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Article

# Direct Measurement of the Cortical Tension during the Growth of Membrane Blebs

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ABSTRACT Mechanics is at the heart of many cellular processes and its importance has received considerable attention during the last two decades. In particular, the tension of cell membranes, and more specifically of the cell cortex, is a key parameter that determines the mechanical behavior of the cell periphery. However, the measurement of tension remains challenging due to its dynamic nature. Here we show that a noninvasive interferometric technique can reveal time-resolved effective tension measurements by a high-accuracy determination of edge fluctuations in expanding cell blebs of filamin-deficient melanoma cells. The introduced technique shows that the bleb tension is ~10–100 pN/$\mu$m and increases during bleb growth. Our results directly confirm that the subsequent stop of bleb growth is induced by an increase of measured tension, possibly mediated by the repolymerized actin cytoskeleton.

## INTRODUCTION

Cellular membrane blebbing is a well-known and highly dynamic phenomenon that occurs throughout the lifecycle of many cell types. Cell blebs have been described in the context of apoptosis (1), cell division (2), cell spreading (3), and notably in cell motility (4). The time course of a bleb (its growth and retraction) has been carefully characterized (5–8), but a dynamic and time-resolved measurement of the tension is still missing. Blebs are driven by hydrostatic pressure generated by cortical tension, which is maintained by the contractile acto-myosin cortex that is physically linked to the membrane. The actin cortex is composed of a dense actin network that is connected to the plasma membrane and gives mechanical rigidity to the cell periphery. This connection limits the mobility of the membrane and prevents the formation of blebs in most cell types, despite the presence of hydrostatic pressure. However, if the actin cortex ruptures, or if the cortex to membrane connection is lost, the pressure pushes the membrane outwards, and a cell bleb grows.

Experimental techniques such as micropipette aspiration or the fluctuation analysis used here measure the effective tension. In the general case, the effective tension consists of both a contribution from the membrane and the actin cortex. Because the two structures are mechanically coupled, the fluctuations of the cell edge contain contributions from both. In principle, the membrane and the cortex have different tension, but it remains difficult to experimentally dissect the contribution from each component. Under steady-state conditions, the hydrostatic pressure that pushes against the plasma membrane is balanced by the mechanical rigidity of the actin cortex to which the membrane is connected. However, if this balance is broken by a local fracture of the actin cortex or by a local detachment of the actin cortex from the plasma membrane, hydrostatic pressure pushes the membrane outward to form a fast-expanding membrane bleb. Until a new actin cortex repolymerizes in the growing bleb, no mechanical support structure hinders its expansion, and the growth is determined by the pressure driving it and the friction forces of both the flowing fluid and membrane that enter the expanding bleb (9,10).

Techniques like micropipette aspiration made it possible to measure the tension of the cell cortex and to relate this tension to the growth velocity and final size of cell blebs (8). These experiments confirm that the mechanical origin of the hydrostatic pressure that drives bleb extension is the active contractility of the cellular acto-myosin cortex. Tension within the bleb is an important mechanical parameter for bleb dynamics. For a better characterization of the mechanical events during bleb growth, direct access to the bleb tension is required.

It is also important to have access to both the membrane tension and the cortex tension, which are not necessarily equal. Although in previous work it was possible to derive an estimation of the bleb tension and directly measure the cortex tension away from the bleb position (7,8), direct and time-resolved measurements at the site of blebbing is required to understand the mechanics of bleb formation. Such measurements are experimentally challenging using the micropipette aspiration method, because the structure is highly dynamic. Hence, we introduce what we believe to be a new method, one that provides direct insight into the effective tension during bleb growth.

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In addition to the micropipette aspiration technique, membrane tethers have been successfully used to determine the membrane tension in blebs (11). In these measurements it became clear that the measured membrane tension in blebs is lower than tension at sites of no bleb appearance. Although this finding seems to challenge the prediction of higher tension in growth-arrested blebs, it is important to note that the pure membrane tension differs from the effective tension of the combined membrane and actin cortex system. Technically, the micropipette aspiration also includes the cortex that is aspirated together with the membrane, whereas in tether-forming experiments, only membrane is pulled. Tether measurements include both membrane tension and membrane to cortex adhesion energy. The tether formation technique is therefore well suited to determine the membrane tension and other parameters such as contributions from the cytoskeletal adhesion energy. However, it does not probe the mechanics of the underlying actin cortex. In this view, these first investigations of blebs did indeed suggest a decrease of adhesion energy in the bleb membrane if compared to the nonblebbing cell cortex.

The method we introduce in this article uses membrane fluctuations to estimate the evolution of tension in blebs by measuring before, during, and after repolymerization of the actin cortex. Measurements are performed in filamin-depleted M2 melanoma cells, a common model system for membrane blebs because they continuously produce blebs all over their surface (12) and have been well studied previously (5,7). To determine the tension during bleb growth and growth arrest, we measure the membrane movement by a laser-based interferometric method capable of determining the membrane position with subnanometer precision and at submillisecond time resolution (13,14). This allows observation of the thermal fluctuations of the membrane during bleb growth. Separation of timescales is used to differentiate between the high frequency fluctuations that are considered in equilibrium and the low-frequency, active movement.

