Abstract
Deformability of epithelial tissues plays a crucial role in embryogenesis, homeostasis, wound healing, and disease. The deformability is determined by the mechanical balance between active force generation and passive response of cells. However, little is known about how multiple cells in epithelial tissues passively respond to external forces. Using a 3D vertex model, we performed computational simulations of longitudinal tension and compression tests of an epithelial tube. Under tension, the tube extended with necking as exhibiting cell rearrangements that play a role in reducing local stiffness of the tube. On the other hand, under compression, the tube buckled with kinking without cell rearrangements. The cell rearrangements occurred when apical and basal cell surfaces stored elastic deformation energies. These results illustrate the variance of deformation modes of epithelial tissues in the single cell level as well as the importance of cell rearrangements in regulating epithelial deformability.

Keywords: 3D vertex model, Multicellular dynamics, Epithelial mechanics, Cell rearrangement, Tension and compression test

1. Introduction
Epithelial tissues dynamically deform in 3D space in various processes of development, homeostasis, wound healing, and disease. These deformations are determined by the mechanical balance between active force generation and passive response of cells. Especially, example of the passive response includes roles of decreasing the stiffness of retinal tissues in epithelial invagination during optic-cup formation (Eiraku et al. 2011). However, whereas the active force generation of cells such as actomyosin contractilities has been well studied (Haigo et al. 2003; Sawyer et al. 2010), the passive mechanical response of cells are not properly understood.

Stress environments of epithelial tissues vary such as tension and compression. For example, actomyosin contractions generate tension state by generating pulling forces on the surrounding cells (Stendahl and Thomas 1980), whereas cell proliferation can generate compression state by generating pushing forces (Kondo and Hayashi 2013). These stress environments cause various 3D deformations in the cell and tissue levels, such as cell intercalations in epithelial plane during convergence–extension (Heisenberg et al. 2000) and buckling of epithelial sheets during invagination (Sweeton et al. 1991; Oda and Tsukita 2001).

It remains to be elucidated how cells passively respond to external forces, even though we know macroscopic deformation modes of epithelial tissues as a homogeneous material. The deformation of epithelial tissues includes multicellular dynamics within tissues, including changes in cell shape and configuration in 3D space. Based on inner structures of epithelial tissues, they may provide a different deformability from a continuum.
To address 3D dynamics of multiple cells, 3D vertex models are gradually gaining more attention as a robust tool due to their applicability to various phenomena. We have improved the 3D vertex model in terms of kinematic and mechanical expressions of multicellular behaviors (Okuda et al. 2013a, 2015a,b; Hashimoto et al. 2018). This model enables in-silico mechanical tests of 3D multicellular tissues at the single cell level while taking into account cell deformation, rearrangement, and their apicobasal differences.

In this study, we perform computational simulations of tension and compression tests of epithelial tissues using the 3D vertex model. To focus on general mechanical properties of epithelial tissues, for simplification, we employ a tube structure as a tissue morphology. The tension and compression tests reveal how the deformability of epithelial tissues depends on their stress environments. We also show how epithelial deformability is determined based on cellular deformations and rearrangements.

2. Cell-based modeling of epithelial dynamics

2.1. Multicellular structure and dynamics

In the 3D vertex model, an individual cell shape is represented by a polyhedron comprising the arbitrary number of polygons. Each polygon expresses the boundary face between neighboring cells, and comprises the arbitrary numbers of vertices and edges. Since all polygons are shared by neighboring polyhedrons, all vertices and edges compose a single network that expresses the entire structure of a 3D cell aggregate (Fig. 1a, b, c). In the network, as a topological constraint, each vertex is connected to exactly four edges, by which each vertex coordinate corresponds to a meeting point of exactly six boundary faces.

