© 2015. Published by The Company of Biologists Ltd | Journal of Cell Science (2015) 128, 3687-3693 doi:10.1242/jcs.174623

OPINION

Cell adhesion strength from cortical tension – an integration of concepts

Rudolf Winklbauer*

ABSTRACT

Morphogenetic mechanisms such as cell movement or tissue separation depend on cell attachment and detachment processes, which involve adhesion receptors as well as the cortical cytoskeleton. The interplay between the two components is of stunning complexity. Most strikingly, the binding energy of adhesion molecules is usually too small for substantial cell–cell attachment, pointing to a main deficit in our present understanding of adhesion. In this Opinion article, I integrate recent findings and conceptual advances in the field into a coherent framework for cell adhesion. I argue that active cortical tension is best viewed as an integral part of adhesion, and propose on this basis a non-arbitrary measure of adhesion strength – the tissue surface tension of cell aggregates. This concept of adhesion integrates heterogeneous molecular inputs into a single mechanical property and simplifies the analysis of attachment–detachment processes. It draws attention to the enormous variation of adhesion strengths among tissues, whose origin and function is little understood.

KEY WORDS: Cell adhesion, Adhesion strength, Cortical tension, Tissue surface tension

Introduction

For half a century, the importance of cell adhesion in morphogenesis was embodied in the differential adhesion hypothesis, which posits that differences in adhesion strength underlie cell sorting, boundary formation and tissue positioning (Steinberg, 1963). Although a masterpiece of lucid critique, the early objection of Harris (1976) that the differential contractility of cells would be a more likely cause of these phenomena was almost without practical impact. More recently, however, experimental findings (Krieg et al., 2008) and theoretical considerations (Brodland and Chen, 2000; Manning et al., 2010) rehabilitated Harris' original conjecture with a modification – instead of treating contractility as an alternative mechanism, it is described to act together with adhesion to determine the mutual attachment of cells. A further, crucial challenge to our conventional concept of adhesion came with the recognition that the binding energies of adhesion molecules are too small to account for the observed degrees of cell–cell attachment (Youssef et al., 2011; Maître et al., 2012; Amack and Manning, 2012; Stirbat et al., 2013; David et al., 2014).

So what actually is cell adhesion, and how can it be quantified? In this Opinion article, I will argue that the modulation of mechanical tension at the cell cortex is not an additional, confounding factor, but an integral, non-separable part of cell–cell adhesion. Such an extended notion of cell adhesion (Amack and Manning, 2012) bears on the concept of adhesion strength, which is notoriously difficult to define. I suggest here that the equilibrium adhesion energy per unit area, expressed for example as tissue surface tension, can be exactly defined and is a rational, non-arbitrary measure of adhesion strength. The strict definition of adhesion and adhesion strength should remove some of the confusion that presently surrounds these concepts, and at the same time stimulate further research, particularly when cell–cell adhesion is being compared between different tissues or biological processes.

Cortical tension, a sustained contraction of the cortical cytoskeleton

Isolated cells in suspension typically round up in a manner that is reminiscent of drops of liquid (Fig. 1A). There, cohesive forces between molecules act in all directions within the droplet, but inwards at the surface. This gives rise to a tension that tends to minimize the surface area. To increase the area, reversible work proportional to the added area and to the surface tension σ has to be expended; this defines σ as the surface free energy per unit area. A formally similar, yet mechanistically different tension causes the rounding of cells (Evans and Yeung, 1989).

A sustained contraction of the cortical cytoskeleton – cortical tension – has been suggested to be the main factor that generates tension at the cell surface (Evans and Yeung, 1989). Cortical tension is largely but not exclusively based on actomyosin contraction (Pasternak et al., 1989; Tinevez et al., 2009; Stewart et al., 2011) and depends on the density of the cortex, as well as on its structure and composition (Salbreux et al., 2012; Clark et al., 2014). Interaction of the cortex with the cell membrane also generates tension, for example, by regulating membrane reservoirs that are required for rapid changes in surface area (Sens and Turner, 2006).

Thus, in fundamental contrast to inanimate liquids, tension at the cell surface is maintained by the permanent expenditure of metabolic energy to support contraction of the cortex. This allows for the flexible spatio-temporal regulation of cortical tension to drive cell shape changes and motility. In fact, controlled cortex contractility is a main engine of morphogenesis that is involved in cytokinesis, blebbing locomotion of single cells, or epithelial cell rearrangement through junction remodeling (Salbreux et al., 2012; Clark et al., 2014; Sens and Turner, 2006; Lecuit and Lenne, 2007). However, the cortex control mechanism can also be used to rapidly restore the resting tension at the cell surface after surface stretching or shrinking. Keeping tension constant in this way, cell cortex behavior mimics the surface tension of a liquid, although the molecular basis is completely different.

Cortical tensions of cells have been measured by using various techniques (Box 1). At the lower end of the scale are human neutrophils with cortical tension of 0.02 mJ/m^2 (Lomakina et al., 2004), closely followed by zebrafish gastrula cells (Krieg et al., 2008). The values for cortical tension in macrophages or

Department of Cell and Systems Biology, University of Toronto, 25 Harbord Street, Toronto, Ontario M5S 3G5, Canada.

*Author for correspondence (r.winklbauer@utoronto.ca)

