Effective Cell Membrane Tension is Independent of Substrate Stiffness

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Abstract

Most animal cells are surrounded by a cell membrane and an underlying actomyosin cortex. Both structures are linked with each other, and they are under tension. Membrane tension and cortical tension both influence many cellular processes, including cell migration, division, and endocytosis. However, while actomyosin tension is regulated by substrate stiffness, how membrane tension responds to mechanical substrate properties is currently poorly understood. Here, we probed the effective membrane tension of neurons and fibroblasts cultured on glass and polyacrylamide substrates of varying stiffness using optical tweezers. In contrast to actomyosin-based traction forces, both peak forces and steady state tether forces of cells cultured on hydrogels were independent of substrate stiffness and did not change after blocking myosin II activity using blebbistatin, indicating that tether and traction forces are not directly linked with each other. Peak forces on hydrogels were about twice as high in fibroblasts if compared to neurons, indicating stronger membrane-cortex adhesion in fibroblasts. Finally, tether forces were generally higher in cells cultured on hydrogels compared to cells cultured on glass, which we attribute to substrate-dependent alterations of the actomyosin cortex and an inverse relationship between tension along stress fibres and cortical tension. Our results provide new insights into the complex regulation of membrane tension, and they pave the way for a deeper understanding of biological processes instructed by it.

Introduction

The cell membrane forms the interface between the interior of a cell and its environment. As a lipid bilayer surrounded by aqueous solutions, cell membranes are usually under tension. Different external forces are thought to contribute to membrane tension, including osmotic pressure and interactions with the force generating cytoskeleton. The plasma membrane is supported by the underlying actomyosin cortex, and molecular linkers form tight connections between the two layers.

Many cellular processes, such as the regulation of cell morphology, migration, division, stem cell fate choice, and endo- and exocytosis, are regulated by membrane tension¹⁻⁶. Despite its biological importance⁷⁻⁹, membrane tension is still difficult to quantify. In plane membrane tension is defined as the force per unit length acting tangentially on the membrane. However, because of the membrane's coupling to cytoskeletal elements, measurements of the 'effective' membrane tension usually contain contributions of the underlying actomyosin cortex and other force-generating elements, as membrane-cytoskeleton adhesion has to be overcome^{10,11}.

The actomyosin cortex is coupled to the extracellular environment through integral membrane proteins such as integrins and cadherins. It generates tensile forces which are exerted on the environment. These forces, and hence cytoskeletal tension, increase with substrate stiffness^{12,13}, suggesting that the measured effective membrane tension should increase on stiffer substrates. However, the regulation of membrane tension by substrate mechanics is currently poorly understood.

Results and discussion

We here measured the effective membrane tension of cells cultured on substrates of varying stiffness by pulling thin membrane tethers from the cell using a membrane-adherent bead held in an optical trap (OT)^{2,14,15} (Fig. 1a, b; Fig. S1). Throughout a pull, the force on the tether can be continuously monitored, resulting in a characteristic force curve (Fig. 1c). The force rises quickly after initiation of the pull and reaches a peak before decreasing sharply. We attribute this peak force (PF) to the initial local detachment of plasma membrane from the actomyosin cortex (Fig. 1a). Subsequently, the tether is pulled until it reaches a set length. During this elongation process, the plasma membrane slides over the actin cortex while bonds between the membrane and the lipid binding proteins located in or at the cortex are broken and quickly re-established¹⁶. Following the elongation phase, the bead is held in a stable position and the steady-state force (SSF) is measured (Fig. 1b). The SSF is thought to reflect a combination of the in-plane plasma membrane tension and the membrane-cortex adhesion, which is in turn influenced by the cortical tension^{16,17}.

Tether forces are independent of hydrogel stiffness but PFs on glass depend on cell type

To test the effect of substrate mechanics and cortical tension on membrane tension, we performed tether pulls in fibroblasts, which possess a fully developed actomyosin cortex, and in neuronal axons, whose sub-membranous cytoskeleton is characterized by actin rings spaced by spectrin tetramers. Cells were cultured on glass as well as on hydrogels within a stiffness range adjusted to match their natural environment. For fibroblasts, hydrogel stiffnesses ranged from

100Pa (very soft; similar to subcutaneous adipose tissue), to 10kPa (stiff; already in the stiffness range of many fibrotic tissues)¹⁸. For neurons, the highest substrate stiffness used was 1kPa, corresponding to the highest stiffness experienced by these cells *in vivo*¹⁹.