Dynamic changes in cell configuration are expressed by operating the topology of the network as described in our previous study (Okuda et al. 2013a). In this operation, the network topology and the numbers of vertices and edges are
Elastic property of cells is expressed as a function of the current volume of the study (Okuda et al. 2017). Specifically, elastic property of cells is expressed by the total energy, exerted on the where \( \nabla \) is the gradient in the orthogonal coordinates. The left hand side of Eq. (1) indicates a viscous friction force exerted on the \( j \)th vertex, where \( \eta_i \) is the viscous friction coefficient of the \( j \)th vertex and \( \dot{v}_{ij} \) is a local velocity around the \( j \)th vertex. The right hand side of Eq. (1) indicates an energetic force acting on the \( j \)th vertex, where \( U \) is the total energy. Elastic property of cells is expressed as a function of \( U \), and viscous property is expressed as functions of \( \eta_i \) and \( \dot{v}_{ij} \).

2.2. Viscoelastic behaviors of epithelial cells

Viscoelastic behaviors of epithelial cells are expressed by functions of \( U, \eta_i, \) and \( \dot{v}_{ij} \) as described in our previous study (Okuda et al. 2017). Specifically, elastic property of cells is expressed by the total energy, \( U \), in Eq. (1). Here, a current volume of the \( j \)th cell is represented by \( v_j \), a lateral surface area of the \( j \)th cell by \( s_{lj} \), an apical perimeter length of the \( j \)th cell by \( p_{ajl} \), and an basal surface area of the \( j \)th cell by \( s_{bj} \), respectively. As a function of these variables, \( U \) is described as follows:

\[
U = \sum_{j} \frac{1}{2} k_{v} \left( \frac{v_j}{v_{eqj}} - 1 \right)^2 + \sum_{j} \frac{1}{2} k_{a} p_{ajl}^2 + \sum_{j} k_{s} s_{lj} + \sum_{j} k_{b} s_{bj},
\]

where \( \sum_{j} \) is the summation across all cells. The first term indicates a volume elastic energy, where \( k_{v} \) and \( v_{eqj} \) are the volume elastic coefficient and reference volume of the \( j \)th cell. The second term indicates a contractile energy of the actomyosin belt along the cell circumference on the apical side (Sweeton et al. 1991; Oda and Tsukita 2001) (Fig. 1c), where \( k_{a} \) is the area energy density of individual cells. This function has been often employed to express epithelial dynamics (Farhadifar et al. 2007; Hannezo et al. 2014; Okuda et al. 2013b). The third term indicates the lateral surface energy, where constant \( k_{s} \) is the area energy density of the lateral surface. The fourth term is a basal surface energy, where constant \( k_{b} \) is the area energy density of the basal surface. Because variables \( v_{eqj}, s_{lj}, p_{ajl}, \) and \( s_{bj} \) are functions of \( r_{i} \), total energy \( U \) is a function of vertex location vectors \( r_{i} \).

Viscous property of cells is expressed using functions of \( \eta_i \) and \( \dot{v}_{ij} \) in Eq. (1) as employed in our previous study (Okuda et al. 2015c). In this model, friction \( \eta_i \) is defined as the summation across all \( \eta \) cells: \( \sum_{j} \eta_{i} = \sum_{j} \eta_{i} \), where constant \( \eta_{i} \) is a viscous friction of vertices from a cell. Velocity \( \dot{v}_{ij} \) is defined as the average velocity of the surrounding cells, where the velocity of the \( j \)th cell is defined as the average velocity of the vertices composing the \( j \)th cell. Based on this formulation, Eq. (1) satisfies the Galilean invariance and \( \eta \) reflects a viscous property of cells (Okuda et al. 2015c).

2.3. Parameter setting

By regarding a cell embedded in epithelium as a free body, an equilibrium shape of the cell is determined by the force balance according to the energy function, \( U \). By assuming an incompressibility, the cell equilibrium shape is determined by the force balance of the surface energies described by the second, third, and fourth terms of Eq. (2). In particular, the spontaneous curvature of the epithelial sheet is given by the ratio of the apical to basal surface energy densities. By assuming a frustum shape as the apical surface of the cell, the apical surface energy density can be estimated as \( 2\pi k_{a} \). Because our previous study suggested that the epithelial tube deforms qualitatively in the same manner independent on the spontaneous curvature (Okuda et al. 2017), for simplification, we assume that the spontaneous curvature of the epithelial sheet is flat so that we set the same value of effective energy density both on the apical and basal surfaces \( (2\pi k_{a} = \kappa_{b}) \). Moreover, we assume that the energy density on the lateral side is the average value of those in the apical and basal sides as \( \kappa_{a} \leq (2\pi k_{a} + \kappa_{b}) / 2 \). From these assumptions, we obtain the relationship: \( 2\pi k_{a} = \kappa_{a} + \kappa_{b} = \kappa_{a} \).

