Tissue cohesion and the mechanics of cell rearrangement

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ABSTRACT

Morphogenetic processes often involve the rapid rearrangement of cells held together by mutual adhesion. The dynamic nature of this adhesion endows tissues with liquid-like properties, such that large-scale shape changes appear as tissue flows. Generally, the resistance to flow (tissue viscosity) is expected to depend on the cohesion of a tissue (how strongly its cells adhere to each other), but the exact relationship between these parameters is not known. Here, we analyse the link between cohesion and viscosity to uncover basic mechanical principles of cell rearrangement. We show that for vertebrate and invertebrate tissues, viscosity varies in proportion to cohesion over a 200-fold range of values. We demonstrate that this proportionality is predicted by a cell-based model of tissue viscosity. To do so, we analyse cell adhesion in Xenopus embryonic tissues and determine a number of parameters, including tissue surface tension (as a measure of cohesion), cell contact fluctuation and cortical tension. In the tissues studied, the ratio of surface tension to viscosity, which has the dimension of a velocity, is 1.8 μm/min. This characteristic velocity reflects the rate of cell-cell boundary contraction during rearrangement, and sets a limit to rearrangement rates. Moreover, we propose that, in these tissues, cell movement is maximally efficient. Our approach to cell rearrangement mechanics links adhesion to the resistance of a tissue to plastic deformation, identifies the characteristic velocity of the process, and provides a basis for the comparison of tissues with mechanical properties that may vary by orders of magnitude.

KEY WORDS: Tissue surface tension, Tissue viscosity, Cell rearrangement, Cell adhesion

INTRODUCTION

Among multicellular organisms, animals are unique in their ability to perform rapid cell rearrangements based on a combination of dynamic, flexible cell adhesion and the modulation of the cytoskeleton at cell contacts. This feature is essential for many morphogenetic processes, and is extensively studied at the subcellular level during epithelial cell rearrangement. It also underlies the liquid-like behaviour of tissues (Gordon et al., 1972; Forgacs et al., 1998; Gordon et al., 2002; Mombach et al., 2005). To understand the basic mechanics of cell rearrangements in liquid-like tissues, the relationship between tissue cohesion and viscosity needs to be clarified.

As a first step in this analysis, we show that for different tissues of the Xenopus gastrula, and for other tissues from various species, these two parameters are directly proportional to each other. We then determine a number of additional cellular parameters, in part derived from an analysis of cell adhesion, and use these to show that a recent model of tissue viscosity (Marmottant et al., 2009) predicts and explains the linear relationship between tissue surface tension and viscosity. These findings have far-reaching implications for the mechanics of cell rearrangement. We argue that rearrangements are characterised by a constant rate, and that tissues are tuned for maximally efficient cell movement over a wide range of cohesiveness.

RESULTS

Relationship between tissue surface tension and tissue viscosity

We quantified cohesion and viscosity for various regions of the Xenopus gastrula. Tissue explants, after having rounded up under the influence of surface tension, deviate from a spherical equilibrium shape due to gravity. We used this effect to determine tissue surface tension $\sigma$ (Del Rio and Neumann, 1997; David et al., 2009; Luu et al., 2011) as a measure of cohesion (Box 1, Fig. 1A, Table 1; supplementary material Fig. S2, Table S1 and mathematical analyses, Section 1).

To measure tissue viscosity $\eta$, we analysed the timecourse of surface tension-driven, off-equilibrium shape changes of explants. (a list of the symbols used for the main parameters can be found in supplementary material Table S4). A commonly employed method, the fusion of two explants (Gordon et al., 1972), underestimated viscosity. Explants not only merged by flowing into each other as expected, but also extended protrusions across the space between them and zipped up (supplementary material Fig. S1 and mathematical analyses, Section 2). We therefore used the rounding of explants instead (Gordon et al., 1972) (Fig. 1B; supplementary mathematical analyses, Section 2). Observation started about 20 min after excision, when explants had taken on ellipsoidal shapes, and elastic (von Dassow and Davidson, 2009; Luu et al., 2011) and wounding (Li et al., 2013) reactions had subsided. Rounding was associated with cell rearrangement (Fig. 1C; supplementary material Movie 1) and was followed for over 3 h (supplementary material Movie 2). Surface tension was stable over this period (supplementary material Fig. S2, Movie 3), and evolving explant shapes fit well to the rigorous solution for a viscous liquid (Fig. 1D). Tissue viscosities $\eta$ were calculated from the ratio $\sigma/\eta$ obtained from the rounding rates for individual explants and the average surface tension $\sigma$ of the examined tissue, since a parallel determination of $\sigma$ for each explant was technically not feasible (Fig. 1E, Table 1; supplementary material Table S1). This slightly overestimates viscosities.

For Xenopus gastrula tissues, surface tension and viscosity are directly proportional over a 10-fold range of values (Fig. 1E). Even more strikingly, when published data from various other tissues are...
Box 1. Cell adhesion and cortical tensions

In an isolated cell, cortical tension minimises the cell surface by rounding it up into a sphere. For cells to attach to each other, cortical tension $\beta$ at the exposed surfaces of two adjacent cells has to be balanced by a reduced tension $\beta^*$ in each cell at the area of contact, as shown in the drawing. For this equilibrium, $\beta^*/\cos(\theta)$, where $\theta$ is the contact angle between the cells. Thus, without any reduction of tension at contacts, i.e. $\beta^*=\beta$, the contact angle is zero and cells touch at one point only. By contrast, complete reduction to $\beta^*=0$ gives $\theta=90^\circ$, and two cells attach maximally. These cellular tensions are linked to a tissue-level parameter—the tissue surface tension $\sigma$—by the approximation $\alpha=\beta^*/\beta$ (Brodland et al., 2009; Manning et al., 2010) (see supplementary mathematical analyses, Section 1). This allows us to calculate $\beta^*$ from measurements of $\alpha$ and $\theta$.