Journal of Cell Science (2015) 128, 3687-3693 doi:10.1242/jcs.174623

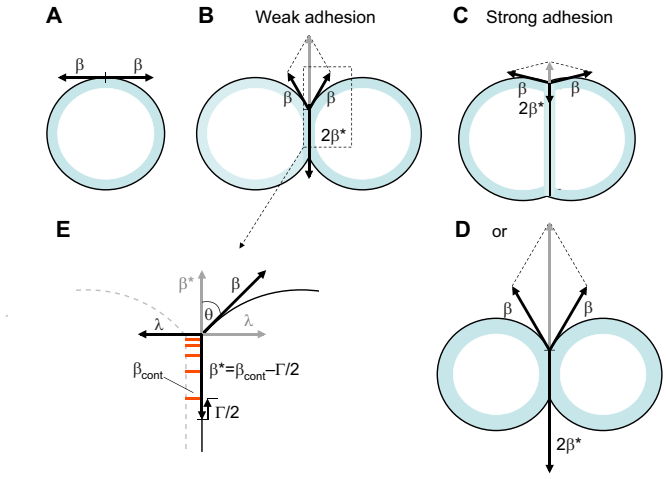

Fig. 1. Mechanical tensions in cell adhesion. Shown here is a single cell (A), and weakly adherent (B) and strongly adherent (C,D) cell pairs at force equilibrium. The cell cortex is represented as a light blue layer, which is reduced at cell–cell contacts. Strong adhesion can be due to a stronger relative reduction of cortical tension at cell contacts (C) or an increased overall cortical tension, symbolized here by an increased cortex layer thickness (D). In B–D, mechanical linkage of cells is assumed. This linkage is explicitly shown in the more detailed depiction of the equilibrium of tensions at contacts (dashed frame in B is shown in E). For a better overview, one side of the contact is shown fully; in the second cell (gray dashed line), tensions would present a mirror image of the first one. β, cortical tension at free cell surface; β*, residual tension in a cell at contact area, which equals the reduced cortical tension at contacts β_{cont} minus the adhesion tension Γ/2. Link tension λ and contact angle θ are indicated. β can be decomposed into two components (gray arrows and symbols) that are balanced by β* and λ, respectively. Red dashes symbolize adhesion molecule bonds that link the two cells.

carcinosarcoma cells are an order of magnitude higher (Lam et al., 2009; Bergert et al., 2012), and the upper end of the scale as presently known is defined by *Dictyostelium* amoeba, which show cortical tensions of up to 4.1 mJ/m^2 (Pasternak et al., 1989).

Reduction of tension at contacts between adherent cells

When two adhesive cells touch, they will spontaneously spread onto each other until an equilibrium is attained where the tension in the contact area is lower than the tension at medium-exposed cell surfaces, the latter being essentially cortical tension β (Fig. 1B–E). At the interface, two processes contribute to tension reduction. First, in adhesive cells, a potential source of surface free energy is present in the form of unligated adhesion molecules. Upon binding in trans to molecules on adjacent cells, a defined amount of binding energy is released per molecule. The total energy released per unit contact area, or adhesion tension Γ (Γ/2 per cell) (Brodland and Chen, 2000; Manning et al., 2010), increases the contact area because the more molecules that bind, the lower the remaining potential energy is. By contrast, cortical tension shrinks the contact area, and therefore any reduction of cortical tension at the area of contact, from β at the free surface to a lower value in the contact region of, say, β_{cont}, will favor attachment – it will add to the effect of the adhesion tension and further lower the residual tension β* according to $\beta^*=\beta_{cont}-\Gamma/2$ (Fig. 1E) (Brodland and Chen, 2000; Manning et al., 2010). In this way, at a given cortical tension, the contact area will be larger the more tension is reduced at the interface (Fig. 1B,C). Paradoxically, as discussed in the following section, the adhesion tension Γ is usually not large enough to provide for significant adhesion.

Box 1. Measuring cell cortical tension and tissue surface tension

Determining the cortical tension of single cells, and the tissue surface tension of cell aggregates, allows us to understand adhesion quantitatively. Cortical tension is estimated in the micropipette aspiration assay by determining the pressure required to suck a cell into a micropipette, the opening of which is smaller than the cell diameter. It is assumed that the cell interior behaves as a fluid and that its cortical tension resists the induced deviation of cell shape from a sphere (Evans and Yeung, 1989). In atomic force measurement indentation experiments, cortical tension is calculated from the apparent stiffness of the cell surface, as determined from its resistance to indentation with a small probe (Krieg et al., 2008). Tissue surface tension is usually measured using cell aggregates or tissue explants that have reached an equilibrium state. Cell positions still fluctuate randomly, but all large-scale net movements have ceased. In parallel plate compression measurements, cell aggregates that have rounded up to an approximate spherical shape under the influence of surface tension are slowly compressed to a predetermined extent between two plates. The force applied to the plates is measured. After the initial compression movement has stopped, the applied force approaches a constant equilibrium level where it is balanced by the surface-tension-dependent restoring force, which tends to round up the aggregate again. From this force and the geometry of the compressed aggregate, the tissue surface tension is calculated (Davis et al., 1997). Axisymmetric drop shape analysis can also be applied to cell aggregates to determine their tissue surface tension. If sufficiently large and heavy, aggregates that attempt to round up are deformed by gravity to take on a drop shape where, at equilibrium, gravity-induced hydrostatic pressure in the aggregate is balanced by its surface tension. From the specific weight of the tissue tested, and the aggregate size and profile, its tissue surface tension can be calculated (David et al., 2009). Measured in this way, tissue surface tension corresponds to the equilibrium adhesion energy per unit area, and therefore the adhesion strength as defined in the text.

Molecular binding energies and cell adhesion

The binding energy of cadherins, the main adhesion molecules in most cell–cell contacts, is about 5×10^{-20} J per cadherin pair (Sivasankar et al., 1999). This is generally insufficient to overcome contractile tensions and to account for observed degrees of cell attachment. For zebrafish gastrula cells, adhesion tension (i.e. Γ/2) is indeed only 2–7% of the cortical tension that these cells experience (Maître et al., 2012). Similarly, cadherin density would have to be 100-fold higher than that typically observed to account for the power output during the adhesion-driven shape changes of toroid cell aggregates (Youssef et al., 2011). In embryonic carcinoma cell aggregates, adhesion tension contributes to 30% of the reduction of tension at cell contacts; although substantial, this is still not sufficient to account quantitatively for the observed adhesion (Stirbat et al., 2013). In conclusion, although adhesion tension contributes to variable degrees to the reduction of tension at cell–cell contacts, it is rarely sufficient to mediate adhesion. Adhesion tension by itself would result in very small contact areas, with cells barely touching, similar to solid beads that are coated with purified adhesion molecules. Therefore, for any substantial adhesion to be achieved, a reduction of cortical tension at contacts is required (Fig. 1). In this sense, cell contractility is not just an additional factor that modulates adhesion, but an integral part of cell–cell adhesion.

The cortex at cell–cell contacts

Two of the main contributors to cortical tension, F-actin density and myosin activity, are often downregulated at cell contacts, for example in aggregating Madin-Darby canine kidney cells

(MDCKs) (Yamada and Nelson, 2007; Toret et al., 2014), in carcinoma cells (Hidalgo-Carcedo et al., 2011) and in zebrafish gastrula cell pairs brought into contact through micropipette manipulation (Maître et al., 2012). In *Xenopus* gastrula cells, the intensity of staining of the fluorescently labeled F-actin cortex diminishes within minutes of contact during re-aggregation (David et al., 2014). To be consistent with these observations, the high cortex density at the surface relative to its lower density at internal cell–cell borders in zebrafish gastrula explants (Krieg et al., 2008) and MDCK cell sheets (Reffay et al., 2014) should not be interpreted as an upregulation of cortex density at the tissue surface, but instead as its downregulation at internal cell contacts to promote adhesion.