To solve Eq. (1), parameter values were normalized by unit length \( (v_{ref})^{\frac{1}{3}} \), unit energy \( 10\kappa_{a} (v_{ref})^{\frac{1}{3}} \), and unit time \( 0.4\kappa_{a} / \kappa_{1} \). Here, factors 10 and 0.4 are just for simplification. Hereafter, all parameters are described as dimensionless quantities.

Based on these formulations, there are two variables of physical parameters: \( k_{v} \) and \( T \). By introducing a characteristic surface area of a cell as \( (v_{ref})^{\frac{1}{3}} \), a characteristic surface energy of a cell, represented by \( \kappa_{1} (v_{ref})^{\frac{1}{3}} \), is estimated to be 0.1. To assume an incompressibility of individual cell volumes, \( k_{v} \) should be much larger than \( \kappa_{1} (v_{ref})^{\frac{1}{3}} \). Hence, \( k_{v} \) is set to be 20. To analyze deformation characteristics, variable \( T \) is varied. Details of numerical implementations and simulation procedure are described in Appendix.
2.4. Simulation condition

The tissue structure is set to be a cylindrical tube of a monolayer cell sheet, aligned along the z-axis on the x-y-z orthogonal coordinates (Fig. 1a). This tube is composed of a total number of 1,000 cells, where about 70 cells are aligned along the z-axis and about 14 cells are aligned along the perimeter. Reference cell volume \(v_{eq}\) was randomly set to satisfy that their average is \(v_{ref}\). The inside of the tube is defined as the apical surface (Fig. 1b, c), on which the apical contractility, described by the second term of Eq. (2), is exerted.

As a boundary condition, an external tensile force, represented by \(T\), is exerted on the ends of the tube along the z-axis (Fig. 1a). About 100 cells located at each end are selected as the loaded region (colored by dark gray in Fig. 1a, b), over which \(T\) is equally distributed. Additionally, the center of the individual loaded regions are fixed on the z axis (Fig. 1a).

Computational simulations were performed according to the following processes: First, we gave a set of physical parameter values such as \(k_b\) and \(v_{eq}\). Second, we calculated the relaxation process of tissue deformations under unloaded condition, and obtained an initial condition under equilibrium. Third, we performed tension and compression tests by varying \(T\).

3. Results

3.1. Epithelial tube exhibits cell rearrangements under tension and tissue buckling under compression

To investigate mechanical characteristics of epithelial tissues, we performed the tension and compression tests of an epithelial tube, and varied the external force, \(T\). As a result, epithelial dynamics drastically differed with \(T\). Under the condition with the high tension (\(T = 2.0\)), the tube extended with necking and broke away at the narrow part of the neck (Fig. 2a), whose process was accompanied by frequent cell rearrangements (Fig. 2b, c). On the other hand, under the condition with the high compression (\(T = -0.6\)), the tube kinked to buckle without cell rearrangements (Fig. 2d).

To quantify these behaviors, we measured the axial length strain of the tube, as shown in Fig. 3a. Here, the strain is defined as \(L(t)/L(0) - 1\), where \(L(t)\) is the axial length between the centers of the loaded regions at the time \(t\), as shown in Fig. 1a. The strain converged to a constant under the condition with the low absolute value of the external force \((\leq 0.05)\), and diverged to break its structure under the condition with the high absolute value of the external force \((T \leq -0.06\) and \(2.0 \leq T\)). Additionally, the number of cell rearrangements has been measured within the range where the tube strains converged to constants \((-0.05 \leq T \leq 1.8\)) as shown in Fig. 3b. Interestingly, the number of cell rearrangements increased with the absolute value of the external force under tension but not under compression. Therefore, the epithelial tube extended with cell rearrangements under tension and buckled without cell rearrangements under compression.

