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Mechanobiology of the cell surface: Probing its remodeling dynamics using membrane tether pulling assays with optical tweezers

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Abstract: Mammalian cell surfaces consist of the plasma membrane supported by an underneath cortical cytoskeleton. Together, these structures can control not only the shape of cells but also a series of cellular functions ranging from migration and division to exocytosis, endocytosis and differentiation. Furthermore, the cell surface is capable of exerting and reacting to mechanical forces. Its viscoelastic properties, especially membrane tension and bending modulus, are fundamental parameters involved in these responses. This viewpoint summarizes our current knowledge on how to measure the viscoelastic properties of cell surfaces employing optical tweezers-based tether assays, paving the way for a better understanding of how cells react to external mechanical forces, with a glance on their remodeling dynamics and possible consequences on downstream cellular processes.

Introduction

Mammalian cell surfaces are extremely dynamic and complex structures, mainly composed of a lipid bilayer membrane supported by a handful of proteins and other accessory molecules. A set of polymerizing proteins make up the underneath cell cortex, involved in maintaining cell shape and integrity, and allowing cell movement, division, and tissue morphogenesis (Chugh and Paluch, 2018). In most eukaryotic cells, the cortex is a well-conserved actin-based network composed of F-actin filaments, myosin, and actin-binding proteins, and thus is called the actomyosin cortex (Chugh and Paluch, 2018; Svitkina, 2020).

The cell membrane and its associated actomyosin cortex, also known as the membrane-cytoskeleton complex (MCC), are important regulators of cell functions, from migration and shape/size determination to molecule-presenting and signaling (Salbreux *et al.*, 2012). Besides interacting with plenty of biochemical stimuli, the MCC exerts and reacts to mechanical forces from its environment (Salbreux *et al.*, 2012).

In this context, MCC's viscoelastic properties, especially membrane tension and bending modulus, are fundamental parameters involved in their interaction with the intra- and extracellular spaces (Pontes *et al.*, 2017a). In this viewpoint manuscript, we present how these properties are measured, their implications on cell functions, as well as a detailed description of the membrane tether extraction experiment using optical tweezers (OT), the gold-standard tool to perform these measurements (Pompeu *et al.*, 2021). Finally, we also discuss how membrane tether-pulling assays can be used to probe cell surface remodeling dynamics together with possible consequences on downstream cellular processes.

Main Text

OT are described as single-beam gradient force optical traps that explore the property of photons being able to transfer momentum to small particles in focused laser beams. Reflection and refraction of light by a transparent particle near the laser beam with a Gaussian intensity profile causes a change in the photons' momentum that is translated as force, attracting it to the focus point. There, the particle experiences a balance of forces that maintains it trapped. These conditions describe a successfully employed OT (Ashkin *et al.*, 1986).

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Several biological transparent objects can be trapped with OT, including viruses, bacteria or even suspended cells (Ashkin and Dziedzic, 1987). However, the most suitable way to interact with biological systems using OT is to capture dielectric transparent microspheres with the focused laser beam (Ashkin *et al.*, 1986; Neuman and Block, 2004). It is possible to use these trapped microspheres as handles, attaching them to cell surfaces, and then displacing the microscope stage. This procedure will produce forces (in the piconewton range, pN) on the membrane and can also be used to measure its reaction, given that any displacement in the microsphere position relative to its focal point (Δx in nanometers, nm) causes a restoring force in the opposite direction. This is predicted by Hooke's Law ($F = -\kappa\Delta x$) for an ideal spring extended at a distance Δx and stiffness κ (pN/nm), the latter obtained by proper OT calibration methods (Pompeu *et al.*, 2021; Dutra *et al.*, 2014; Neuman and Block, 2004). Such robust methodology can be applied, for example, to measure the forces that MCC produces on optically trapped microspheres.

Over the years, OT and other micromanipulation tools, such as atomic force microscopy (AFM), traction force microscopy, magnetic twisting cytometry and micropipette aspiration have been employed to exert forces on MCCs to characterize their mechanical responses (Moeendarbary and Harris, 2014). Membrane tether-pulling is one of the most common assays in this regard. Tether extractions were first performed in red blood cells, using a flow channel experiment where cells previously attached to a coverslip were subjected to fluid shear stress until they began to form membrane tubes connected to the substrate (Hochmuth *et al.*, 1973). An improvement was later introduced using micropipettes. On one side of the red blood cell a portion of its surface was aspirated, and on the opposite side a microsphere (held by another micropipette) was attached to the cell surface and subsequently removed to generate a membrane tether (Hochmuth and Evans, 1982; Hochmuth *et al.*, 1982). However, this assay could not be easily applied to adherent cells. Thus, an OT-based membrane tether pulling method was created (Dai and Sheetz, 1995) and has been widely applied to extract tethers from cells to determine their membrane tension and bending modulus (Ayala *et al.*, 2017; Hissa *et al.*, 2017; Hissa *et al.*, 2013; Pontes *et al.*, 2013; Pontes *et al.*, 2017a; Pontes *et al.*, 2017b; Pontes *et al.*, 2011; Soares *et al.*, 2020; Farias *et al.*, 2020; Gomez *et al.*, 2020).