Cell adhesion is, within limits, proportional to cadherin expression levels (Foty and Steinberg, 2005; Krieg et al., 2008), and in aggregating *Xenopus* gastrula cells, the reduced cortex density at cell–cell contacts is indeed inversely related to experimentally manipulated cadherin levels – decreased cadherin expression leads to lowered adhesion and increased cortex density at contacts, and increased cadherin expression has the opposite effects (David et al., 2014).

This reduction of cortex strength upon contact is, at least in part, a direct effect of cadherin signaling. Cortex tension modulation through cadherins in aggregating embryonic carcinoma F9 cells is mediated by α-catenin, a component of the core cadherin–catenin complex (Stirbat et al., 2013). A possible mechanism involves the suppression of Arp2/3-dependent actin polymerization through α-catenin at cell contacts (Drees et al., 2005). The GTPase Cdc42 is also activated in response to cadherin adhesion (Chu et al., 2004), and a pathway involving Cdc42, atypical protein kinase C (aPKC), the Par3–Par6 cell polarity complex and RhoA downregulates the actomyosin cortex at contacts between cancer cells to promote their cohesion (Hidalgo-Carcedo et al., 2011). Finally, in aggregating MDCK cells, the scaffold protein Elmo2 transiently recruits the Rac guanine nucleotide exchange factor (GEF) Dock1 to nascent cell–cell contacts. Dock1 activates the small GTPase Rac, which in turn attenuates RhoA activity at adhesion sites and promotes the dissolution of the actomyosin cortex (Toret et al., 2014).

Given the complexity of the cell cortex, with its more than 60 adaptor proteins and over 70 regulatory components (Zaidel-Bar, 2013), diminishing its bulk should not be the sole mechanism to reduce cortical tension. In fact, cases of increased F-actin density at cell–cell contacts have been reported (for example, see Chu et al., 2004), and in mouse oocytes, reduction of cortical tension at cell contacts is associated with higher F-actin density, yet an exclusion of myosin II from contacts (Chaigne et al., 2013). Different subpopulations of cortical F-actin might serve different functions during adhesion (Cavey et al., 2008; Eghiaian et al., 2015), with some roles actually requiring the upregulation of F-actin. For example, as discussed below, cadherins also function to mechanically link adjacent cells by connecting their cortices, particularly at the periphery of cell contact areas. Consequently, F-actin might be enriched in this region (Yamada and Nelson, 2007; Maître et al., 2012).

Adhesion molecules mechanically link cells

Lowering cortex tension at cell–cell contacts is not sufficient for attachment. This only leads to the bulging of a cell in the region of weakened cortical tension, such as that observed when cells are pulled apart rapidly and cortical tension at the former interface is not restored quickly enough (Maître et al., 2012). Thus, cells need to be physically linked (Maître et al., 2012). I define here the link tension λ as acting perpendicular to the contact surface at its periphery (Fig. 1E). Given its orientation, it does not affect the size of the contact area. It balances the horizontal component of cortical tension β just as the residual tension β* balances the vertical component of β. To understand its mechanics, the peeling of a cell off its substratum is instructive (Décavé et al., 2002). Here, in a zone at the detaching margin of the cell, adhesion molecules are elastically stretched by an applied force. If the force remains below a threshold, the zone is stable and peeling does not occur. This suggests that the link tension can be defined as the total elastic energy of the stretched adhesion molecules per unit area in the corresponding zone of a cell pair.

Cadherins can bear this link tension. They connect to cortical F-actin, and through binding in trans, they link the cortices of apposed cells, particularly at the periphery of contact areas. Single cadherin pairs can withstand pulling forces of 40 pN (Leckband and Prakasam, 2006; Bajpai et al., 2008); for comparison, a myosin II molecule pulls at the cadherin pair with a force of only 1 pN (Fukui, 1993). Despite the considerable force required for the rupture of cadherin bonds, however, the binding energy of cadherins of 5×10^{-20} J is small and generates only a small adhesion tension Γ, because the cadherin–cadherin bond is stretched only very little before it ruptures – i.e. the force is exerted only over a very short distance (Bajpai et al., 2008; Bell, 1978; Pierres et al., 2007). Thus, although the link tension might satisfy an intuitive notion of cell–cell adhesion, by itself it would attach cells only at minute contacts, as argued above. The combination of high rupture force and low binding energy of cadherin bonds raises the question of how the strength of an adhesion should actually be defined.

The strength of cell adhesion

When two cells attach to each other, part of their free surface is replaced by an interface whereby in each cell, the residual tension ($\beta^*=\beta_{cont}-\Gamma/2$) at the cell contact area is lower than the cortical tension β at the remaining exposed surface (Fig. 1). Thus, the overall surface free energy of the system is generally diminished as a result of adhesion. To separate the two cells again, work has to be expended to eventually restore their initial energy level. This indicates that the adhesion energy of a cell pair should be defined as the difference in total surface free energy between the non-attached and attached state. Notably, this definition does not imply a specific process of attachment or separation. Instead, two equilibrium states are compared – those of cells before and after attachment.

However, applying this concept to the actual separation of cells is complicated. Basically, cells are deformed when they are pulled apart; consequently, the residual tension β* at the interface is no longer balanced and the contact zone contracts (Fig. 2A,B). To compensate, the surface of the cell that is not in contact with the other cell is stretched, and overall, new surface area with the original surface free energy density β is generated to replace a similar area of surface with the energy density β* (Fig. 2A,B). In an idealized case, pulling is infinitely slow so that cells are always infinitely close to the force equilibrium of the non-deformed state. In this case, the required work would equal the equilibrium adhesion energy defined above – i.e. the difference in surface free energy (β−β*) multiplied by two times the initial contact area, or, equivalently, the pulling force integrated over the separation distance. After separation, this reversible work is available as free energy to drive processes such as the re-adhesion of the cells.

