A scaling law for epithelial tissue rheology

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This manuscript was compiled on September 12, 2023

Predictive mechanistic understanding of morphogenesis absolutely requires quantitative knowledge of the mechanical properties of embryonic tissues. Our lab has recently established a set of experimental tools that have allowed us to directly probe tissue material properties in the living early embryo of *Drosophila melanogaster*. Here, we develop a simple quantitative theory explaining our central experimental observation: when subjected to concentrated constant pulling force, epithelium deforms following a power law with an exponent of $1/2$. Our analysis explains this finding in terms of two physical effects: (1) actin turnover and (2) geometric nonlinearity due to the floppy topology of the cellular network, and has additionally allowed us to measure the effective spring constant of a cellular edge.

tissue rheology | power law | *Drosophila* | epithelium

Animal morphogenesis is a process whereby simple epithelial sheets form organs with complex shape and function. Much recent research, both experimental and theoretical, has been devoted to understanding the physical basis of morphogenesis. Unfortunately, quantitative measurements of tissue material properties are not available for most developmental model systems. For this reason, theoretical studies of tissue morphogenesis often rely on untested speculative assumptions and may therefore lack in predictive power.

A popular model system to study animal morphogenesis is early embryo of the fruit fly *Drosophila melanogaster*. At the blastula stage, the embryo is a ball of epithelial cells. Subsequently, in a stereotyped sequence of folding events called gastrulation, the embryo becomes multilayered. Recent experimental studies examined material properties of embryonic tissues during the blastula stage (1–3). Strikingly, when constant concentrated force was applied to the cellular layer, displacement followed a power law with an exponent of $1/2$. Here, we establish and further test a minimal quantitative theory explaining this observation.

Results

Several previously reported experiments probed mechanical responses of the early *Drosophila* embryo epithelium. In these experiments, an embryo was injected with ferrofluid droplet, positioned within the epithelial layer. Using a magnet, the droplet was subjected to constant external force over a time period of approx. 1 min, causing epithelial tissue to stretch. Droplet position as a function of time was then extracted from imaging and was observed to follow a power law with an exponent of $1/2$. We repeated previous measurements from (1) applying external pulling force for approx. 3 min, Fig. 1. Notably, power law dependence persists over almost three decades in time. In general, power law scaling is indicative of scale-free dynamics and is directly informative about its underlying physical nature.

To understand the physical nature of the observed scaling, we developed a minimal model of epithelial tissue. We describe cells as hexagons, whose edges are linearly elastic springs with spring constant k. The tissue is a network of cells confined to the surface of an ellipsoid, representing the surface of the embryo, Fiure 2a. Cell and embryo dimensions were chosen as previously established from microscopy. Rest lengths of cell edges are dynamic, relaxing to their respective instantaneous lengths on a timescale τ:

$$\tau\dot{\mathcal{L}}_{0,i} = -(\mathcal{L}_{0,i} - \mathcal{L}_i) \quad , \qquad [1]$$

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M.I.C. and K.D. designed research; performed research; analyzed data; and wrote the paper. N.R. performed research.

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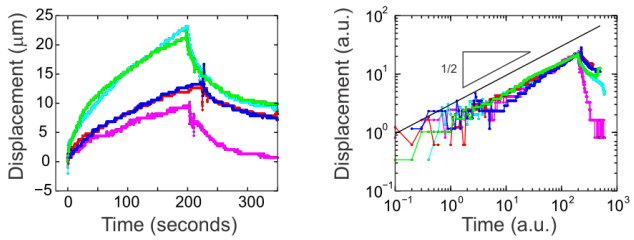

Fig. 1. Power law response in tissue pulling experiments. Left panel: when a ferrofluid droplet inserted into the cellular layer is pulled with constant force, displacement scales like time to the power $1/2$. The force was applied for approx. 200 seconds, compared to approx. 60 sec in the previously reported experiments. Right panel shows the same five traces on a log-log plot.

where $\mathcal{L}_i(t)$ is the length of the i:th cell edge, and $\mathcal{L}_{0,i}(t)$ is its rest length. To simulate external pulling force from ferrofluid droplet, constant force of magnitude F is applied to one vertex of the cellular network. Dynamics of each cell vertex is given by the force balance

$$\eta \dot{\mathbf{r}}_i = \mathbf{f}_i \quad , \qquad [2]$$

where $\mathbf{f}_i$ is total force on the i:th vertex comprising the following contributions: (1) elastic force from adjacent cell edges, (2) "soft force of constraint" confining the vertex to the surface of the ellipsoid, and (3) active force due to external pulling. The last contribution is only applied to a single vertex assumed to be in contact with the droplet. Finally, the node being pulled interacts with edges through excluded volume interactions such that it drags along with it any edges that it encounters in its path. Simulation results are shown in Figs. 2d,e. It is readily seen that the displacement of the vertex that is being pulled follows a $1/2$ power law, in agreement with experimental findings.