Briefly, in this assay, an optically trapped microsphere is attached to the MCC and then withdrawn when the microscope stage is set to move (in the xy direction). The trapped microsphere position (Δx) is recorded over time and converted into force. During this process, a thin membrane tether is formed. Why does it form? When a perpendicular force is applied to a membrane bilayer, a catenoid-shaped structure initially appears, but is then replaced by a thin membrane tube, because the membrane is always under tension and therefore tends to minimize its surface area. In theory, the minimum surface area would be reached when almost the entire membrane gets retracted to its original situation, leaving only an infinitesimally thin tube. For such a narrow tube to occur, the membrane curvature would dramatically increase; but due to membrane bending rigidity (also known as bending modulus–resistance of a membrane to bend), a tether with radius R is formed. Thus, the balance between membrane tension and bending rigidity generates a tube with a given radius (R) maintained by a certain force (F_t) (Fig. 1) (Derenyi *et al.*, 2002; Powers *et al.*, 2002):

$$\textit{membrane tension} = F_t/4\pi R;\ \textit{bending modulus} = F_t R/2\pi.$$

Experimentally, during tether extractions, if one measures the tether radius and the force to maintain the tether, it is possible to determine the cell membrane tension and bending modulus (Pontes *et al.*, 2017a; Derenyi *et al.*, 2002; Powers *et al.*, 2002). Based on the same principles, AFM cantilevers can also be used to pull membrane tubes (in the z direction) (Diz-Muñoz *et al.*, 2016), but it does not always provide the optical capacity to observe tether formation.

Several studies have demonstrated that these physical parameters, measured with tether extraction experiments, are not only cell-type specific (for more information, see Table 1 in Pontes *et al.*, 2017a), but also depends on the MCC, more specifically on the lipid composition (Hissa *et al.*, 2017; Hissa *et al.*, 2013; Khatibzadeh *et al.*, 2012), the actomyosin cortex organization (Pontes *et al.*, 2011; Masters *et al.*, 2013; Diz-Muñoz *et al.*, 2016; Ayala *et al.*, 2017) and, more strikingly, the membrane-cortex attachment (Nambiar *et al.*, 2009; Diz-Muñoz *et al.*, 2010; Bergert *et al.*, 2021). The membrane tension and bending rigidity of a cell is thus a combination which includes the tension and bending rigidity of the plasma membrane itself plus the membrane-cortex attachment (Dai and Sheetz, 1999; Pontes *et al.*, 2013).

Moreover, other studies have shown the importance of these parameters, particularly membrane tension, as capable of orchestrating a series of cellular functions ranging from endocytosis (Boulant *et al.*, 2011; Bucher *et al.*, 2018; Sinha *et al.*, 2011; Del Pozo *et al.*, 2021; Djakbarova *et al.*, 2021), exocytosis (Gauthier *et al.*, 2011; Bretou *et al.*, 2014; Masedunskas *et al.*, 2011) and phagocytosis (Masters *et al.*, 2013) to migration (Pontes *et al.*, 2017b; Hetmanski *et al.*, 2019), polarity (Houk *et al.*, 2012; Graziano *et al.*, 2019) and differentiation (Bergert *et al.*, 2021; de Belly *et al.*, 2021). High tension impairs endocytosis, exocytosis, phagocytosis and the overall migration but maintains cell polarity by confining signals to the leading edge of cells. In addition, tension decrease was correlated with a decrease in membrane-cytoskeleton attachment, increased endocytosis and enhanced ERK signaling, which allows exit from naïve to primed pluripotency in embryonic stem cells (Bergert *et al.*, 2021; de Belly *et al.*, 2021). A more detailed description of how membrane tension controls these and other cellular processes are better reviewed in Pontes *et al.* (2017a) and Sitarska and Diz-Muñoz (2020). In contrast, little is known about how bending rigidity can alter cellular events.

In order to determine these two physical properties, it is necessary to measure both the tether force and radius (Pontes *et al.*, 2017a; Derenyi *et al.*, 2002; Powers *et al.*, 2002). Measuring the tether force and/or radius individually does not allow an absolute estimation of mechanical parameters, although most studies consider indirect measurements with the tether force alone.