In actual cell separation experiments, significant cell deformation occurs, with higher separation rates requiring larger deformation. In turn, larger deformation requires a larger force to be applied and,

Journal of Cell Science (2015) 128, 3687-3693 doi:10.1242/jcs.174623

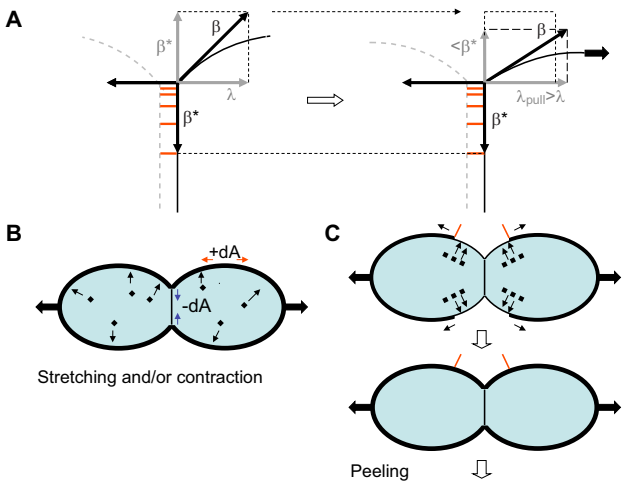

Fig. 2. Cells can be separated by pulling – contact area contraction versus peeling. (A) Tensions before the onset of pulling (left) when cell surface is spherical (see Fig. 1B) and during pulling (right), when the cell surface becomes ellipsoidal in outline (see B). Symbols are as in Fig. 1. For comparison of changes in positions, tips of arrows are connected by fine (initial positions, left) and coarse (changed positions, right) dashed lines. The magnitude of the cortical tension β remains constant. However, upon cell deformation, the upward component of β no longer balances β*, and the link tension λ increases, potentially exceeding the rupture force of the adhesion molecules (red dashes). (B,C) Two possible modes of cell separation through pulling. (B) The free surface of the cell and its cortex (bold line) is stretched to continuously add new surface area (exemplarily shown at +dA, red arrows), whereas the contact surface (thin line) shrinks (−dA, blue arrows). The small black squares illustrate that the cortex is constantly rebuilt during stretching by adding material to maintain its strength. (C) Adhesion bonds between adjacent cells fail, and cells are peeled off each other. The former contact area is exposed where tension had been reduced from β (bold cell outline) to β* (thin contour line between thin red lines). A cortex with tension β is rebuilt locally (indicated by the small squares and arrows). The continuous process has been depicted as occurring stepwise. In both modes (B and C), metabolic energy is used to restore or maintain cortical tension β.

hence, increased work of separation, implying that separation force and adhesion energy are rate dependent. Here, the additional deformation energy is not available for re-adhesion but is dissipated when cells round up after detachment. As a further complication, the mode of cell separation might change. During cell pulling, link tension λ is increased (Fig. 2A), and the bonds that mechanically couple the two cells might fail. In this case, cells will be peeled apart; instead of contraction of the contact zone, apposed cell surfaces separate and become exposed (Fig. 2C). Peeling commences when the applied force exceeds a threshold (Décavé et al., 2002; Griffin et al., 2004). Because the link tension increases with cell stretching, and hence with the rate of pulling cells apart, the onset of peeling should be rate dependent.

During peeling, adhesion molecules are stretched elastically until they eventually rupture, after which the elastic energy is dissipated (Décavé et al., 2002). The whole cell surface is also deformed. This is most strikingly observed when thin membrane tubes, or tethers, are extracted during rapid cell separation (Maître et al., 2012; Griffin et al., 2004; Hosu et al., 2007; Tabdanov et al., 2009). Overall, energy dissipation might consume the main part of the work of separation. For instance, in *Dictyostelium* cells that detach from a substratum, the irreversible work is estimated to be 100-fold in excess over that of the reversible work (Décavé et al., 2002). During peeling, the low cortex tension at the gradually exposed former contact region returns to the higher cortical tension β of its original, surface-exposed state through the expenditure of metabolic energy (Fig. 2C). Likewise, when separation occurs through contact-area constriction and the simultaneous, compensating expansion of the exposed cell surface, the cortex must constantly be rebuilt as it is stretched so as to maintain its strength (Fig. 2B). This active maintenance of contractility levels is an essential feature of cortical tension mechanics.

Taken together, the separation of cell pairs elicits an intuitive notion of adhesion strength, but the actual work required to pull cells apart is strongly context dependent and variable. This limits the interpretation of quantitative methods for the analysis of cell adhesion, which are based on cell–cell separation procedures (Kashef and Franz, 2015). However, if performed under near-equilibrium conditions, pulling cells apart would allow the measurement of adhesion energy as the difference in total surface free energy between the non-attached and the attached state of cells, thus providing a robust definition of adhesion strength. Fortunately, this equilibrium adhesion energy is related to a macroscopic and easily obtainable property of cell aggregates, tissue surface tension, the measurement of which does not require cell separation.

Tissue surface tension as a measure of adhesion strength

Cell aggregates or tissues often show liquid-like behavior and can be characterized by their surface tension (Steinberg, 1978; Graner, 1993). In liquids, the surface tension is directly linked to the attraction between the constituent elements – i.e. its cohesion. To separate a body of inanimate liquid into two parts, cohesive forces between molecules have to be overcome and two new surfaces have to be created, each with a surface free energy density (i.e. surface tension) σ. Thus cohesion – the work required for separation per unit area – equals two times the surface tension.

By analogy, to cleave a tissue into two parts, adhesion between layers of cells has to be overcome. Separating two cell layers generates two new surfaces, each with a surface free energy density β, from a previous cell–cell interface with an energy density 2β* (Fig. 3A). The difference in total surface free energy between the

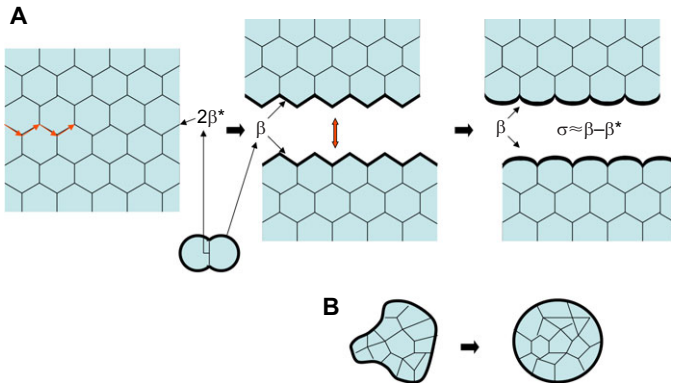

Fig.3. Origin and effect of tissue surface tension. (A) Sections through a tissue before (left) and after cleaving it into two parts (right). Tissue surface tension arises from the difference in cortical tensions between cell–cell contacts and free cell surfaces, as in cell pairs. Within the tissue, tensions at cell interfaces are 2β* (right) – i.e. β* per cell (compare Fig. 1B and E). When the tissue is separated into two fragments along a fracture line (small red arrows), the newly exposed surface assumes the free surface cortical tension β on each side of the gap (middle). See cortical tensions in cell pair for reference (bottom). Exposed cells take on a rounded surface shape (right); this only minimally changes the overall surface area. The difference between β and β* corresponds to the tissue surface tension, σ. (B) Tissue surface tension tends to minimize the surface area of cell aggregates, leading, for example, to the rounding up of tissue explants.