Cell aggregation is mediated by adhesion molecules such as cadherins. Cadherin engagement has two effects. First, release of the binding energy $\Gamma$ favours adhesion by reducing tension at contacts. Second, tension cadherin engagement has two effects. First, release of the binding energy $\Gamma$ has two effects. First, release of the binding energy $\Gamma$ favours adhesion by reducing tension at contacts. Second, tension in the cortical cytoskeleton is also reduced, to a degree that depends on $\beta^*/\beta$. In an isolated cell, cortical tension minimises the cell surface by rounding it up into a sphere. For cells to attach to each other, cortical tension $\beta$ at the exposed surfaces of two adjacent cells has to be balanced by a reduced tension $\beta^*$ in each cell at the area of contact, as shown in the drawing. For this equilibrium, $\beta^*/\cos(\theta)$, where $\theta$ is the contact angle between the cells. Thus, without any reduction of tension at contacts, i.e. $\beta^*=\beta$, the contact angle is zero and cells touch at one point only. By contrast, complete reduction to $\beta^*=0$ gives $\theta=90^\circ$, and two cells attach maximally. These cellular tensions are linked to a tissue-level parameter—the tissue surface tension $\sigma$—by the approximation $\alpha=\beta^*/\beta$ (Brodland et al., 2009; Manning et al., 2010). Importantly, for cadherins the binding energy $\Gamma$ is small compared with cortical tensions (Maître et al., 2012; Youssef et al., 2011) (supplementary mathematical analyses, Section 2), and $\beta^*$ corresponds practically to the reduced cortical tension $\beta^*$.

With binding energy $\Gamma$ being negligible, the strength of adhesion, or cohesion, is determined by the exposed surface cortical tension $\sigma$ of a cell, and by the degree of its reduction at contacts. Tensions $\beta$ and $\beta^*$, as well as surface tension $\sigma$, all have the dimension of energy/area, and separating two cells corresponds to generating new surface with surface energy $\beta$, which requires an energy per unit area of new surface of $\alpha=\beta^*/\beta$. Thus, tissue surface tension is a measure of the fact that cells adhesion energy per area or, equivalently, of tissue cohesion. The value for cohesion or adhesion energy is twice the relative surface tension value, as two surfaces, each with a surface energy corresponding to surface tension, are generated when a tissue is split into two. However, we may use these terms interchangeably when the difference is not relevant, and will colloquially refer to adhesion energy as the strength of cell adhesion.

Given the importance of tension reduction at cell contacts, we define a relative adhesiveness $\alpha$ as the reduction in tension at contacting interfaces, relative to exposed surface cortical tension, $\alpha=\beta^*/\beta$. For $\beta^*/\beta=0$ there is essentially no adhesion, and to the degree that $\Gamma$ is negligible, $\alpha=0$; if $\beta^*/\beta=1$ maximal adhesion ensues and $\alpha=1$. With $\alpha=\beta^*/\beta=\alpha(\beta,\beta^*)$, the strength of adhesion can be expressed as $\sigma=\alpha\beta$. Moreover, as $\alpha=\beta^*/\beta=1-(\beta^*/\beta)=1-\cos(\theta)$, the relative adhesiveness $\alpha$ depends only on the contact angle, and can be simply determined from its measurement.

Including in the analysis, surface tension and viscosity each vary by three orders of magnitude, and proportionality between the two parameters holds for viscosity values ranging between 2 and 200 kPa s (Fig. 1F; supplementary material Table S2). The ratio $\alpha/\eta$ has the dimension of a velocity and, in the proportional range, for tissues from cnidarians, fish, frog, chicken and mouse, this characteristic velocity $v$ is $v_c=1.8\times0.9$ nm/min. We will refer to this subclass of liquid-like tissues thus defined as $v_c$ tissues. The value of $v_c$ is reminiscent of common cell migration velocities; we will return to this observation below.

Intuitively, an increase of viscosity in proportion with cohesion might be expected. However, this is not a mechanical necessity: first, above 200 kPa s viscosity increases without an increase in surface tension (Fig. 1F); second, viscosity and surface tension can easily be decoupled (Fig. 1G). C-cadherin is essential for gastrula cell adhesion (Heasman et al., 1994), and C-cadherin morpholino antisense oligonucleotides (C-cad-MOs) (Ninomiya et al., 2012) reduced surface tension but, counterintuitively, increased viscosity. Expression of a mutant protocadherin, M-PAPC (Kim et al., 1998), or of a cytoskeletal regulator, the kinase Pak1 (Bokoch, 2003), did not change surface tension, but altered viscosity. Thus, proportionality between cohesion and viscosity is not a trivial feature, but appears to be maintained in $v_c$ tissues. To elucidate the link between these two mechanical properties, we will derive viscosity from cell-level parameters. For this purpose, we will first analyse cell adhesion, in particular its dependence on cortical tension.

**Cadherin-dependent cell adhesion**

In recent studies, an intimate link between cell adhesion and cell cortical tension became apparent (Box 1) (Brodland et al., 2009; Manning et al., 2010; Youssef et al., 2011; Maître et al., 2012). The cytoskeleton of a cell cortex is usually under tension, which causes isolated cells to take on a spherical shape. Adhesion molecules such as cadherins link cells together, but, as it turned out, the binding energy released upon cadherin engagement is insufficient to overcome the rounding effect of cortical tension. Instead, the cortical tension $\beta$ itself has to be reduced at contact sites in order for cells to spread on each other (Box 1; supplementary mathematical analyses, Section 3). This implies that the strength of adhesion is mostly determined not by the cadherin binding energy, but by the magnitude of basic cortical tension of a cell, and by how much this tension is reduced at cell-cell contacts (Box 1).

Cell cortex tensions are also related to tissue surface tension (Brodland et al., 2009; Manning et al., 2010), and we calculated cortical tension $\beta$ and the reduced tension at contacts $\beta^*$ from the surface tension $\sigma$ and the contact angle between adjacent cells (Box 1). Moreover, we defined a relative adhesiveness $\alpha$ to write surface tension as $\sigma=\alpha\beta$: surface tension, which represents tissue cohesion, is the product of a dimensionless factor $\alpha$ and the cortical tension at free cell surfaces. $\alpha$ varies between 0 and 1 and expresses the relative reduction of cortical tension at cell contacts (Box 1). Thus, two components contribute in principle to the variation of surface tension between tissues: surface tension is increased or decreased by an increase or decrease in cortical tension $\beta$ or in relative adhesiveness $\alpha$.

Relative adhesiveness $\alpha$ only depends on the contact angle between cells and is easily measured (Box 1). In normal gastrula tissues, $\alpha$ is 0.6-0.8 (Fig. 2A, Table 1), indicating a fairly constant reduction of tensions at contacts to about a quarter of exposed-surface cortical tension. As expected from $\alpha$ being constant between tissues, cortical tension $\beta$ increased with increasing surface tension (Table 1; supplementary material Table S1). Differences between the surface tensions of normal tissues were thus primarily due to differences in cortical tension. However, variation of relative adhesiveness $\alpha$ could be induced by altering cadherin expression. C-cad-MO injection diminished surface tension (supplementary material Fig. S3) by...
decreasing relative adhesiveness while cortical tension remained constant (Table 1, Fig. 2B). In endoderm, lower cadherin density, as compared with ectoderm, was also accompanied by lower relative adhesiveness (supplementary material Fig. S3; Table 1), but overall the modulation of cadherin-dependent relative adhesiveness was less important in normal tissues than changes in cortical tension.