Peak forces were about twice as high in fibroblasts if compared to neuronal axons (Fig. 1e, h). In both cell types, PFs were similar across all tested hydrogel substrates. In neurons, also PFs measured in axons cultured on glass were similar to those seen on hydrogels (Fig. 1h). In fibroblasts, however, we found significantly lower PFs when cultured on glass compared to those cultured on hydrogels (Fig. 1e).

SSFs were significantly higher for fibroblasts and neurons cultured on hydrogels compared to those cultured on glass (Fig. 1f, i). Similar to PFs, there was also no significant change in the SSFs among the cells cultured on differently stiff hydrogels.

In contrast, actomyosin-based traction forces scale with substrate stiffnesses for both neurons and fibroblasts²². Also in our culture conditions, cellular traction forces significantly increased on stiffer substrates in both cell types within the investigated stiffness range (Fig. 1j, k), while tether forces were independent of hydrogel substrate stiffness (Fig. 1d-i). These data indicated that traction forces and tether forces are not tightly coupled.

Tether forces on hydrogels but not SSFs on glass are independent of actomyosin contractility

Cellular cortical tension is mediated by actomyosin contractility²⁰. In order to further examine the interplay between cortical tension and membrane tension, we measured tether forces in

fibroblasts cultured on glass and 10 kPa hydrogels, where we would expect the highest cortical tension²², following treatment with the myosin inhibitor blebbistatin. Blebbistatin treatment had no effect on tether forces in fibroblasts cultured on hydrogels (Fig. 2). While PFs also did not change significantly after blocking actomyosin contractility in fibroblasts cultured on glass, SSFs increased on glass following blebbistatin treatment²¹ (Fig 2b).

Discussion

Here we found that the effective membrane tension, which is thought to scale with in-plane membrane tension, does not change as a function of hydrogel substrate stiffness within a physiologically relevant stiffness range in either fibroblasts or neurons (Fig. 1d-i). In contrast, actomyosin-based traction forces increased on stiffer substrates (Fig. 1j-k), suggesting that membrane tension is not linked to cellular contractility²⁸. In line with this thought, the application of the myosin II blocker, blebbistatin, did not alter the effective membrane tension in cells cultured on hydrogels (Fig. 2).

However, we found differences in effective membrane tension between cells cultured on hydrogels and glass. In fibroblasts, both PFs and SSFs were significantly lower in cells grown on glass in comparison to those grown on hydrogels (Fig. 1d-f). On glass, the application of blebbistatin led to a significant increase in effective membrane tension; both PFs and SSFs rose to levels found in fibroblasts cultured on hydrogels (Fig. 2). In neurons, SSFs were also significantly lower on glass than on hydrogels, whereas PFs on glass were similar to those found on hydrogels of different stiffnesses (Fig. 1g-i).

Peak forces as a readout of membrane-cortex adhesion

Our results suggest that the largest contribution to the measured PF is the initial force required to detach the membrane from the cortex, which is strongly related to membrane-cortex adhesion (in analogy to attempting to open Velcro from the middle). Fibroblasts have a fully developed actomyosin cortex, and proteins such as ezrin/radixin/moesin (ERM) proteins can connect membrane proteins to the actin cytoskeleton throughout the cell surface. Neuronal axons, on the other hand, have a highly ordered, periodic actin-spectrin network underneath their membrane²³ (Fig. 3b), and much less area for ERM proteins to connect the membrane to the actin rings, suggesting that membrane-cortex adhesion in axons is very low. Hence, this difference in the structure of the cell cortex may explain why PFs are about twice as high in fibroblasts than in neuronal axons cultured on physiologically stiff hydrogels.

The cortical structure, and hence likely also membrane-cortex adhesion, of most cell types varies depending on, for example, the mitotic state^{24,20} or the cell spread area²⁵. The actin cytoskeleton in fibroblasts changes drastically between hydrogel and glass surfaces (Fig. S2). Glass is orders of magnitude stiffer than hydrogels, and it has different surface properties. These mechanical and chemical differences between the substrates may not only lead to changes in the actin cytoskeleton but also cause changes in membrane-cortex adhesion, explaining why PFs in fibroblasts are higher on hydrogels than on glass. In neurons, where the actin-spectrin network is preserved on the different substrates and membrane-cortex adhesion is likely low in either environment, PFs were similar on hydrogel and glass substrates.

Interpretation of Steady State Forces

The significantly lower SSFs on glass compared to hydrogels in both cell types could result from changes in either the in-plane membrane tension and/or the adhesion between cortex and plasma membrane. As depicted in Figure 3, these differences in SSFs could be explained by a purely mechanical model²⁶.