Journal of Cell Science (2015) 128, 3687-3693 doi:10.1242/jcs.174623

separated and the initial state is equal to the difference ($\beta-\beta^*$) multiplied by two times the initial contact area. We arrive thus at the same equilibrium adhesion energy that we used to define adhesion strength in cell pairs. Of practical interest is usually the density of this enery at the tissue or aggregate surface, and as in liquids, this corresponds to the tissue surface tension σ (Brodland and Chen, 2000; Manning et al., 2010). The tissue surface tension is not exactly equal to $\beta-\beta^*$ because cells change shape when they become exposed at the surface of the cell layer (Fig. 3); however, these surface area changes are only small, and $\sigma\approx\beta-\beta^*$ has been demonstrated to be a valid approximation (Manning et al., 2010).

The actual separation of cell layers by teasing them apart is sensitive to pulling rates and energy dissipation, in a similar manner to that discussed above for cell pair separation (Gonzalez-Rodriguez et al., 2013). However, tissue surface tension is usually determined using cell aggregates that have reached an equilibrium state where net cell movements and associated changes in cell–cell contacts have ceased (Box 1). In this way, tissue surface tension measures the equilibrium adhesion energy per unit area, and therefore the adhesion strength as defined above.

The classic differential adhesion hypothesis (Steinberg, 1963) also quantifies adhesiveness by using tissue surface tension (Foty and Steinberg, 2005), although the origin of adhesion from the interplay of adhesion molecule function and cortical tension modulation has not been explored. Nevertheless, the predictions of this theory regarding explant rounding (Fig. 3B), cell sorting and the engulfment patterns of aggregates that differ in adhesiveness still hold, except for minor deviations (Brodland and Chen, 2000; Manning et al., 2010; Stirbat et al., 2013).

Tissue surface tension measurements have generated a valuable set of data that illustrate the enormous 1000-fold variation of adhesiveness. At the lower end of the scale, a tissue surface tension of 0.05 mJ/m^2 is found for the *Xenopus* gastrula endoderm (David et al., 2014). Mesodermal and ectodermal tissues from *Xenopus*, *Rana* and zebrafish gastrulae also have low tissue surface tensions, ranging from 0.15 to 0.8 mJ/m^2 (Davis et al., 1997; Schötz et al., 2008; David et al., 2014). Tissues from chick embryo organs cover a middle range, from 1.6 mJ/m^2 for retina cell aggregates to 20 mJ/m^2 for limb bud tissue (Foty et al., 1996; Forgacs et al., 1998). It is unknown whether these higher values are a characteristic of avian tissues, or are related to organogenesis or increase in embryonic size. Even higher tissue surface tensions occur in aggregates from various cancer cell lines, which reach a staggering 56 mJ/m^2 for ependymoma cells (Hegedüs et al., 2006).

From a definition of tissue surface tension, and hence adhesion strength, as $\sigma\approx\beta-\beta^*$, it is obvious that adhesion strength can be increased in two ways. Thus, at a given cortical tension β, decreasing the residual tension at contacts, β^*, increases the tissue surface tension. As argued above, β^* is inversely proportional to cadherin density – i.e. the higher the cadherin expression level, the stronger the reduction of cortical tension at contacts. The expected correlation between cadherin expression levels and tissue surface tension has indeed been found for aggregates of L cells that had been transfected to express various amounts of cadherin (Foty and Steinberg, 2005), and for zebrafish and *Xenopus* gastrula tissue (Schötz et al., 2008; David et al., 2014).

However, the ability to increase tissue surface tension by decreasing β^* is limited – at $\beta^*=0$, tissue surface tension is maximal and corresponds to β. In other words, tissue surface tension is always smaller or, at most, equal to the free surface cortical tension β of the respective cells. To further increase adhesiveness, β itself has to be increased. It has been argued that β^* is usually about a quarter of the initial cortical tension β and that differences in surface tension between tissues are in fact mainly due to differences in β (David et al., 2014). Possibly, the same cadherin density induces the same fractional reduction of cortical tension at contacts, yet when starting from different initial levels, this generates different levels of surface tension and adhesion strength. The function of cadherin would then be a catalytic one.

Adhesion strength, a unifying framework for morphogenesis

Many morphogenetic processes involve cell attachment and detachment events, and adhesion strength must be a parameter in their tissue mechanical analysis. For example, cell rearrangement amounts to the exchange of cell neighbors, often involving a constriction of the boundary between two cells until the cells are separated (Lecuit and Lenne, 2007; Marmottant et al., 2009). Although one can focus on the underlying activity of the cytoskeleton and adhesion molecules dynamics, the process can also be viewed from an adhesion strength standpoint – regulated changes of cortical tension alter adhesion locally and temporally so as to promote cell rearrangement. This change in perspective brings into view general patterns of rearrangement processes. For example, the resistance to movement – tissue viscosity – is proportional to adhesion strength in a wide range of tissues, the surface tensions of which vary by more than 200-fold, and the velocity at which cells rearrange is surprisingly constant in these tissues (David et al., 2014).

The concept of adhesion strength also unifies the description of tissue boundary formation. The separation of two cell populations can be viewed as being based on low adhesion across the boundary, but increased actomyosin density and cytoskeletal tension at the boundary have also been implicated, for example at compartment boundaries in *Drosophila* (Dahmann et al., 2011; Fagotto, 2014). The concept of adhesion strength presents both mechanisms as two aspects of the same process – cortical tension increases at the boundary as adhesion is reduced, and vice versa.