Cortical tension is actomyosin dependent (Pasternak et al., 1989; Tinevez et al., 2009; Stewart et al., 2011) and is regulated by myosin activity (Maître et al., 2012) and the amount of F-actin at cell contacts (Krieg et al., 2008; Yamada and Nelson, 2007; Hidalgo-Carcedo et al., 2011). In ectoderm cells in the process of attachment, the actin cortex was diminished within minutes of contact (Fig. 2C). In already established cell pairs, the ratio of F-actin at contacting versus exposed surfaces decreased with increasing, cadherin-dependent adhesiveness \( \alpha \) (Fig. 2D,E). Our results suggest that cadherin reduces tension at cell contacts through a downregulation of cortex thickness or density (Clark et al., 2013), with the residual cortex being sufficient for cadherin anchoring (Maître et al., 2012). Such a catalytic role of cadherins in adhesion can be mediated by \( \alpha \)-catenin (Stirbat et al., 2013). It is the reduced tension \( \beta^* \) at contacts that becomes relevant in the following section for the calculation of tissue viscosity (see Fig. 2H).

### Viscosity as a function of tissue surface tension

Marmottant et al. (2009) modelled tissue flow by starting from the plastic flow of foams. In a foam, the liquid walls surrounding the air-filled cells minimise their surface due to liquid-air interfacial tension. A small stress leads to a small elastic deformation of the
into the equation of Marmottant et al. (2009) yielded (Box 2): Substituting these parameters measured the amplitudes and frequencies of contact area fluctuations from time-lapse recordings (Fig. 2F, Table 1; supplementary material Table S1 and mathematical analyses, Section 4). We also measured the sizes of interstitial gaps (Box 2, Table 1; supplementary material). Cell contact lengths fluctuated substantially (Fig. 2F; supplementary material Movie 4), with barriers in tissues are partly overcome by metabolically driven cell transitions, energy is dissipated and the non-reversible expansion of new walls, a process termed T1 transition (Box 2). At these transitions, energy is indeed largely determined by the cell rearrangement foam; it returns to its initial state when the stress is relieved. However, with increasing stress more and more air cells begin to rearrange by the concordant shrinkage and disappearance of existing walls and the expansion of new walls, a process termed T1 transition (Box 2). At these transitions, energy is dissipated and the non-reversible rearrangements amount to a plastic flow. The resistance to this flow is treated as occurring through T1 cell rearrangements, with the energy barrier being proportional to the tension at cell-cell contacts.

An important difference between tissues and foams is that energy barriers in tissues are partly overcome by metabolically driven cell boundary fluctuations. In explants, cell contact lengths fluctuated substantially (Fig. 2F; supplementary material Movie 4), with occasional spontaneous cell rearrangements (Fig. 2G). This effect leads to Newtonian tissue flow at sufficiently low stress and, together with the energy barrier, it determines viscosity (Marmottant et al., 2009) (Box 2). We calculated tensions at cell contacts \( \beta^* \) as described above, and contact areas from cell sizes and the sizes of interstitial gaps (Box 2, Table 1; supplementary material Fig. S4, Table S1 and mathematical analyses, Section 4). We also measured the amplitudes and frequencies of contact area fluctuations from time-lapse recordings (Fig. 2F, Table 1; supplementary material Table S1). Substituting these parameters into the equation of Marmottant et al. (2009) yielded (Box 2):

\[
\eta = \frac{1.375 \cdot d^2}{r} \cdot \frac{q}{f} \cdot \left( \frac{\beta}{\sigma} - 1 \right). \tag{1}
\]

The first term in Eqn (1) amalgamates all dimensionless factors and contains the cell radius \( r \) and the correction for interstitial gaps \( d \), linking viscosity to tissue geometry. The second term uses the relative amplitude \( q \) (Box 2) and the frequency \( f \) to describe contact area fluctuations. The third term expresses the mechanical tension \( \beta^* \) as the difference between cell cortical and tissue surface tension.

We calculated the average viscosity \( \eta \) from Eqn (1) for several gastrula tissues, and found good agreement with measured values (Table 1). We also used a calculation of error propagation to estimate how the within-tissue variation of measured parameters translates into that of viscosity, and found that calculated and measured standard deviations were similar (Table 1). The overall agreement between calculation and measurement suggested that viscosity is indeed largely determined by the cell rearrangement process. Cytoplasmic flow during cell deformations at and between T1 events could contribute to viscosity. However, *Xenopus* egg cytoplasmic viscosity is 10-30 mPa·s (Valentine et al., 2005), which is orders of magnitudes lower than measured tissue viscosities.

Eqn (1) can be used to analyse the proportionality between tissue surface tension and viscosity. For gastrula tissues, the fluctuation term is nearly constant, as the fluctuation amplitude has only a small effect on viscosity (supplementary material Fig. S5) and the fluctuation frequency is similar in all tissues (Table 1; supplementary material Table S1). Cell size differs significantly between endoderm and other tissues, but in general we expect that cell radius will vary much less than viscosity, i.e. less than 1000-fold. We therefore propose that it is the third term, the tension term of Eqn (1), that is mainly responsible for the proportionality of cohesion and viscosity in \( \eta \) tissues.

With the tension term \( \beta^* = \beta - \sigma \), viscosity is obviously not proportional to surface tension directly, but to the difference between cortical and surface tension. Therefore, a requirement for proportionality is that cortical and surface tension themselves vary