Particularly on very stiff glass substrates, fibroblasts developed strong stress fibres. Recent experiments suggested that perturbations of stress fibres lead to an increase in tension of the nearby cortex²⁷. Thus, the contraction of stress fibres on glass might release tension in the cortex and the membrane between focal adhesion sites (Fig. 3a), explaining why SSFs in fibroblasts were lower on glass than on hydrogels (Fig. 1f), and why blebbistatin treatment, which relaxes stress fibres, led to a significant increase in SSFs on glass but not on hydrogels (Fig. 2b).

However, blebbistatin treatment does not only affect stress fibres but also cortical tension. The lack of a measurable change of SSFs after blebbistatin treatment in fibroblasts cultured on hydrogels suggests either that the influence of the cortical tension on the SSFs is negligible, or that the cortical tension changes very little under this treatment.

In developing neurons, growth cones at the leading tips of growing axons pull on their axons. The magnitude of forces generated by growth cones increases with substrate stiffness (Fig. 1k), suggesting that tension in the actin-spectrin cortex increases near the growth cone on stiffer substrates and thus affects the plasma membrane tension in a similar way as described above for fibroblasts (Fig. 3).

Conclusion

We found that effective membrane tension of cells cultured on hydrogels is independent of substrate stiffness and traction forces. This robustness suggests that membrane tension might either not be influenced by cortical tension, or it is actively maintained within a certain range. Another possibility is that traction forces transmitted to the cortex might dissipate quickly so that overall cortical tension would not scale with local traction forces²². If cortical and membrane tension are coupled, changes in membrane tension on the different hydrogels would happen only locally²⁹ near focal adhesions and cannot be seen when probed further away. Future technological developments enabling highly resolved membrane tension measurements will shed light on these important unresolved questions.

Author Contributions

J. Mc H., E. K., and K. A. performed tether pulling experiments. J. Mc H. analysed the results from OT experiments. E. K. cultured 3T3 fibroblasts and prepared hydrogels. S. K. F. dissected and cultured *Xenopus laevis* RGCs and prepared hydrogels. A.D. performed TFM experiments with 3T3 fibroblasts. R. G. performed the TFM experiments with neurons and all TFM analyses. E. K. and E. P. performed the immunostainings. J. Mc H. and E. K. designed experiments with 3T3 fibroblasts. J. Mc H., S. K. F., K. A., and U. F. K. designed pulling experiments with *X. laevis* RGCs. S. K. F., E. K., U.F.K. and K. F. conceived the project. E. K., J. Mc H., S. K. F., U.F.K. and K.F. wrote the manuscript.

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Figures

Figure 1: Peak and steady state tether forces provide different insights into cell properties. *a*) When an OT pull is initiated, the local membrane-cortex adhesion first needs to be overcome, resulting in the peak force. *b*) After tether extension, the bead is held stationary, and the steady state force, which scales with membrane tension, is recorded. *c*) A typical force curve recorded during a tether pull shows a peak (see *a*)) at the beginning of the pull and a steady state force when the tether is held at its maximum extension (see *b*). (*d-f, j*) 3T3 fibroblasts and (*g-i, k*) Xenopus retinal ganglion cell axons on different substrates; shear moduli are provided in Pa. *d*) Fluorescence image of a lipid tether pulled from a 3T3 fibroblast. *e*) Peak tether forces (PFs) and *f*) steady state tether forces (SSFs) of fibroblasts

291 *are similar on all hydrogel substrates irrespective of their stiffness and higher compared to on glass. g)*
 292 *Fluorescence image of a lipid tether pulled from an axon. h) PFs in axons are similar on all hydrogel*
 293 *and glass substrates. i) SSFs are similar on all hydrogels and higher if compared to glass. j-k) Traction*
 294 *forces measured for (j) 3T3 fibroblasts and (k) neuronal axons. Both cell types exerted higher traction*
 295 *forces on stiff hydrogels compared to softer ones.*
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Figure 2: The effect of actomyosin contractility on PFs and SSFs in fibroblasts. a) Blebbistatin application did not significantly alter PFs on glass and hydrogel substrates. b) SSFs are unaffected in cells growing on hydrogels but significantly increase on glass substrates after blebbistatin treatment.

Figure 3: Toy model of substrate type-dependent effective membrane tension. a) Schematic representation of the cell cortex and stress fibres in fibroblasts. The strong contraction of stress fibres on glass substrates might relax the tension in the cortex and thus reduce membrane tension. The relaxation of these stress fibres, on the other hand, might increase the cortical tension. The top model illustrates the tension in untreated fibroblasts grown on glass. The bottom shows the tension in the relevant components in fibroblasts on glass treated with blebbistatin or in untreated fibroblasts grown on hydrogels. b) The axon has a periodic actin ring-spectrin network underneath its membrane²³, which is likely rather static and poorly adhered to the membrane.