Journal of Biomechanics 46 (2013) 1705–1713

Contents lists available at SciVerse ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Apical contractility in growing epithelium supports robust maintenance of smooth curvatures against cell-division-induced mechanical disturbance

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ARTICLE INFO

Article history:
Accepted 31 March 2013

Keywords:
Tissue morphogenesis
Multicellular dynamics
Three-dimensional vertex model
Reversible network reconnection model
Developmental biomechanics

ABSTRACT

In general, a rapidly growing epithelial sheet during tissue morphogenesis shows a smooth and continuous curvature on both inner cavity (apical) and basement membrane (basal) sides. For instance, epithelia of the neural tube and optic vesicle in the early embryo maintain continuous curvatures in their local domains, even during their rapid growth. However, given that cell divisions, which substantially perturb the local force balance, frequently and successively occur in an uncoordinated manner, it is not self-evident to explain how the tissue keeps a continuous curvature at large. In the majority of developing embryonic epithelia with smooth surfaces, their curvatures are apically concave, because of the presence of strong tangential contractile force on the apical side. In this numerical study, we demonstrate that tangential contractile forces on the apical surface play a critical role in the maintenance of smooth curvatures in the epithelium and reduce irregular undulations caused by uncoordinated generation of local pushing force. Using a reversible network reconnection (RNR) model, which we previously developed to make numerical analyses highly reproducible even under rapid tissue-growth conditions, we performed simulations for morphodynamics to examine the effect of apical contractile forces on the continuity of curvatures. Interestingly, the presence of apical contractile forces suppressed irregular undulations not only on the apical side but also on the basal surface. These results indicate that cellular contractile forces on the apical surface control not only the shape at a single cell level but also at a tissue level as a result of emergent mechanical coordination.

1. Introduction

During development of multicellular organisms, tissues deform into complex organ shapes in a three-dimensional (3D) manner. The complex shaping is achieved by the balance of mechanical forces, which are generated by cell activities, such as cell contraction and cell proliferation. However, how these events at the cell level eventually lead to the structure formation at the tissue level remains elusive in most cases of organ development.

An epithelial sheet is a basic structure that is frequently seen in many aspects of organogenesis, and commonly has smooth surface curvatures on both apical and basal sides. Such smooth curvatures can be observed in, for instance, the neural tube and optic vesicle in the early embryo, which give rise to the brain and retina, respectively (Nishimura et al., 2012; Weaver and Hogan, 2001; Zolessi and Arruti, 2001; Eiraku et al., 2011, 2012). However, it is not simple to explain how these smooth surface curvatures are maintained in these rapidly growing tissues, because the force balance among cells is locally changed by cell divisions, which successively occur with spatiotemporally random timing. Our previous numerical investigation suggested that cell proliferation tends to induce irregular undulation of sheet-shaped tissue (Okuda et al., in press-a, in press-b). To maintain the smooth epithelial curvatures during successive rounds of cell proliferation, the configurations of proliferating cells need to be constrained in the plane of the epithelial sheet.

The smooth epithelia in such embryonic structures as the neural tube and optic vesicles are apically concave. One common feature found in the apical end of these epithelia is that actomyosin is homogeneously and densely accumulated as a circumferential belt in each cell (Nishimura et al., 2012; Weaver and Hogan, 2001; Zolessi and Arruti, 2001; Eiraku et al., 2011, 2012). This circumferential actomyosin belt is known to generate tangential contractile force on the apical surface at the cell level, and

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http://dx.doi.org/10.1016/j.jbiomech.2013.03.035

contributes to deformation in cell shape and rearrangement in geometrical patterns (Lecuit and Lenne, 2007; Lecuit et al., 2010). This implies that the contractile force may affect 3D tissue shapes and configurations, e.g., the curvatures of epithelial sheets. Consistent with this idea, previous Drosophila studies have shown that local control of actomyosin causes a change in tissue curvature via the apical constriction during mesoderm invagination (Martin et al., 2010). A similar observations were reported in the vertebrate studies of eye cup formation (Weaver and Hogan, 2001; Zolessi and Arruti, 2001; Eiraku et al., 2011, 2012) and neural tube formation (Nishimura et al., 2012).

On the basis of these reports, we became interested in the idea that tangential contractile force on the apical surface maintains smooth curvatures of epithelium by preventing it from undulating irregularly during tissue growth. In rapidly growing epithelia, substantial mechanical disturbances are given internally by irregular pushing forces locally induced by cell divisions. The question is whether the contractile force along the apical actin belt at the cell level is sufficient to overcome these uncoordinated perturbations for smoothening the epithelial structure at the tissue level.

To analyze multicellular dynamics during the 3D morphogenesis, 3D vertex models have been proposed (Honda et al., 2004). A conventional 3D vertex model has been successfully used for simulating tissue morphogenesis, such as the polarization process in the preimplantation mouse embryo (Honda et al., 2008) and tissue extensions driven by cellular intercalations (Honda et al., 2008). However, the conventional 3D vertex model has a limitation for simulating complex deformations because of problems in the reconnection of vertices. To resolve them, we have developed a reversible network reconnection (RNR) model (Okuda et al., in press-a), and successfully applied it to the simulation study of complex deformations induced by successive rounds of cell proliferation (Okuda et al., in press-b). Thus, the RNR model provides a useful tool for analyzing morphogenesis of epithelium with high cell proliferation.