### Table 1. Tissue parameters

<table>
<thead>
<tr>
<th>Tissue</th>
<th>( \eta_{cal} = \sigma \times F \times T ) (kPa·s)</th>
<th>( \eta_{meas} ) (kPa·s)</th>
<th>s.d. calculated ( ^{a} )</th>
<th>s.d. measured</th>
<th>( \alpha = \sigma / \beta )</th>
<th>( \nu = \alpha / \eta ) (µm/min)</th>
<th>Efficiency ( k_e )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ecto</td>
<td>19.0</td>
<td>26.4</td>
<td>8.2</td>
<td>14.1</td>
<td>0.66</td>
<td>1.7</td>
<td>1.3</td>
</tr>
<tr>
<td>high</td>
<td>5.0</td>
<td>5.3</td>
<td>1.6</td>
<td>2.7</td>
<td>0.74</td>
<td>1.4</td>
<td>1.9</td>
</tr>
<tr>
<td>C-cad-MO</td>
<td>14.2</td>
<td>16.3</td>
<td>5.3</td>
<td>10.6</td>
<td>0.38</td>
<td>1.4</td>
<td>0.3</td>
</tr>
<tr>
<td>EPC</td>
<td>4.4</td>
<td>4.3</td>
<td>2.0</td>
<td>3.3</td>
<td>0.60</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>CM</td>
<td>6.2</td>
<td>6.9</td>
<td>3.7</td>
<td>4.6</td>
<td>0.75</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td>PCM</td>
<td>5.4</td>
<td>5.0</td>
<td>2.2</td>
<td>1.6</td>
<td>0.75</td>
<td>1.7</td>
<td>1.8</td>
</tr>
<tr>
<td>LEM</td>
<td>4.5</td>
<td>7.2</td>
<td>3.0</td>
<td>3.2</td>
<td>0.75</td>
<td>1.9</td>
<td>1.6</td>
</tr>
<tr>
<td>endo</td>
<td>1.8</td>
<td>1.6</td>
<td>1.3</td>
<td>0.9</td>
<td>0.56</td>
<td>2.2</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Parameters were determined for normal tissues (rows 5-8) and for normal ectoderm (rows 1, 2) and variously treated ectoderm (rows 3, 4). For further details of these and for additional measured data (s.d., sample sizes, significance levels) see supplementary material Tables S1 and S3. ecto, ectoderm (see Fig. 1A); CM, chordamesoderm; PCM, prechordal mesoderm; LEM, leading edge mesendoderm; endo, endoderm. C-cad-MO, ectoderm injected with C-cad-MO at 10 ng/embryo; EPC, ectoderm injected with EPC mRNA.

*Measured for ecto low or C-cad-MO, respectively.

*Surface tension values for mesoderm tissues pooled.

\(^{a}\)Standard deviation estimated from error propagation calculation.
proportionally, i.e. that relative adhesiveness \( \alpha = \sigma / \beta \) remains constant. This is indeed the case for normal gastrula tissues (Table 1). When, instead, cortical tension \( \beta \) is kept constant, the tension term increases with decreasing surface tension \( \sigma \). This counterintuitive relationship explains why C-cadherin knockdown reduced tissue cohesion but increased viscosity (Table 1): cortical tension at cell boundaries is less strongly reduced during adhesion (i.e. \( \beta^* \) is higher), hence cells can be separated more easily (i.e. cells are less adhesive), whereas energy barriers for cell rearrangement are higher, in proportion to the increased \( \beta^* \) (see Fig. 2H).

**Interpretation of the constant \( v_c = 1.8 \mu m/min \): flow in response to external stress**

Eqn (1) explains how proportionality between tissue surface tension and viscosity can be achieved, but not why proportionality is actually implemented in \( v_c \) tissues. It is also unclear whether the constant \( v_c \) takes on the value of 1.8 \( \mu m/min \) ‘accidentally’, or whether this reflects some basic property of cell movement in all \( v_c \) tissues. To derive the cell biological meaning of \( v_c \), we will relate tissue flow to cell-level events. We will argue that 1.8 \( \mu m/min \) is the rearrangement velocity of a cell in all tissues, and that \( v_c \) tissues are...
Box 2. Tissue viscosity

Marmottant et al. (2009) describe the viscosity $\eta$ of a tissue due to cell rearrangement as:

$$\eta = \frac{\xi \exp(\Delta x T_1/\xi)}{2 IV \xi c^2}$$

We substituted into this equation parameters that we could determine experimentally. $\Delta x_{\text{T1}}$ is the energy barrier for a T1 event (see drawing), and is given by the tension at cell-cell contacts multiplied by the contact area (Marmottant et al. 2009). As shown in Box 1, the tension at contacts is $2\sigma_{\text{T1}}$ (tensions of both cells added) and with $\sigma=\beta-\Delta\sigma$ to replace $\beta$ we obtain $2\sigma=2(\beta-\sigma)$. Regarding contact area, the surface of a cell increases about 1.1-fold when the initially spherical cell is squeezed into tight contact with neighbours to assume an approximately dodecahedral shape of the same volume. Moreover, the number of contacts per cell in a random foam of equally sized cells is 13.4 (Weaire and Rivier, 2009), and we approximated contact area per cell pair $a_{\text{ram}}$ by dividing total cell surface area by 13.4. From the radii of dissociated cells, we calculated the cell surface area of tightly packed cells, and from this we estimated the average contact area under maximal contact (no gaps) $a_{\text{ram}}=0.32a^2$. To correct for the reduction in contact area by interstitial gaps between cells, we determined gap sizes (supplementary material Fig. S4B-D and mathematical analyses, Section 4). The ratio of the diameter of the corrected contact area to the diameter corresponding to $a_{\text{ram}}$ is $dnd/d_{\text{ram}}$, and hence $d/a_{\text{ram}}=d^2$.

In tissues, cell boundary fluctuations partly overcome T1 energy barriers and are thus essential determinants of tissue viscosity (Marmottant et al. 2009). $\xi$ is the amplitude of energy fluctuations at cell contacts. In analogy to Marmottant et al. (2009) we propose that $\xi$ is proportional to contact area fluctuations by $\xi=(qa^2)/(2\beta)$, with $q$ being an average relative area change derived from the standard deviation $s$ of contact lengths $d_c$ (i.e. diameter of contact area). For small $s$, $d_{\text{ram}}=d/(2^{1/2})$, while $d_{\text{ram}}=d_{\text{ram}}=4a^2/d_d$. For larger $s$, as in chondroderm (supplementary material Table S1), $\xi$ is overestimated by about 20%, which barely affects viscosity estimates (see supplementary material Fig. S5). $c$ is the attempt frequency of fluctuation-driven rearrangement (Fig. 2F). Cell volume $V$ (in the equation of Marmottant et al.) is given by the radius $r$ of dissociated cells; the relative deformation during a rearrangement step for a hexagonal array is $d/c=0.423$.

Inserting these parameters into the above equation of Marmottant et al (2009) yields Eqn (1), which connects tissue viscosity $\eta$ to surface tension $\sigma$. Viscosity and surface tension are also linked through the ratio $\nu_{\text{s}}=\nu_{\text{c}}$, with $\nu$ having the dimension of a velocity. This characteristic velocity takes on the constant value $\nu_{\text{c}}$ for some tissues.

tuned for maximal efficiency of rearrangement, which in turn implies proportionality between surface tension and viscosity. We will first consider tissue flow in response to external stress; for example, the compression of a cell aggregate between plates or explant rounding driven by tissue surface tension.