In this work, we focus on the growth phase, and show that a separation of timescales allows determination of the fluctuation amplitude even during the rapid extension phase of the cell bleb. A simplified equilibrium model for thermal fluctuations at short timescales allows extraction of an effective tension from the data that increases systematically during bleb growth. Although the blebbing phenotype of the M2 cells suggests strong mechanical differences to other, nonblebbing cells, it is interesting to see that the measured tension in the bleb membrane is only slightly smaller than previously determined values for the cell cortex in nonblebbing cells, such as L929 fibroblasts (8).

## MATERIALS AND METHODS

### Cell culture and Cytochalasin D application

Filamin A-deficient M2 cells (15) are cultured in DMEM (1 ×) + GlutaMAX medium (Gibco, Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS Gold; PAA, GE Healthcare, Pasching, Austria) under standard conditions (37°, 5% $CO_2$, maximal humidity) and passaged every 3–4 days at 80–90% confluence. For passaging, cells are washed with phosphate-buffered saline and trypsinized (TrypLE Express (1 ×) + Phenol red; Gibco, Life Technologies, Grand Island, NY) at 37° for 10 min. Trypsin is inactivated by FBS-containing media and the cell solution is centrifuged for 3 min at 1000 relative centrifugal force for subsequent medium exchange, resuspension, and seeding in new flasks. Cells are counted using an automated cell counter (Countess; Invitrogen, Carlsbad, CA). Twenty-four hours before measurements, 40,000 cells are seeded on 18 × 18 $mm^2$ coverslips. Immediately before imaging, coverslips are placed on a glass slide, sealed, and the measurements are performed for up to 1 h.

Cytochalasin D (CD) (Sigma Aldrich, St. Louis, MO) was applied at 2.5 $\mu$M 30 min before starting the experiment.

### Experimental setup and data acquisition

The membrane position was detected with a recently developed interferometric detection method using an NIR laser (YLM, 1064 nm; IPG Photonics, Oxford, MA) with a low average laser power of 40 $\mu$W per laser focus at the sample, to avoid optical trapping effects (14). The position of the laser beam in the sample plane is controlled using an *xy* acousto-optical deflector (AOD) device (MT80-A1,5-1064 nm; AA Opto Electronic, Orsay, France), that allows a fast repositioning of the laser focus with a switching time of 10 $\mu$s. As sketched in Fig. 1 *A*, the beam first passes the AOD, and is subsequently imaged into the back focal plane of a 60× NA = 1.2 water immersion objective (UPLSAPO 60×W/IR; Olympus, Rungis, France) using a 1:2 telescope and a dichroic mirror. After interaction with the sample, the scattered and unscattered light is collected by a long-distance water immersion objective (U LUMPL FL 60×W/IR, 60×, NA 0.9; Olympus) that also serves as condenser. Subsequently, the light is imaged onto a quadrant photodiode (QPD) using a 2:1 telescope. The signal from the QPD (InGaAs QPD G6849; Hamamatsu Photonics, Massy, France) is preprocessed by an analog circuit that delivers the asymmetry of the light in the *x* and *y* directions and the total sum of the signal (Öffner MSR-Technik, Plankstadt, Germany). Briefly, the asymmetry is calculated by $\Delta x = ((Q2 + Q4) - (Q1 + Q3))/\text{sum}$, $\Delta y = ((Q1 + Q2) - (Q3 + Q4))/\text{sum}$, and $\text{sum} = Q1 + Q2 + Q3 + Q4$. Here $Q_i$ corresponds to the light intensity that is measured at the four different photodiodes of the detector. These signals are digitized at 500 kHz using multichannel DAQ cards (NI-DAQ 6363 PCIe; National Instruments, Austin, Texas) and further processed using the softwares LABVIEW (National Instruments) and MATLAB (The MathWorks, Natick, MA). The detection method works reliably if the bleb edge is in the focal volume of the laser, which has a diameter of ≈500 nm. However, due to the rapid growth, the bleb may leave this area quickly (timescale, 1 s). To ensure recording of the bleb during movement, we rapidly scan the laser over 50 positions normal to the bleb edge with a step size of 100 nm between each scan point. Every 500 $\mu$s, a full scan is completed, leading to an effective acquisition frequency of 2 kHz. Data acquisition and trap position are synchronized by using a common 500 kHz TTL trigger generated by the acquisition card. Careful calibration was performed to assure synchronization of laser positioning and data acquisition. The resulting $\Delta x$, $\Delta y$, and sum signals are recorded as a function of the laser position for later analysis.

### Experimental protocol and edge detection

For experiments, M2 cells are plated on uncoated coverslips one day before observation. Cells are sealed in a chamber formed between two coverslips. An average distance of ≈500 $\mu$m between the coverslips ensures that cells are not compressed between the glass faces. After mounting the sample on the microscope, an isolated cell is observed in video microscopy. The temperature during the experiment is typically between 29° and 31°, and