To evaluate the tissue dynamics resulting from the simulations, we discuss the values of \(T\) employed in the simulations. For instance, endothelial cells whose diameter varies between 8 and 12 \(\mu\)m behave as a solid sphere with cortical tension of about 2,200 pN/\(\mu\)m (Hochmuth 2007). In this case, the unit values of length and force are estimated to be about 8 \(\mu\)m and 200 nN. Therefore, the maximum external force \((T = 2.0)\) in this model can be estimated as about 400 nN. On the other hand, it has been reported that Madin-Darby canine kidney (MDCK) epithelial cells generate about 100 nN of traction force (Maruthamuthu et al. 2011). Hence, the values of \(T\) employed in the simulations are in the similar order to those measured in experiments, and thereby certainly include plausible values for living tissues.

3.2. Cell rearrangements reduce epithelial stiffness under tension but not under compression

To reveal the role of cell rearrangements in epithelial tube deformations, we fixed cell configurations by removing the operators described in Fig. 1d, and compared the results between the conditions with and without cell rearrangements (Fig. 4a, b). Under the high tension condition \((T = 2.0)\), while the tube with cell rearrangements formed the neck to be broken away, those without cell rearrangements did not (Fig. 4a). Moreover, the axial strain of the tube with cell rearrangements was lower than those without cell rearrangements under the low tension condition \((1.6 \leq T \leq 1.8)\), but it is not the case under compression (Fig. 4b). Thus, cell rearrangements reduced the effective stiffness of epithelial tissues under tension, whereas they did not affect not under compression.

3.3. Apical and basal surfaces store mechanical energies under tension but not under compression

What causes the difference in the deformation modes in the single cell level? To qualify this question, we measured the mechanical energies of apical and basal surfaces as shown in Fig. 5. Apical and basal surfaces stored energies under tension but not under compression. The apical cell surfaces lined by the actomyosin belt stored energies more than the
Fig. 2 Dynamics of the epithelial tube under tension and compression. a, b, c Tissue and cellular dynamics of the epithelial tube under the high external tension ($T = 2.0$). Some of cells within the epithelial tube are shown in b, where individual cells are marked by different colors. The topology of cell-cell contacts among cells A, B, and C in b are shown in c, where cells A and C detached from each other. d Tissue dynamics of the epithelial tube under the high external compression ($T = -0.06$). In a and d, cell surfaces in the test regions are colored by local mean curvatures to emphasize the tissue morphologies. In a, the triangle indicates a neck structure, at which the tube broke away. In d, the triangle indicates a kink structure, at which the tube buckled.

basal cell surfaces, and the cell strain under tension is much larger than those under compression. Thus, external forces were exerted on individual cell surfaces under tension, but not under compression.

4. Discussion

The significant difference between an epithelial tissue and a simple elastic body is whether there are the changes in their inner structures, i.e., cell rearrangements, which generates the fluidity of cells within a tissue and causes plastic
Fig. 3  Tissue strain and cell rearrangements of the epithelial tube.  
a Axial length strain of the tube, $\gamma_l$, as a function of time, $t$.  
b Frequency of cell rearrangements, $n_r$, as a function of the bias of the external force, $T$, within the range where the tube strains converged to constants ($-0.05 \leq T \leq 1.8$).

Fig. 4  Comparison of epithelial tube dynamics under the conditions with and without cell rearrangements.  
a Axial length strain of the tube, $\gamma_l$, as a function of time, $t$.  
b Axial length strain of the tube, $\gamma_l$, as a function of the bias of the external force, $T$, within the range where the tube strains converged to constants ($-0.05 \leq T \leq 1.8$ in the case with cell rearrangements and $-0.05 \leq T \leq 2.0$ in the case without cell rearrangements).

Fig. 5  Average of surface energy on the apical and basal surfaces, $\langle \kappa_a p_a^2 \rangle$ and $\langle \kappa_b p_b \rangle$, as a function of external force, $T$, within the range where the tube strains converged to constants ($-0.05 \leq T \leq 1.8$ in the case of allowing cell rearrangements and $-0.05 \leq T \leq 2.0$ in the case of fixing cell configurations).
deformations. The regulation of cell rearrangements plays a crucial role in forming and maintaining tissue and organ structures. However, while deformations of elastic tubes have been well studied (Karamanos 2002), little is known about those of epithelial tubes. Therefore, understanding mechanics of epithelial tubes requires mechanical analyses in the single cell level.