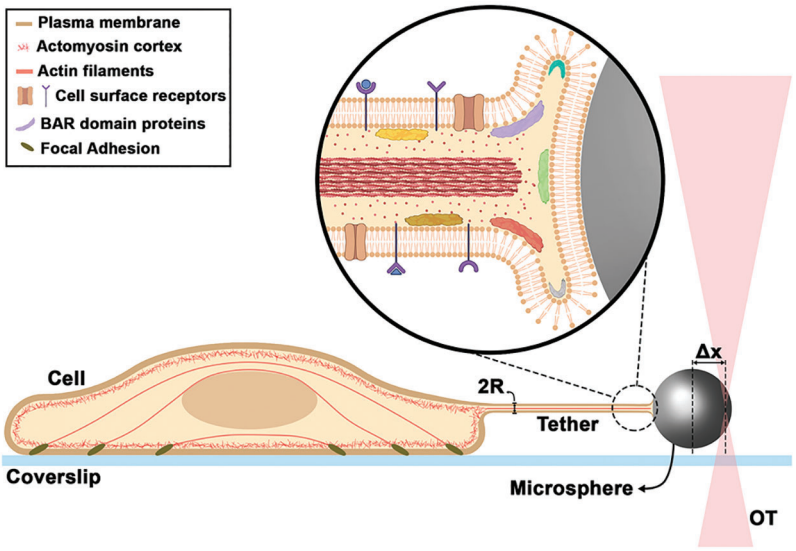

FIGURE 1. Schematic of a tether extraction experiment with OT, highlighting the nanotube internal organization. Δx is the trapped microsphere position and R is the tether radius.

Tether force can be measured during OT-tether extraction experiments, as already mentioned. However, measuring the tether radius (50–150 nm) is a bigger challenge, as its size is typically below the resolving limit of conventional optical microscopes (~250 nm) (Fig. 1). Therefore, a correlative microscopy-based method was established (Pontes *et al.*, 2013; Pontes *et al.*, 2011; Pompeu *et al.*, 2021). In this method, a tether is extracted via OT and the force required to perform tether extraction is obtained during the experiment, while the tether radius is later measured by scanning electron microscopy (SEM). The most challenging step is to perform the SEM images of tethers. Regardless of the difficulties inherent to correlative experiments, this is currently the most reliable method to determine the mechanical properties of cell membranes (for a step-by-step procedure, see Pompeu *et al.*, 2021). New optical microscopy methods integrated with OT are needed in order to measure tether radius concomitant with its generation and without other steps, such as fixation for SEM. A proposed method based on quantitative phase imaging, known as spatial light interference microscopy (SLIM) (Wang *et al.*, 2011) combined with OT appears to be very promising in this regard (Lu and Anvari, 2020; Sarshar *et al.*, 2016). Tether radii between 55 and 110 nm were measured for ovarian cancer cells using this method (Lu and Anvari, 2020). Also, stimulated emission depletion (STED) microscopy and AFM have been used; however, for AFM the tether needs to be adhered to the substrate and, as a result of this adhesion, the tube morphology gets slightly deformed (Lamour *et al.*, 2020). And for STED combined with OT, the initial study was carried out in giant unilamellar vesicles (Roy *et al.*, 2020) and no application in cells has been performed so far.

In addition to the mechanical characterization of cell surfaces, membrane tether-pulling can also probe how a cell is able to dynamically remodel its surface in response to an external force. Contrary to some observations (Raucher *et al.*, 2000; Gabella *et al.*, 2014) tethers from adherent cells have been shown to present F-actin inside (Pontes *et al.*, 2011; Pontes *et al.*, 2013; Bornschlögl *et al.*, 2013; Leijnse *et al.*, 2020), which is probably coming from the actomyosin cortex. Moreover, studies of tether-pulling from mast cells (Farrell *et al.*, 2013) and neuronal axons (Datar *et al.*, 2015) found evidence of dynamic saw-tooth-shaped force peaks, with slow rises and sharp decays, arising beyond the tether force plateau region when tethers were kept stretched. Possible explanations for such observations are based on actin polymerization/depolymerization dynamics, together with the action of molecular motors. While the slow rises in force were attributed to the polymerization of F-actin, the decays were associated with depolymerization and/or active rearward movement due to molecular motors such as myosin II (Farrell *et al.*, 2013; Datar *et al.*, 2015). In addition, recently, in a manuscript yet to be published (Leijnse *et al.*, 2020), the presence of actin inside tethers was confirmed not only from the initial moments of extraction, but also increasing after a few minutes post-extraction. The authors also demonstrated that the dynamics of force peaks may be associated with twists and buckles of the F-actin inside the tether, such as those happening in filopodia (Leijnse *et al.*, 2020; Leijnse *et al.*, 2015).

All the experimental evidences described above point to membrane tethers-pulling not only as a strategy to measure the mechanical properties and their variations according to different situations to which cells are exposed, but also as a tool to follow the dynamic rearrangement of cell surfaces. Important consequences of such method could be the elucidation of several molecular mechanisms of protein-membrane interactions and particularly how proteins are able to shape membranes. Also important is the activation/deactivation of local membrane proteins, such as ion channels or other cell receptors after an external pulling force is applied, together with their effects when membrane curvature increases. All proposed observations would be influenced by bending rigidity. A combination of OT and fluorescence microscopy, as previously highlighted (Arbore *et al.*, 2019), can help the field to advance. A schematic summarizing some of the findings described in this viewpoint together with future implications is presented in Fig. 1. Further studies exploring such possibilities could greatly improve our understanding of the role of forces acting on cell surfaces together with their consequences in several downstream cellular processes.

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