In a sense, cortical tension acts as a common language for cells, which mechanically integrates the myriad of factors affecting adhesion. The complex composition of the cortex (Nelson, 2008; Priya and Yap, 2015), with some 170 structural and regulatory proteins engaged in multiple interactions (Zaidel-Bar, 2013), explains the notorious sensitivity of cell adhesion to cytoskeletal perturbations. For example, interfering with a selection of cytoskeletal-interacting proteins, such as phosphatidylinositol-4,5-bisphosphate 3-kinase, Pak1, myosin II, α-catenin or RhoA, in all cases, affects tissue surface tension (Stirbat et al., 2013; David et al., 2014). On the positive side, this sensitivity offers opportunities for regulatory inputs on adhesion, in addition to the expression and turnover of adhesion molecules themselves. For example, signaling cascades that originate from interactions between Eph receptors and ephrin ligands can impact on cytoskeletal effectors (Murai and Pasquale, 2003), thus modulating cell adhesion and leading, for example, to cell repulsion. In principle, however, otherwise required changes in cortex contractility could also have pleiotropic 'non-intended' side effects on adhesion. Such mechanistically neutral differences in adhesiveness could be common, given the abundance of sensitive cortex components. In a given case, it might be difficult to demonstrate that an observed adhesion difference is indeed non-functional, but this possibility should not be neglected.

Conclusions

In this Opinion article, I argue for two main propositions – first, that cell adhesion and the modulation of cortical tension at cell contacts

are aspects of the same process; and second, that the equilibrium adhesion energy, measured as tissue surface tension of cell aggregates or tissues, is a non-arbitrary measure of adhesion strength. The revision of the concept of cell adhesion that is presently taking place and that is explicated here was prompted by the recent realization that the binding energy of cadherins is not sufficient to promote substantial cell–cell attachment (Youssef et al., 2011; Maître et al., 2012; Amack and Manning, 2012; Stirbat et al., 2013; David et al., 2014). Instead, the induced downregulation of cortical tension at cell–cell contacts is essential, providing an intimate link between adhesion and cytoskeletal dynamics, and a deeper understanding of tissue surface tension.

The concept of adhesion presented here engenders several predictions, but most importantly, it helps to clarify a number of issues around cell adhesion, cell cortex contractility and morphogenesis. To start with, it can guide a more systematic characterization of adhesion molecules. Although cadherins have been shown to perform all three functions involved in adhesion (Maître and Heisenberg, 2013) – generating adhesion tension, supporting link tension and catalyzing cortical tension regulation – their relative contributions to these tasks could vary depending on the contexts. Moreover, contributions could differ for different adhesion molecules.

A dominance of one of these roles could explain the spatial arrangement of adhesion molecules, for example, their segregation into specialized structures, such as desmosomes. In these adhesive plaques, desmosomal cadherins are densely packed (Al-Amoudi et al., 2007), and rupturing a desmosome would require the breaking of many cadherin bonds simultaneously. This arrangement emphasizes the link function of adhesion molecules, and permits desmosomes to withstand high mechanical stresses. Similarly, spot-like adherens junctions at the ends of actomyosin cables integrate stress components in epithelia (Lecuit and Yap, 2015). A different specialization is exemplified by cell adhesion through carbohydrate–carbohydrate interactions. Here, the high density of binding sites in large polysaccharides at the cell surface can accumulate high binding energies and potentially generate a strong adhesion tension (Bucior and Burger, 2004; Garcia-Manyes et al., 2006), which might diminish the need for cortical tension reduction.

The adhesion mechanism predicts that in aggregates, the residual tension β^* at cell contacts is always lower than the cortical tension β at the aggregate surface. Moreover, the tissue surface tension σ of a cell aggregate, as the difference between these tensions, must be lower than the cortical tension β of the cells. For example, in the L1 cells used to induce surface tension that is proportional to cadherin expression levels (Foty and Steinberg, 2005), cortical tension would have to be in excess of 6 mJ/m^2, the maximal tissue surface tension generated in the experiments. This would be close to the maximal cortex tension measured so far – i.e. 4.1 mJ/m^2 (Pasternak et al., 1989). However, the observed tissue surface tension of 20 mJ/m^2 for chick limb bud tissue (Forgacs et al., 1998) predicts a cortical tension that is at least five times higher than the presently known maximum.

Such predictions motivate additional measurements of tissue surface and cell cortical tensions, to expand the database for analyses of adhesion strengths. It would be interesting to study how the unusually high cortical tensions, if indeed found as predicted, are generated molecularly. Likewise, one would like to know whether cadherins indeed act catalytically to reduce cortical tensions upon cell contact over a 1000-fold range of tensions, as implied by the range of measured tissue surface tensions, or whether different molecules or completely different, presently unknown, mechanisms become involved at some point. Analyzing the functional aspects of adhesion strength differences would also benefit from additional comparative data – for example, is embryonic growth associated with increased adhesiveness to support larger structures? What functional consequences does the extremely high adhesiveness of some tumor cell aggregates have, if any?

Adhesion is a main concept in morphogenesis, and the ongoing, detailed analysis of all aspects at the molecular level is essential. My discussion of general mechanical features of cell–cell adhesion and adhesion strength is intended to provide an overall framework for a quantitative analysis of adhesion mechanisms and their functions in morphogenesis and tissue homeostasis, hopefully making the daunting complexity of adhesion processes more manageable.

Acknowledgements
I thank Ashley Bruce, Rodrigo Fernandez-Gonzales, Tony Harris and Teresa Zulueta for critical reading of the manuscript, all lab members for discussions and Olivia Luu for help with the figures.

Competing interests
The author declares no competing or financial interests.

Funding
Work of the author is supported by the Canadian Institutes of Health Research [grant number MOP-53075].