According to Marmottant et al. (2009), the occurrence of T1 events, i.e. the fraction of cells rearranging at any time point, increases in proportion to the applied stress. We therefore model tissue flow assuming that, for normal, moderate stress levels, only a fraction of cells is moving directionally at any time, with individual cells translocating at a fixed velocity relative to their neighbours for the duration of a T1 event, and pausing between events. This rearrangement velocity $V_r$ depends on the contraction and expansion rates of cell boundaries during T1 rearrangements, and on a geometric factor that accounts for the orientation of cell boundaries relative to the applied stress (Box 3).

Contraction and expansion rates were similar in all gastrula tissues and not significantly affected by treatments such as cadherin inhibition (Table 2), although a slight tendency to increase with cell size was noted (correlation coefficient 0.186). The average rate of contraction or expansion was 2.3 µm/min. During Drosophila germ band extension, myosin II-controlled contraction rates of 2.5 and 1.6 µm/min have been measured (Rauzi et al., 2010), and, for a similarly rearranging Xenopus chondroderm cell, rates varied around 2.6 µm/min (Shindo and Wallingford, 2014). Rates are of the same order in cell contractions during micropipette aspiration-induced cell rearrangements in aggregates (Guevorkian et al., 2011).

We propose that the similarity of these rates reflects a constraint of the molecular mechanisms of cell boundary dynamics, which sets a time-scale for rearrangement. From the rate for Xenopus tissues, we estimated a rearrangement velocity $V_r$ of 1.8 µm/min (Box 3, Table 2), and expect it to be similar in other tissues.

The two characteristic velocities that we have identified, $V_r$ and $\nu_{\text{c}}$, are of the same magnitude, and we asked how they are related. We had defined $\nu=\sigma/\eta$. To express $V_r$, similarly, we used the equation for tissue movement of a Newtonian fluid, which relates the applied stress to viscosity and to the velocity gradient generated in the fluid (Box 4). The average speed of a cell layer relative to an adjacent layer, which depends on $V_r$, was used to derive the velocity gradient. Moreover, we stressed the strain as a fraction or multiple $p$ of a standard stress conveniently defined in terms of tissue surface tension (Box 4). This gave $V_r(\sigma\eta/(1/k))$, and thus $\nu=\nu_{\text{c}}V_r$, with $k$ denoting the fraction of cells moving at standard stress. In other words, the ratio $\nu$ corresponds to the rearrangement velocity of 1.8 µm/min multiplied by the fraction of cells moving under standard conditions. When $\nu=\nu_{\text{c}}=1.8$ µm/min, all cells should be moving; i.e. $k=1$; at lower $\nu$ values, the fraction of moving cells at a comparable stress is correspondingly lower, i.e. $k<1$. Apparently, $k_\alpha$ measures the efficiency of rearrangement, and $\nu_{\text{c}}$ is tuned for maximal efficiency. The constancy of $\nu$ implies a constant ratio $\sigma/\eta$ for these tissues, i.e. proportionality.

For normal gastrula tissues $\nu$ is indeed around 1.8 µm/min, and with similar values calculated for $V_r$, we obtained an average $k_\alpha$ of 1.0 (range 0.8-1.3; Table 1), suggesting maximal efficiency of movement. However, C-cadherin knockdown led to a $\nu$ of only 0.3 µm/min and hence to a $k_\alpha$ of 0.2 (Table 1). Similarly, the adhesion-defining C-cadherin construct EPAC reduced $\nu$ to 0.8 µm/min and $k_\alpha$ to 0.6 (Table 1). In such tissues, a stress considerably higher than standard stress would be required to generate the same shear rate. Notably, the stress that drives tissue flow is normally much lower than the standard stress. In the gastrula, the actual average cell velocity in tissues is 0.2-0.5 µm/min instead of 1.8 µm/min (supplementary mathematical analyses, Section 5), i.e. the fraction of cells rearranging at any time is only 0.1-0.3. Since for these tissues $k_\alpha>1$, the effective driving stress should be one-tenth to one-third of the standard stress.

To see how the efficiency factor $k_\alpha$ is determined, we derived Eqn (S1) (supplementary mathematical analyses, Section 6), which relates $k_\alpha$ to relative adhesiveness $\alpha$. It shows that for gastrula tissues to have a $k_\alpha=1$ requires that $0.6<\alpha<0.8$ (supplementary mathematical analyses, Section 6), in agreement with the measured values for relative adhesiveness. Apparently,
Box 3. Cell rearrangement velocity

In the schematic drawing, it is seen that a cell moves about one cell diameter (2r) during a T1 step of duration T. During the process, the boundary shown in red contracts and the blue boundary expands. The rearrangement velocity \( V_r \) of a cell depends on the contraction rate \( c_1 \) of a respective cell boundary during the first phase of a T1 transition and the expansion rate \( c_2 \) of the new boundary during the second phase. Both rates are very similar, and we used the harmonic mean of \( c_1 \) and \( c_2 \) to describe boundary length changes (Table 2).

To derive \( V_r \) from \( c \), we considered that the movement of a cell relative to its neighbour during a T1 event occurs maximally at velocity \( V_{\text{max}} = c \cos 30^\circ \), assuming an angle of 120° at cell edges, as shown in the drawing. With random packing, planes of cell boundaries are variably oriented relative to the direction of stress, and contribute to different degrees to velocity. To correct for this effect, we considered a tilted cell group, as shown on the righthand side of the drawing, to estimate the minimal velocity \( V_{\text{min}} \) during T1. At sixfold rotational symmetry, the orientation of the cell pair shown relative to the direction of stress maximally deviates by 30° in a plane perpendicular to the cell edge, in which case the component of \( V_{\text{max}} \) in the direction of the overall resultant movement is \( V_{\text{max}} \cos 30^\circ \). Applying this also to the remaining, perpendicular plane, the minimal velocity contributed by unfavourably oriented contacts will be \( V_{\text{min}} = V_{\text{max}} \cos 30^\circ \) and with \( V_{\text{max}} = c \cos 30^\circ \), \( V_{\text{min}} = c \cos 30^\circ \). We approximated \( V_r \) by the average of \( V_{\text{max}} \) and \( V_{\text{min}} \) to obtain \( V_r = 1.8 \mu\text{m/min} \) (Tables 1 and 2). This coarse estimate can be refined; for example, by considering that cells often elongate to varying degrees in the direction of movement. This effect should tend to increase \( V_r \). With \( k \) the fraction of cells moving via T1 transitions at a given instant, or equivalently with \( k \) the likelihood of a cell moving at that instant, the long-term average relative velocity is \( V_r = k V_r \).

only a narrow range of \( \alpha \) values is compatible with maximal efficiency of rearrangement: at low \( \alpha \), \( k_r \) is expected to be smaller than 1. In fact, C-cadherin knockdown reduced \( \alpha \) to 0.38, for which Eqn (S1) predicts a \( k_r \) of 0.3, close to the value of 0.2 that was determined independently from \( V_r \) and \( V_c \) (Table 1).