This study revealed that an epithelial tube provides the different deformation modes in the single cell level under tension and compression; while the tube extended with frequent cell rearrangements under tension, it buckled without cell rearrangements under compression (Fig. 2, 3). Both extension under tension as well as buckling with kinking under compression can be also observed in a simple elastic tube. The drastic difference from the elastic body to the multicellular body is cell rearrangements that effectively reduced the tissue stiffness to cause necking under tension (Fig. 4). The frequency of cell rearrangements depended on whether external forces are exerted on individual cell surfaces or not (Fig. 5). Therefore, based on the interaction between the entire tissue deformation and local cell rearrangements, cell rearrangements provide the variance of deformation modes of epithelial tissues in the single cell level.

Cell rearrangements reducing the stiffness of epithelial tissues occurred under tension but not under compression; while external tension force balanced with the elastic force by the extension of individual cell surfaces, external compressive force balanced with the elastic force by the buckling of the entire tube. This analogy may be generally applicable for thin epithelial tissues but not for other thick tissues. This is because it is relatively hard for thick tissues to exhibit buckling. In thick tissues, cell rearrangements may also reduce the stiffness of tissues under compression as well as under tension. This predicted property can be experimentally tested using the deformable elastic cell culture chamber combined with a uniaxial stretch device (Tamura et al. 2007). Thus, this study provides the new insight into epithelial dynamics and suggests the testable experiment.

The frequency of cell rearrangements can be regulated by contractile structures on cell surfaces, as reported in our previous study (Okuda et al. 2017); the circumferential actin belt lining adherens junctions tends to suppress cell rearrangements more than the actin mesh beneath the apical membrane. Therefore, in biological systems, the stiffness of epithelial tissues may be regulated by subcellular contractile structures via cell rearrangements.

While this study simplified the stress condition to be a longitudinal tension and compression, biological tissues are exposed under various stress environments such as in 3D twisting forces. Moreover, cells involve various mechanical behaviors such as viscoelastic property, cell division, and cell apoptosis, which may also contribute to their macroscopic mechanical characteristics. These cell behaviors can be taken into account in the 3D vertex model (Okuda et al. 2013c, 2016). Such stress environments and cell behaviors are challenging topics as the future work.

5. Conclusion

Epithelial mechanics plays a crucial role in various multicellular phenomena through the passive deformation in response to external forces. This study suggested that cell rearrangements provide the variance of deformation modes of epithelial tissues in the single cell level. Particularly under tension, cell rearrangements, reducing tissue stiffness, play a key role in determining a deformation mode of epithelial tubes. Further studies will reveal how the regulation of mechanical characteristics via cell rearrangements contributes to forming and maintaining tissues and organ structures.

Appendix: Numerical implementation and simulation procedure

The time integration of Eq. (1) was numerically performed using the Euler method with time step of $\Delta t_v$. Vertex velocities in Eq. (1) were solved by convergent calculations using the iterative method, in which the mean residual error is to be under the threshold value, $R_{th}$ (Okuda et al. 2015c). According to the reversible network reconnection model (Okuda et al. 2013a), local network patterns were reconnected when each edge included in a local pattern became shorter than a threshold value, $\Delta l_{th}$. Trials for applying the reconnection rule were conducted for each edge and each trigonal face at each time interval of $\Delta t_r$. Numerical parameters are shown in Table 1.

Based on the assumptions in Sect. 2.3, the number of physical parameters is narrowed down to only $T$. Hence, the epithelial tube is brought into equilibrium before the tests by calculating dynamics under the condition with $T = 0$.

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Table 1 Numerical parameters

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<th>Symbol</th>
<th>Value</th>
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<td>$\Delta t$</td>
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<td>$R_{th}$</td>
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References


Okuda, S., Inoue, Y., Eiraku, M., Sasai, Y., and Adachi, T., Apical contractility in growing epithelium supports robust...