In this study, we use the RNR model to evaluate the contribution of the contractile force, generated by circumferential actomyosin belt, to the maintenance of smooth curvatures of epithelium. By simulating the morphodynamics of epithelial sheets with cell proliferation with the RNR model, we demonstrate that the presence of apical contractile force is critical for preventing the epithelium from undulating irregularly during tissue growth.

2. Reversible network reconnection simulation

2.1. Modeling multicellular dynamics

Multicellular dynamics during tissue morphogenesis was mathematically expressed using the RNR model (Okuda et al., in press-a). In the RNR model, the shape of a cell is represented by a polyhedron. This polyhedron includes vertices and edges that are shared by neighboring polyhedrons. These vertices and edges comprise a network that represents the entire shape of the aggregate. In this network, each vertex is connected to four edges. Moreover, neighboring polyhedrons are compartmentalized by these polygonal faces. The shape of a polygonal face that has four or more edges is defined as radially arranged triangles composed of each edge and the center point of the polygonal face (Okuda et al., in press-a).

To express the multicellular dynamics within aggregates, an equation for the motion of the ith vertex was introduced as follows:

$$\eta \frac{d\boldsymbol{r}_i}{dt} = -\frac{\partial U}{\partial \boldsymbol{r}_i}. \quad (1)$$

The left-hand side of Eq. (1) is a frictional force that is exerted on the ith vertex, where η is a friction coefficient and $\boldsymbol{r}_i$ is the position vector of the ith vertex. The right-hand side of Eq. (1) is a conservative force, where U represents the potential energy of cells. In addition, cell rearrangements within an aggregate are expressed by reconnecting local network patterns (Okuda et al., in press-a). In this network reconnection, because cells are strongly connected by tight junctions located near their apical surfaces (Tsukita et al., 2001), it is assumed that each cell cannot lose its apical surface.

Cell proliferation was expressed using a cell proliferation model (Okuda et al., in press-b), in which cell proliferation was characterized both by cell division (increase in cell number) and cell growth (increase in cell volume) (Fig. 1a). In addition, cell division was represented by dividing a single polyhedron at a plane. Direction of the dividing plane is locally regulated to the longest axis of cell shape in the plane of epithelial sheet normal to the apicobasal direction (Fig. 1b). Details of the cell division manner are described in Supplementary information S1, which are similar to those of the local regulation used in our previous study (Okuda et al., in press-b).

Cell mechanical properties and reference states were simply expressed using the potential energy, U, (Fig. 1c) as follows:

$$U = \sum_i^{\text{cell}} (u_i^{\text{cv}} + u_i^{\text{cs}} + u_i^{\text{ch}} + u_i^{\text{ac}}), \quad (2)$$

where $\sum_i^{\text{cell}}$ indicates the summation for all cells. Here u_i^{cv} is the cell volume elastic energy, u_i^{cs} is the cell surface elastic energy, u_i^{ch} is the cell height elastic energy, and u_i^{ac} is the apical contractile energy. Functions of these potential energies are described in Supplementary information S2.

2.2. Simulation conditions

To investigate the effects of apical contractility on the continuity of curvatures in growing epithelia, we simulated the morphodynamics of tissues consisting of proliferating cells. To solve Eq. (1), parameter values were normalized by unit length (l), unit time (τ), and unit energy (k_BT). Here l and τ were set at $l = (v_0)^{1/3}$ and $\tau = \tau_{\text{ave}}^{\text{cellcycle}}$.

As an initial condition, we chose a spherical shell configuration, in which proliferating cells were located in a monolayer sheet. This is because a spherical shell with a homogeneous curvature is appropriate for analyzing mechanical effects of cell behavior on a tissue curvature. In addition, such spherical shell configurations can be observed in cavitation processes of stem cell aggregates, and hemispherical shell configurations can be observed in processes of regeneration of an optic-cup (Eiraku et al., 2011, 2012). The number of proliferating cells under this initial condition was set to $n_0^c = 258$. An apical surface was inside the spherical shell. Each cell time, t_i^c, for the initial condition was randomly determined.

The standard deviation of cell division times, $\tau_{\text{sd}}^{\text{cellcycle}}$, was set at $0.1\tau_{\text{ave}}^{\text{cellcycle}}$. Numerical integration of Eq. (1) over time used the Euler method with a time step of Δt. Local network patterns were reconnected when each edge included in a local pattern became shorter than a threshold value, Δl_{th}. Trials for applying the reconnection rule were conducted for each edge, and each trigonal face at each of the $\Delta t_r/\Delta t$ steps ($\Delta t_r \geq \Delta t$). All model parameters are shown in Table 1.

3. Results

3.1. Apical contractility supports to maintain smooth curvatures of epithelial sheet

To investigate the effects of apical contractility on the maintenance of the smooth epithelial curvatures, we simulated the