Interpretation of the constant \( \nu^2 \) flow driven by internally generated stress

An important difference between foams and tissues is that tissues can actively generate internal stress by mechanochemical processes to drive controlled cell rearrangements. To see whether the concepts derived above for external stress apply here as well, we now consider active cell intercalation, as seen for example during *Xenopus* convergent extension (Keller et al., 2008; Shindo and Wallingford, 2014) or *Drosophila* germ band extension (Bertet et al., 2004). We replace the externally applied stress by an active, regulated constriction of cell boundaries that is superimposed on the random fluctuations. The boundaries are preselected according to their proper spatial orientation by some patterning mechanism. For example, in convergent extension and germ band extension, anterior and posterior contacts of cells are determined to contract by an activin signalling gradient (Ninomiya

Table 2. Measured parameter values for boundary contraction and extension

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>( c_1 )</th>
<th>s.d. (n)</th>
<th>( c_2 )</th>
<th>s.d. (n)</th>
<th>( c )</th>
<th>( V_r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ecto (high)</td>
<td>1.97</td>
<td>1.21 (35)</td>
<td>2.44</td>
<td>1.27 (35)</td>
<td>2.18</td>
<td>1.65</td>
</tr>
<tr>
<td>ecto</td>
<td>1.76</td>
<td>1.08 (32)</td>
<td>2</td>
<td>1.29 (28)</td>
<td>1.89</td>
<td>1.44</td>
</tr>
<tr>
<td>CM</td>
<td>2.08</td>
<td>1.22 (40)</td>
<td>2.21</td>
<td>1.72 (49)</td>
<td>2.15</td>
<td>1.63</td>
</tr>
<tr>
<td>PCM</td>
<td>2.24</td>
<td>1.28 (55)</td>
<td>2.37</td>
<td>1.25 (43)</td>
<td>2.29</td>
<td>1.74</td>
</tr>
<tr>
<td>LEM</td>
<td>2.43</td>
<td>1.47 (40)</td>
<td>2.31</td>
<td>1.53 (39)</td>
<td>2.35</td>
<td>1.78</td>
</tr>
<tr>
<td>endo</td>
<td>3.01</td>
<td>1.74 (42)</td>
<td>2.93</td>
<td>1.48 (28)</td>
<td>2.95</td>
<td>2.23</td>
</tr>
<tr>
<td>Total</td>
<td>2.27</td>
<td>1.4 (244)</td>
<td>2.36</td>
<td>1.46 (222)</td>
<td>2.35</td>
<td>1.78</td>
</tr>
<tr>
<td>C-cad-MO</td>
<td>1.79</td>
<td>0.92 (21)</td>
<td>1.87</td>
<td>0.92 (22)</td>
<td>1.83</td>
<td>1.39</td>
</tr>
<tr>
<td>EPAC</td>
<td>2.02</td>
<td>1.30 (27)</td>
<td>1.80</td>
<td>0.99 (23)</td>
<td>1.90</td>
<td>1.44</td>
</tr>
</tbody>
</table>

\( c_1 \), contraction rates and \( c_2 \), expansion rates of cell-cell boundaries, calculated from the data sets used to determine frequency and amplitude of boundary fluctuations, for all boundary length changes >3 \( \mu \)m. c, harmonic mean of contraction and expansion rates; \( V_r \), relative cell velocity during T1 event; all rates in \( \mu \)m/min.; (n), number of measurements. Total: all \( c_1 \) and all \( c_2 \) measurements from untreated tissues were pooled, respectively; and \( c_1 \) \( V_r \) and \( k_r \) were calculated from the pooled data. The two-tailed t-test was used to calculate significance levels for differences between individual tissues and the pooled data. No significant differences were found for \( c_2 \); for \( c_1 \), \( P=0.047 \) for ecto and \( P=0.003 \) for endo, all other \( P \)-values are >0.224. Pooled data (total) for \( c_2 \) and \( c_1 \) are not significantly different from each other (\( P=0.512 \)). C-cad-MO rates are not significantly different from total (\( P=0.121 \) and \( P=0.122 \), respectively). For tissue type, see Table 1.
**Box 4. Tissue flow**

According to Marmottant et al. (2009), tissues behave like Newtonian fluids below a critical stress, i.e. their shear movement is described by $\tau = \eta (dV/dx)$, where $\tau$ is an applied stress and $dV/dx$ a velocity gradient (see drawing, left part). To generate in different tissues the same shear rate, or velocity gradient, the applied stress must in each case be proportional to the viscosity of the tissue, $\sim \eta$. For $\nu$, tissues in which viscosity is proportional to surface tension (i.e. $\eta = \sigma$), this stress must be proportional to tissue surface tension, that is, $\tau = \sigma$.

This consideration motivated us to express stress in terms of surface tension. Surface tension has the dimension of [energy/area] or equivalently [force/length]; to obtain the dimension of stress, i.e. [force/area], it has to be divided by a length. We defined $d\nu$ as a standard stress $\tau$, with $L$ a characteristic cell length, such as the average centre-to-centre distance between cells (see drawing). The actual applied stress can then be expressed as a fraction or multiple $p$ ($p$ being a dimensionless factor) of the standard stress. $\tau = p\nu = p\sigma L$. The long-term, averaged velocity of cells relative to neighbours, $V$, divided by length $L$, represents the shear rate of the flow, i.e. the average speed of a cell layer moving relative to an adjacent layer (see drawing). With $V = k V_r$ (Box 3), the shear rate becomes $\nu = k V_r / L$. Taken together, we relate stress to shear rate as $p\nu = k \sigma / \eta L$. At standard stress, i.e. at $p=1$, and with $k_c$ denoting $k$ under the condition of standard stress, the equation becomes $\nu = k_c V_r$ and thus $\tau = k_c \sigma V_r = \nu$. We conclude that the ratio $\nu = k_c V_r$ corresponds to the fraction of cells moving multiplied by their rearrangement velocity, and for $\nu = \sigma V_r$, it follows that $k_c = V_r / \sigma$. If $V_r = \sigma V$, then $k_c = 1$. We can interpret $k_c$ as indicating the efficiency of cell rearrangement.