References
Al-Amoudi, A., Díez, D. C., Betts, M. J. and Frangakis, A. S. (2007). The molecular architecture of cadherins in native epidermal desmosomes. *Nature* **450**, 832-837.
Amack, J. D. and Manning, L. M. (2012). Knowing the boundaries: extending the differential adhesion hypothesis in embryonic cell sorting. *Science* **338**, 212-215.
Bajpai, S., Correia, J., Feng, Y., Figueiredo, J., Sun, S. X., Longmore, G. D., Suriano, G. and Wirtz, D. (2008). α-catenin mediates initial E-cadherin-dependent cell-cell recognition and subsequent bond strengthening. *Proc. Natl. Acad. Sci. USA* **105**, 18331-18336.
Bell, G. I. (1978). Models for the specific adhesion of cells to cells. *Science* **200**, 618-627.
Bergert, M., Chandradoss, S. D., Desai, R. A. and Paluch, E. (2012). Cell mechanics control rapid transitions between blebs and lamellipodia during migration. *Proc. Natl. Acad. Sci. USA* **109**, 14434-14439.
Brodland, W. G. and Chen, H. H. (2000). The mechanics of cell sorting and envelopment. *J. Biomech.* **33**, 845-851.
Bucior, I. and Burger, M. M. (2004). Carbohydrate–carbohydrate interactions in cell recognition. *Curr. Opin. Struct. Biol.* **14**, 631-637.
Cavey, M., Rauzi, M., Lenne, P.-F. and Lecuit, T. (2008). A two-tiered mechanism for stabilization and immobilization of E-cadherin. *Nature* **453**, 751-756.
Chaigne, A., Campillo, C., Gov, N. S., Voituriez, R., Azoury, J., Umaña-Diaz, C., Almonacid, M., Queguiner, I., Nassoy, P., Sykes, C. et al. (2013). A soft cortex is essential for asymmetric spindle positioning in mouse oocytes. *Nat. Cell Biol.* **15**, 958-966.
Chu, Y.-S., Thomas, W. A., Eder, O., Pincet, F., Perez, E., Thiery, J. P. and Dufour, S. (2004). Force measurements in E-cadherin-mediated cell doublets reveal rapid adhesion strengthened by actin cytoskeleton remodeling through Rac and Cdc42. *J. Cell Biol.* **167**, 1183-1194.
Clark, A. G., Wartlick, O., Salbreux, G. and Paluch, E. K. (2014). Stresses at the cell surface during animal cell morphogenesis. *Curr. Biol.* **24**, R484-R494.
Dahmann, C., Oates, A. C. and Brand, M. (2011). Boundary formation and maintenance in tissue development. *Nat. Rev. Genet.* **12**, 43-55.
David, R., Ninomiya, H., Winklbauer, R. and Neumann, A. W. (2009). Tissue surface tension measurement by rigorous axisymmetric drop shape analysis. *Colloids Surf. B Biointerfaces* **72**, 236-240.
David, R., Luu, O., Damm, E. W., Wen, J. W. H., Nagel, M. and Winklbauer, R. (2014). Tissue cohesion and the mechanics of cell rearrangement. *Development* **141**, 3672-3682.
Davis, G. S., Phillips, H. M. and Steinberg, M. S. (1997). Germ-layer surface tensions and "tissue affinities" in Rana pipiens gastrulae: quantitative measurements. *Dev. Biol.* **192**, 630-644.
Décavé, E., Garrivier, D., Bréchet, Y., Bruckert, F. and Fourcade, B. (2002). Peeling process in living cell movement under shear flow. *Phys. Rev. Lett.* **89**, 108101.

