 and 45 kPa polyacrylamide (PAA) gels. (D-E) Representative traction maps on 13 and 45 kPa gels. (F) Box plot of integrated intensity of vinculin on 13 and 45 kPa gels (integrated intensity at two ends of the cell of 20% cell length each is considered). Immunofluorescence images showing vinculin on 13 kPa (G) and 45 kPa (H) substrates. Dashed rectangle shows 20 percent of cell length and scale bar for distance is 20 µm.

**Conclusions**

Neither the strain energy nor the maximum traction force generated by fibrillar fibroblasts depends on substrate stiffness. Both fibroblast length and fibrillar force generation depend on formin.

**Acknowledgments**

We thank Benedikt Sabass and Ulrich Schwarz for force reconstruction scripts.

**References**