If movement is driven by active, controlled cell-cell boundary constrictions instead of an external force, the dimensionless factor $p$ becomes the fraction of cells actively engaged in rearrangement at a given time point. To separate two cells, a controlled increase in tension in each cell at the contact zone equivalent to surface tension $\sigma$, i.e. from $\beta$ to $\beta^*$, is sufficient (Fig. 2H). The stress generated in this way at the cell level amounts to $p\nu L$, which corresponds to the standard stress $\tau$, as defined above. The overall stress at the tissue level is approximately proportional to this standard stress, and to the fraction $p$ of cells activated to constrict, that is, $p = k_c \sigma L$, assuming that the contacting boundaries are sufficiently well oriented to the direction of movement. Altogether, this leads to the same equation that describes rearrangement due to external stress, only with $p$ denoting now the fraction of activated cells instead of the fraction of cells prompted to move passively.

et al., 2004) or striped pair-rule gene expression (Zallen and Wieschaus, 2004) to ensure anteroposterior tissue elongation.

To separate two cells in such a controlled T1 event, an increase in cortical tension in the contact area equivalent to surface tension is sufficient: this would correspond to the cell attachment process running in reverse (Box 4, Fig. 2H). Cell boundary constriction can be generated by myosin activation (Lecuit and Lenne, 2007; Skoglund et al., 2008; Shindo and Wallingford, 2014). Alternatively, a transient suspension of the process that normally downregulates cortical density at contacts could ensure cell separation regardless of adhesion strength. Regardless, the stress generated at the cell level will correspond to the standard stress, identifying it as the average stress required per cell to drive a T1 event (Box 4).

In a simple model, the overall stress at the tissue level under these conditions is proportional to the fraction $p$ of cells actively engaged in constriction at a given instant, and the same equation as for the flow due to external stress applies (Box 4). Consequently, when $k_c \approx 1$, essentially all cells attempting a controlled boundary contraction at a given time would indeed be moving through a T1 process, whereas in tissues with low $k_c$, only a small fraction of attempts would be productive. Note that progression through T1 can be stepwise when contractions are pulsed (Skoglund et al., 2008; Shindo and Wallingford, 2014), such that short episodes of cell movement at velocity $V_r$ alternate with pauses, and the overall movement becomes smoother. Irrespective, here too $k_c$ is a measure of the efficiency of cell rearrangement. Overall, the available data suggest that $V_r$, tissues are tuned for maximal efficiency: with a $V_r$ of 1.8 µm/min for these tissues, $k_c \approx 1$ regardless of whether rearrangement is intrinsically controlled or a response to external stress. This propensity for maximal efficiency can explain the constancy of the ratio $V_r$ in many tissues.

Whether the same tissue is deformed by an external stress or by an internal stress of the same magnitude, the same viscosity should be measured in both cases. We confirmed this prediction for chordamesoderm, which lengthens by active cell rearrangement. From its rate of elongation (Ninomiya and Winklbauer, 2008) and from the driving force measured by Moore et al. (1995), we calculated a viscosity of 5.7 kPa·s (supplementary mathematical analyses, Section 7), which is close to the 6.9 kPa·s determined from surface tension-driven explant rounding. Thus, internally generated stress produces the same shear rate as if stress of the same magnitude were applied externally. As a principle, this allows the calculation from simple, external stress-driven movements, such as explant rounding, of mechanical parameters that are relevant for active cell rearrangement, for example through the determination of the characteristic velocity $\nu$.

**DISCUSSION**

We found that the ratio of tissue surface tension to viscosity is remarkably constant over a wide range of values, and identifies the $V_r$ subclass of liquid-like tissues. We further showed that tissue viscosity can be predicted quantitatively from a model of tissue flow based on foam rheology (Marmottant et al., 2009). Building on this model, we argued that cell rearrangement is characterised by a constant derived
from the contraction and expansion rates of cell boundaries during T1 events: these rates translate into the velocity by which individual cells move past each other during a rearrangement step. It is this velocity that corresponds to the ratio of surface tension to viscosity in \( v_c \) tissues. As a consequence, cell rearrangement is predicted to be maximally efficient, i.e. the maximal number of cells is moving simultaneously at a given applied stress.

This analysis implies that movement is due to cell rearrangements via T1 processes, as is indeed common in epithelia. This also seems likely for rearrangements driven by external forces. However, models of active cell intercalation in three-dimensional tissues usually employ the concept of intercellular migration (Gumbiner, 2005), in which cells crawl across each other’s surfaces with the use of locomotory protrusions. For example, this has been the predominant model for convergent extension of the Xenopus chordamesoderm (Keller et al., 2008). However, an epithelial-type T1 mechanism has been proposed recently for this process (Shindo and Wallingford, 2014), and this kind of rearrangement might be more common than previously thought. Moreover, the limiting steps of intercellular migration are probably also cell contact-changing processes, such as membrane detachment and reattachment, the efficiency of which could depend on the ratio \( v \) similarly to cell boundary contraction and expansion.

The present analysis draws attention to the striking variability in mechanical parameters both within and between tissues. Strong within-tissue variation often represents true variability and not measurement errors (von Dassow and Davidson, 2007, 2009). For some parameters, the origin of the variation is obvious. For example, contact angles between cells vary heavily due to cell boundary fluctuations, whereas the measurement error is small. Fluctuation frequency also shows strong within-tissue variability as it does in other systems, e.g. in pulsating actomyosin networks (Martin et al., 2009; David et al., 2010). The measurement of tissue surface tension by different methods (parallel plate compression versus drop shape analysis) and in tissues from different species revealed a strikingly similar variability in each case (Davis et al., 1997; Schütz et al., 2008). The stiffness of a tissue can also vary substantially, without affecting morphogenesis (von Dassow and Davidson, 2007, 2009).

While most mechanical parameters show considerable within-tissue variation, some also differ enormously between different types of tissue whereas others do not. For \( v_c \) tissues, we predict that relative adhesiveness, magnitude of cell boundary fluctuations and velocity of cells in T1 events will be nearly constant. This would keep the ratio \( v \) constant as tissue surface tension and viscosity vary widely. Moreover, constant relative adhesiveness implies a similar effect of cadherins on cortical tension reduction in different \( v_c \) tissues. Furthermore, we predict that the vast range of surface tension values is matched by a similar range of cell cortical tensions, as cortical tension must always be larger than surface tension (Brodland et al., 2009; Manning et al., 2010). A range of cortical tension values consistent with this assumption is indeed observed for \( v_c \) tissues (supplementary material Table S3). As cortical tension is related to the strength of cell-cell adhesion, its variation could be explained by requirements for high or low tissue cohesion. Notably, however, cortical tension also varies strongly among solitary cells (supplementary material Table S3).

