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Mechanics of epithelial tissue formation

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ABSTRACT

A key process in the life of any multicellular organism is its development from a single egg into a full grown adult. The first step in this process often consists of forming a tissue layer out of randomly placed cells on the surface of the egg. We present a model for generating such a tissue, based on mechanical interactions between the cells, and find that the resulting cellular pattern corresponds to the Voronoi tessellation of the nuclei of the cells. Experimentally, we obtain the same result in both fruit flies and flour beetles, with a distribution of cell shapes that matches that of the model, without any adjustable parameters. Finally, we show that this pattern is broken when the cells grow at different rates.

1. Introduction

Multicellular organisms start life as a single fertilized cell. From this modest beginning, they undergo a developmental process that leads to the formation of complex tissues and organs with a wide range of different functions. Although it has long been appreciated that these various components of an organism have very different mechanical properties, the role of mechanical interactions in the developmental process has only become the focus of detailed studies relatively recently. One of the earliest milestones in this field is the seminal work by Discher et al. (2005) and Engler et al. (2006), who showed that identical stem cells, when placed on substrates of different stiffness, differentiate into cells of tissues with the corresponding stiffness. Cells in living multicellular organisms, however, do not exist on a substrate in isolation; instead, they are part of a tissue that consists of both cells and extracellular material and together form a mechanical system (Kasza et al., 2007). Moreover, cells react strongly to both direct mechanical interactions with their neighbors (Gibson et al., 2006; Idema et al., 2013; Kaiser et al., 2018; Schwarz and Safran, 2013; Shawky and Davidson, 2015; Vogel and Sheetz, 2006) and indirect interactions via deformations of a shared substrate (Majkut et al., 2013; Nitsan et al., 2016; Tang et al., 2011). Finally, the interior organization of the cell, in particular the position of the nucleus, is also mechanically coupled to its outside environment (Zemel, 2015). To understand how epithelial tissues develop, we thus need a mechanical model coupling the inside to the outside of the cell.

As a model epithelial tissue, we study the first tissue developed in insect embryos, the epithelial blastoderm. This tissue forms as a single layer on top of the yolk. The nuclei of the fertilized egg first divide a couple of times in the egg's interior, then migrate to the surface where they continue to divide, eventually creating a confluent proto-tissue. This proto-tissue is turned into a proper tissue through invagination of the egg's outer (plasma) membrane, which separates the nuclei into cells (cellularization) (Anderson, 1972; Foe and Alberts, 1983; Handel et al., 2000; Harris et al., 2009; Lecuit, 2004; Mazumdar and Mazumdar, 2002; van der Zee et al., 2015). Already during the syncytial stage (i.e., before cellularization), each nucleus is embedded in a full cellular apparatus, including organelles and a cytoskeleton. We present a model for the formation of the epithelial blastoderm. We also study this tissue formation directly in two model organisms: the fruit fly *Drosophila melanogaster* and the flour beetle *Tribolium castaneum*. We find that the touching boundaries of the (proto)cells correspond closely to a Voronoi tessellation of their nuclei, an effect that becomes more pronounced after cellularization. Although Voronoi tessellations have occasionally been used to describe cellular patterns in epithelial tissues (Bock et al., 2010; Honda, 1978; Kim et al., 2016; Sanchez-Gutierrez et al., 2016; Sharma et al., 2009; Sulsky et al., 1984; Weliky and Oster, 1990), to the best of our knowledge, the fact that the nuclei are located at the centers of the corresponding Voronoi cells has not been shown previously. Tessellations have also been used as a basis for mechanical modeling of cellular tissues, especially in vertex models where forces act on the vertices

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of a lattice (Alt et al., 2017; Barton et al., 2017; Farhadifar et al., 2007; Fletcher et al., 2014; Lin et al., 2017; Okuda et al., 2015; Staple et al., 2010; Weliky and Oster, 1990). In contrast, our model faithfully reproduces the Voronoi tessellation, and matches the experimental data quantitatively on a number of geometric and topological measures, irrespective of the choice of the mechanical parameters of the model. We conclude that the mechanical interactions between the (proto)cells in early embryonic epithelial tissues are directly responsible for the observed geometrical cellular patterns of those tissues.

2. Materials and methods

2.1. Model

We model the cells in two dimensions, treating them as purely mechanical objects. Our cells consist of a nucleus, a radial and stiff microtubule network, and a more flexible actin cortex at the cell perimeter (Gittes et al., 1993). We model the nucleus as a single large bead with radius R_n, and the cortex as a collection of M small beads with radius R_c that surround the nucleus (Fig. 1(a)). The cortical beads initially form a circle around the nuclei. We connect each bead to its two neighbors by a spring with spring constant k_c and rest length $u_c = 2R_c$ to mimic the forces in the actin cortex. Cortical beads that are not connected through these springs interact via the repulsive part of the same potential. Microtubules are modeled as springs that connect the nuclear bead to individual beads in the membrane. To do so, we select at random a fraction $f = 1/6$ of the cortex beads and connect them to the nuclear bead with a spring of spring constant k_{MT} and rest length $u_{MT} = 2R_n$.