Drees, F., Pokutta, S., Yamada, S., Nelson, W. J. and Weis, W. I. (2005). Alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly. *Cell* **123**, 903-915.
Eghiaian, F., Rigato, A. and Scheuring, S. (2015). Structural, mechanical, and dynamical variability of the actin cortex in living cells. *Biophys. J.* **108**, 1330-1340.
Evans, E. and Yeung, A. (1989). Apparent viscosity and cortical tension of blood granulocytes determined by micropipet aspiration. *Biophys. J.* **56**, 151-160.
Fagotto, F. (2014). The cellular basis of tissue separation. *Development* **141**, 3303-3318.
Forgacs, G., Foty, R. A., Shafrir, Y. and Steinberg, M. S. (1998). Viscoelastic properties of living embryonic tissues: a quantitative study. *Biophys. J.* **74**, 2227-2234.
Foty, R. A. and Steinberg, M. S. (2005). The differential adhesion hypothesis: a direct evaluation. *Dev. Biol.* **278**, 255-263.
Foty, R. A., Pfleger, C. M., Forgacs, G. and Steinberg, M. S. (1996). Surface tensions of embryonic tissues predict their mutual envelopment behavior. *Development* **122**, 1611-1620.
Fukui, Y. (1993). Toward a new concept of cell motility: cytoskeletal dynamics in amoeboid movement and cell division. *Int. Rev. Cytol.* **144**, 85-127.
Garcia-Manyes, S., Bucior, I., Ros, R., Anselmetti, D., Sanz, F., Burger, M. M. and Fernandez-Busquets, X. (2006). Proteoglycan mechanics studied by single-molecule force spectroscopy of allotypic cell adhesion glycans. *J. Biol. Chem.* **281**, 5992-5999.
Gonzalez-Rodriguez, D., Bonnemay, L., Elgeti, J., Dufour, S., Cuvelier, D. and Brochard-Wyart, F. (2013). Detachment and fracture of cellular aggregates. *Soft Matter* **9**, 2282-2290.
Graner, F. (1993). Can surface adhesion drive cell-rearrangement? Part I: Biological cell-sorting. *J. Theor. Biol.* **164**, 445-476.
Griffin, M. A., Engler, A. J., Barber, T. A., Healy, K. E., Sweeney, H. L. and Discher, D. E. (2004). Patterning, prestress, and peeling dynamics of myocytes. *Biophys. J.* **86**, 1209-1222.
Harris, A. K. (1976). Is cell sorting caused by differences in the work of intercellular adhesion? A critique of the Steinberg hypothesis. *J. Theor. Biol.* **61**, 267-285.
Hegedüs, B., Marga, F., Jakab, K., Sharpe-Timms, K. L. and Forgacs, G. (2006). The interplay of cell-cell and cell-matrix interactions in the invasive properties of brain tumors. *Biophys. J.* **91**, 2708-2716.
Hidalgo-Carcedo, C., Hooper, S., Chaudhry, S. I., Williamson, P., Harrington, K., Leitinger, B. and Sahai, E. (2011). Collective cell migration requires suppression of actomyosin at cell–cell contacts mediated by DDR1 and the cell polarity regulators Par3 and Par6. *Nat. Cell Biol.* **13**, 49-58.
Hosu, B. G., Sun, M., Marga, F., Grandbois, M. and Forgacs, G. (2007). Eukaryotic membrane tethers revisited using magnetic tweezers. *Phys. Biol.* **4**, 67-78.
Kashef, J. and Franz, C. M. (2015). Quantitative methods for analyzing cell–cell adhesion in development. *Dev. Biol.* **401**, 165-174.
Krieg, M., Arboleda-Estudillo, Y., Puech, P.-H., Käfer, J., Graner, F., Müller, D. J. and Heisenberg, C.-P. (2008). Tensile forces govern germ-layer organization in zebrafish. *Nat. Cell Biol.* **10**, 429-436.
Lam, J., Herant, M., Dembo, M. and Heinrich, V. (2009). Baseline mechanical characterization of J774 macrophages. *Biophys. J.* **96**, 248-254.
Leckband, D. and Prakasam, A. (2006). Mechanism and dynamics of cadherin adhesion. *Annu. Rev. Biomed. Eng.* **8**, 259-287.
Lecuit, T. and Lenne, P.-F. (2007). Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. *Nat. Rev. Mol. Cell Biol.* **8**, 633-644.
Lecuit, T. and Yap, A. S. (2015). E-cadherin junctions as active mechanical integrators in tissue dynamics. *Nat. Cell Biol.* **17**, 533-539.
Lomakina, E. B., Spillmann, C. M., King, M. R. and Waugh, R. E. (2004). Rheological analysis and measurement of neutrophil indentation. *Biophys. J.* **87**, 4246-4258.
Maître, J.-L. and Heisenberg, C.-P. (2013). Three functions of cadherins in cell adhesion. *Curr. Biol.* **23**, R626-R633.
Maître, J.-L., Berthoumieux, H., Krens, S. F. G., Salbreux, G., Jülicher, F., Paluch, E. and Heisenberg, C.-P. (2012). Adhesion functions in cell sorting by mechanically coupling the cortices of adhering cells. *Science* **338**, 253-256.
Manning, M. L., Foty, R. A., Steinberg, M. S. and Schoetz, E.-M. (2010). Coaction of intercellular adhesion and cortical tension specifies tissue surface tension. *Proc. Natl. Acad. Sci. USA* **107**, 12517-12522.
Marmottant, P., Mgharbel, A., Käfer, J., Audren, B., Rieu, J.-P., Vial, J.-C., van der Sanden, B., Maree, A. F.M., Graner, F. and Delanoe-Ayari, H. (2009). The role of fluctuations and stress on the effective viscosity of cell aggregates. *Proc. Natl. Acad. Sci. USA* **106**, 17271-17275.
Murai, K. K. and Pasquale, E. B. (2003). 'Eph'ective signaling: forward, reverse and crosstalk. *J. Cell Sci.* **116**, 2823-2832.
Nelson, W. J. (2008). Regulation of cell–cell adhesion by the cadherin–catenin complex. *Biochem. Soc. Trans.* **36**, 149-155.
Pasternak, C., Spudich, J. A. and Elson, E. L. (1989). Capping of surface receptors and concomitant cortical tension are generated by conventional myosin. *Nature* **341**, 549-551.
Pierres, A., Prakasam, A., Touchard, D., Benoliel, A.-M., Bongrand, P. and Leckband, D. (2007). Dissecting subsecond cadherin bound states reveals an efficient way for cells to achieve ultrafast probing of their environment. *FEBS Lett.* **581**, 1841-1846.
Priya, R. and Yap, A. S. (2015). Active tension: the role of cadherin adhesion and signaling in generating junctional contractility. *Curr. Top. Dev. Biol.* **112**, 65-102.
Reffay, M., Parrini, M. C., Cochet-Escartin, O., Ladoux, B., Buguin, A., Coscoy, S., Amblard, F., Camonis, J. and Silberzan, P. (2014). Interplay of RhoA and mechanical forces in collective cell migration driven by leader cells. *Nat. Cell Biol.* **16**, 217-223.
Salbreux, G., Charras, G. and Paluch, E. (2012). Actin cortex mechanics and cellular morphogenesis. *Trends Cell Biol.* **22**, 536-545.
Schötz, E.-M., Burdine, R. D., Jülicher, F., Steinberg, M. S., Heisenberg, C.-P. and Foty, R. A. (2008). Quantitative differences in tissue surface tension influence zebrafish germ layer positioning. *HFSP J.* **2**, 42-56.
Sens, P. and Turner, M. S. (2006). Budded membrane microdomains as tension regulators. *Phys. Rev. E* **73**, 031918.
Sivasankar, S., Brieher, W., Lavrik, N., Gumbiner, B. and Leckband, D. (1999). Direct molecular force measurements of multiple adhesive interactions between cadherin ectodomains. *Proc. Natl. Acad. Sci. USA* **96**, 11820-11824.
Steinberg, M. S. (1963). Reconstruction of tissues by dissociated cells. *Science* **141**, 401-408.
Steinberg, M. S. (1978). Specific cell ligands and the differential adhesion hypothesis: How do they fit together?. In *Specificity of Embryological Interactions* (*Receptors and Recognition, Series B, Vol.4*, pp. 97–130) (ed. D. R. Garrod). London: Chapman & Hall.
Stewart, M. P., Helenius, J., Toyoda, Y., Ramanathan, S. P., Muller, D. J. and Hyman, A. A. (2011). Hydrostatic pressure and the actomyosin cortex drive mitotic cell rounding. *Nature* **469**, 226-230.
Stirbat, T. V., Mgharbel, A., Bodennec, S., Ferri, K., Mertani, H. C., Rieu, J.-P. and Delanoë-Ayari, H. (2013). Fine tuning of tissues' viscosity and surface tension through contractility suggests a new role for α-catenin. *PLoS ONE* **8**, e52554.
Tabdanov, E., Borghi, N., Brochard-Wyart, F., Dufour, S. and Thiery, J.-P. (2009). Role of E-cadherin in membrane-cortex interaction probed by nanotube extrusion. *Biophys. J.* **96**, 2457-2465.
Tinevez, J.-Y., Schulze, U., Salbreux, G., Roensch, J., Joanny, J.-F. and Paluch, E. (2009). Role of cortical tension in bleb growth. *Proc. Natl. Acad. Sci. USA* **106**, 18581-18586.
Toret, C. P., Collins, C. and Nelson, W. J. (2014). An Elmo-Dock complex locally controls Rho GTPases and actin remodeling during cadherin-mediated adhesion. *J. Cell Biol.* **207**, 577-587.
Yamada, S. and Nelson, W. J. (2007). Localized zones of Rho and Rac activities drive initiation and expansion of epithelial cell cell adhesion. *J. Cell Biol.* **178**, 517-527.
Youssef, J., Nurse, A. K., Freund, L. B. and Morgan, J. R. (2011). Quantification of the forces driving self-assembly of three-dimensional microtissues. *Proc. Natl. Acad. Sci. USA* **108**, 6993-6998.
Zaidel-Bar, R. (2013). Cadherin adhesome at a glance. *J. Cell Sci.* **126**, 373-378.