At its highest level, viscosity increases without a corresponding increase in tissue surface tension. This could be due to high cortical tensions, which are no longer proportionally reduced at contacts but by a fixed amount that corresponds to a limiting, high value of tissue surface tension. Another possibility is that the amplitude and/or frequency of cell boundary fluctuations are decreased to raise tissue viscosity. For example, a slight reduction in the relative fluctuation amplitude \( q \) below 0.5 (supplementary material Fig. S5) would cause a sharp increase in viscosity, generating a tissue with more solid-like behaviour. Lastly, when a stiff extracellular matrix is present, the time required for its enzymatic remodelling could determine flow rates. This condition would mark the transition to tissues that behave essentially as solids and show predominantly elastic responses to mechanical stress even at long time-scales (Levental et al., 2007).

By applying concepts of fluid flow to cell rearrangement, we gained basic insights into the mechanics of cell movement in tissues. In particular, by building on the work of Manning et al. (2010) and Marmottant et al. (2009) we identified cell-level parameters that determine the mechanical properties of tissues relevant for cell rearrangement. We did not characterise the relationship between fluctuation frequency and amplitude, cell boundary contraction rate and cell size in full detail, but these parameters are clearly not independent. Furthermore, cortical tensions at exposed and contact surfaces and relative adhesiveness are connected. We propose that when choosing boundary contraction/expansion rate \( c \), frequency \( f \) and cell radius \( r \) as easily measurable variables to represent the kinetic aspect of cell rearrangement, and cortical tension \( \beta \) and relative adhesiveness \( \alpha \) to represent adhesion strength, these five variables are sufficient to express tissue-level properties, such as viscosity or surface tension, and important tissue characteristics, such as \( v \) and \( k_\alpha \).

In tissues with significant interstitial gaps a respective correction factor would have to be included.

Our analysis draws attention to numerous cell biological questions. For example, it would be interesting to see how the orders-of-magnitude differences in cortical tension between cell types are implemented at the cytoskeletal level, or, on the other hand, which cellular processes limit rates for cell boundary contraction and expansion to a narrow range. The relationship between cadherin density and the reduction of cortical tension (Maître et al., 2012), and the molecular basis of the random cell contact fluctuations, which are so important for the facilitation of cell rearrangement, are currently also not understood. Finally, the concepts developed here can guide our understanding of the large-scale design of tissues of vastly varying mechanical properties, and provide a framework for their analysis and comparison.

**MATERIALS AND METHODS**

**Embryos and explants**

*Xenopus laevis* embryos were obtained, explants were made and cultured in Modified Barth’s Solution (MBS) or dissociated into single cells in Ca\(^{2+}\)/Mg\(^{2+}\)-free MBS, as described (Winklbauer, 1990).

**Measurements of cell and tissue parameters**

Axisymmetric drop shape analysis (ADSA) (Del Rio and Neumann, 1997) was used to determine tissue surface tensions for regions of the early gastrula, as described (Luu et al., 2011). For unknown reasons, surface tension of the ectoderm, but not of the other tissues, alternated between high and low states over the course of many months (although apparently not in a seasonal rhythm). Tissue viscosity was determined by measuring the rate of rounding up of ellipsoidal gastrula explants (Gordon et al., 1972) (for details see supplementary mathematical analyses, Section 1). To view cell rearrangement, rounding explants were slightly compressed under a coverslip to keep sufficient surface area in focus at the high magnification required. As a measure of cell size, the diameter of dissociated single cells was determined. To quantitate cell contact fluctuation, contact lengths were measured at 1 min intervals in time-lapse recordings of explants filmed under indirect illumination. Contact angles between cells were measured in cell pairs or at the periphery of tissue explants (Stirbat et al., 2013) fixed and...
fraught after rounding up for 0.5-1 h. All measurements used the Carl Zeiss Axiovision program; accuracy was ±0.2° (s.d.; n=54) for angles, ±0.28 μm (s.d.; n=21) for lengths. A list of the symbols used for the parameters can be found in supplementary material Table S4.

Microscopy
For immunostaining, embryos were fixed with 2% paraformaldehyde and post-fixed with Dent’s fixative; cryostat sections were immunostained with β-catenin antibody (Santa Cruz Biotechnology, H-102; dilution 1:200). For F-actin staining, dissociated cells were seeded on BSA-coated dishes, fixed with 4% paraformaldehyde/0.1% Triton X-100 and incubated with Alexa Fluor 488 phalloidin (Invitrogen). Pixel intensity was determined using the Carl Zeiss Axiovision program (pixel size 625 nm²). Actin dynamics were viewed in LiveAct mRNA-injected cells using a Carl Zeiss LSM 510 confocal microscope. For transmission electron microscopy, gastrula explants were fixed in 2.5% glutaraldehyde/2% formaldehyde, post-fixed with Dent’s fixative and infiltrated with Spurr resin. Explants were fixed in 2.5% glutaraldehyde/2% formaldehyde, post-fixed with 1% osmium tetroxide and infiltrated with Spurr resin. Electron microscopy samples were then embedded in Spurr’s resin and cut on an Leica ultramicrotome. Sections were post-stained with uranyl acetate and lead citrate.

Microinjections
Embryos were microinjected in 4% Ficoll solution with mRNA or MOs (GeneTools) at the four-cell stage, and kept in 1/10 MBS at 15°C until the required stage. Embryos were injected with previously characterised C-cadherin mRNA or EPAC mRNA (Nimniya et al., 2012), standard control MO, C-cadherin mRNA or EPAC mRNA (Nimniya et al., 2012), and with M-PAPC mRNA (Kim et al., 1998), Pak1 mRNA or LifeAct mRNA. The experiments involving X. laevis embryos conformed to the regulatory standards of the University of Toronto.

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Author contributions
R.W. conceived the study, designed experiments and wrote the manuscript. R.W. and R.D. developed the tools for data analysis. R.W. and R.D. analysed and interpreted the data. R.D., O.L., W.E.D., J.W.H.W., M.N. and R.W. performed the experiments.

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Supplementary material
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Brodtmann et al., 2009, Cellular interface and surface tensions determined from aggregate compression tests using a finite element model. HFSP J. 3, 273-281.


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Author contributions
R.W. conceived the study, designed experiments and wrote the manuscript with contributions from R.D. and the other authors. R.D. developed the tools for data analysis. R.W. and R.D. analysed and interpreted the data. R.D., O.L., W.E.D., J.W.H.W., M.N. and R.W. performed the experiments.

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