We initiate our system by placing N non-overlapping, circular cells at random positions in the plane. To let the cells grow, we allow the rest length of the microtubules and actin filaments to increase linearly over time. Because cells cannot interpenetrate, they exert forces on each other when they touch. These forces counteract the growth of the microtubules, which halts at a given stall force. A microtubule stops extending when the membrane bead it is connected to comes within 99% of the minimal equilibrium distance to a bead of another protocell. In this event we also lock the relative position of the beads. When half of the microtubules have stopped growing, the growth of the actin filaments also stops.

To let the cells divide, we first double the number of beads in the membrane and the number of microtubules connecting them to the nucleus. We then split the nucleus into two daughter nuclei of half the size. Of the cortical beads connected to a microtubule, we select the two beads forming the shortest axis across the cell. We then use this axis to divide the microtubules over the two nuclei (Fig. 1(b)). To help the nuclei separate, an extra spring is positioned between the nuclei, mimicking the interpolar microtubules. The rest length of the interpolar spring is gradually increased from zero to the radius of the nucleus, while the rest length of the other microtubules is reduced with a factor $\sqrt{2}$, so that the total area of the cell remains the same. Once this process is completed, the two axis beads are contracted using a new spring, and when brought together, duplicated and re-connected to complete the division of the cells.

The dynamics of the cytoskeleton and the nuclei are overdamped because the inertia of these small cell components is negligible compared to their viscous drag. Therefore, our equation of motion follows from equating the net force to the drag force, as given by Stokes' law:

$$\mathbf{F}_{i,\mathrm{net}} = 6\pi\eta R_i \mathbf{v}_i, \tag{1}$$

where $\mathbf{F}_{i,\mathrm{net}}$ is the total (net) force on object i, which can be either a nuclear or a cortical bead. The viscosity is denoted by η, R_i is the radius of object i, and $\mathbf{v}_i$ is its velocity.

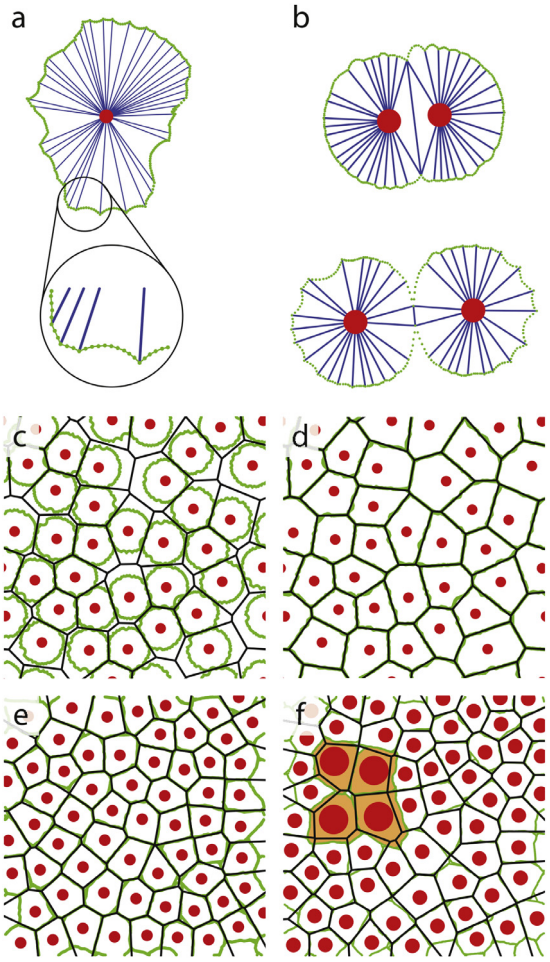

Fig. 1. Mechanical cell model and simulation results. (a) Cells consist of a sphere representing the nucleus (red), connected via microtubules modeled as stiff springs (blue) to the actin cortex, which is modeled as a number of beads connected by weaker springs (green). (b) Cell division. (c) Growing cells at 70% coverage. Where cell boundaries touch, they coincide with the Voronoi boundaries of their nuclei. (d) Growing cells at 100% coverage (no division). (e) Growing and dividing cells at 98% coverage after two divisions. (f) Growing and dividing cells at 98% coverage after two divisions, for the case in which one initial cell (with four daughters, indicated in orange) has a growth rate that is 2.5 × larger than that of the others. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In our simulations, we scale our measure of length by setting $R_c = 1$. For the repulsion between two cortical beads we can define a characteristic time $\tau \equiv 6\pi\eta/k_c$. We non-dimensionalize the units of time and force by setting $\tau = k_c = 1$.

We introduce a quality number Q to quantify the match between the Voronoi tessellation of the nuclei and the actual cells. To do so, we compare the actual area, A_r, of the cells to the area of their corresponding Voronoi cells, A_V. We define Q as:

$$Q = \frac{1}{N}\sum_{i=1}^{N}\left(\frac{A_{r,i} - A_{V,i}}{A_{r,i}}\right)^2, \tag{2}$$

where N is the total number of cells. When the Voronoi tessellation has a perfect match with the actual cells the value of Q is 0. For comparison, the Q number for a random close packing of identical discs is 0.05.

2.2. Experimental system

To be able to concurrently observe the nuclei and the actin cortex of *D. melanogaster* and *T. castaneum*, we required lines in