To understand the origin of the power law scaling, we now turn to simulations representing limits or simplified versions of the original system. Firstly, instead of considering a network of hexagons, consider a network of triangles, Fig. 2d,e. In this case, the asymptotic displacement followed a power law with an exponent of 1, and not $1/2$. This is expected from linear elasticity: a network of triangles is a good approximation of a linearly elastic sheet (4), which effectively behaves as a single elastic spring. Response of a single spring "forgetting" its rest-length on a finite time-scale is asymptotically viscous, such that constant force results in constant speed of displacement. Thus, asymptotically, displacement follows a power law with an exponent of 1.

Why do we then recover an exponent of $1/2$ in the case of hexagons? A network of hexagons is floppy, meaning, in particular, that some deformation can be accommodated without energy cost (5). Such networks can not be quantitatively described by the theory of linear elasticity. A simple physical picture (tentatively) capturing the observed behavior in the floppy case is as follows. First, consider a hexagonal network of springs whose rest lengths are not dynamic. As the ferrofluid droplet moves through such network, it encounters more and more elastic elements that it drags along. This is in stark contrast with the linearly elastic network (of triangles) where the entire network engages in the deformation from the beginning of pulling. Indeed, linearity implies that displacement at any given point is proportional to the displacement of the point being pulled. Hence, in the linear case of triangles, as the droplet moves through the network, all springs stretch more and more. In the floppy case, instead, more and more springs stretch.

To test our simplified physical picture, we analyzed a simplified 1D model meant to capture the conjectured behavior. Consider a 1D lattice of springs, each of initial length zero, see Fig. 2b. Consider a point (or droplet), moving through the spring lattice. As it encounters springs in its path, it engages with one end of the spring, the other end remaining in place, such that the spring stretches out as the point moves past it. Additionally, rest lengths of those springs are dynamic, relaxing on a finite time-scale τ. The dynamics of the system are:

$$\tau \dot{\mathcal{L}}_0(x,t) = \left[(X(t)-x) - \mathcal{L}_0(x,t)\right] H(X(t)-x) \quad , \qquad [3]$$

$$\zeta \dot{X} = F - \bar{k} \int_0^{X(t)} dx' \left[X(t) - x' - \mathcal{L}_0(x',t)\right] \quad . \qquad [4]$$

In Eq. 3, $\mathcal{L}_0(x)$ is the rest length of spring at spatial position x, $X(t)$ is the position of the point pulled through the lattice. Initially, this point is at $x = 0$, i.e. $X(0) = 0$. Note that $X(t) - x$ is instantaneous length of the spring initially attached at position x. H is Heaviside function, reflecting that only springs behind the moving point are stretched. Equation 4 gives the dynamics of the point being pulled and follows from force balance comprising three contributions: (1) external pulling force F, (2) cumulative force from all springs encountered up until the time t (the integral term), and (3) effective viscous friction with the surrounding ($\zeta \dot{X}$, where $1/\zeta$ denotes effective mobility). Note that Eqs. 3, 4 describe 1D spring lattice in the continuum limit, such that $\bar{k}$ reflects the density of elastic elements and has units of spring constant per unit length.

To analyze Eqs. 3, 4, we integrate Eq. 3 with respect to time, substitute the result into 4, and interchange the order of integration in the second term on the r.h.s. to obtain

$$\zeta \dot{X} = F - \bar{k} \left(\frac{X(t)^2}{2} - \int_0^t dt' \int_0^{X(t')} dx' \frac{1}{\tau} e^{-(t-t')/\tau} \left[X(t') - x'\right] \right) \quad . \qquad [5]$$

The integral term can be further simplified to $\int_0^t dt' \frac{1}{\tau} e^{-(t-t')/\tau} X^2(t')/2$. Substituting an ansatz $X(t) \sim t^{1/2}$, dropping all terms that tend to zero asymptotically, and assuming ζ is negligible, we obtain the asymptotic solution to Eqs. 3, 4:

$$X(t) = \sqrt{\frac{2F}{\bar{k}\tau}} t^{1/2} \quad . \qquad [6]$$

To summarize, our simplified 1D model predicts the $1/2$ power law scaling in agreement with simulations from Fig. 2d,e. To check whether this analysis applies to the original network of hexagons that it is meant to approximate, we next map the parameters of the full system to parameters of the simplified 1D model (Eqs. 3, 4). Consider a cylinder, whose radius is that same as that of the ellipsoid in Fig. 2a. Suppose evenly

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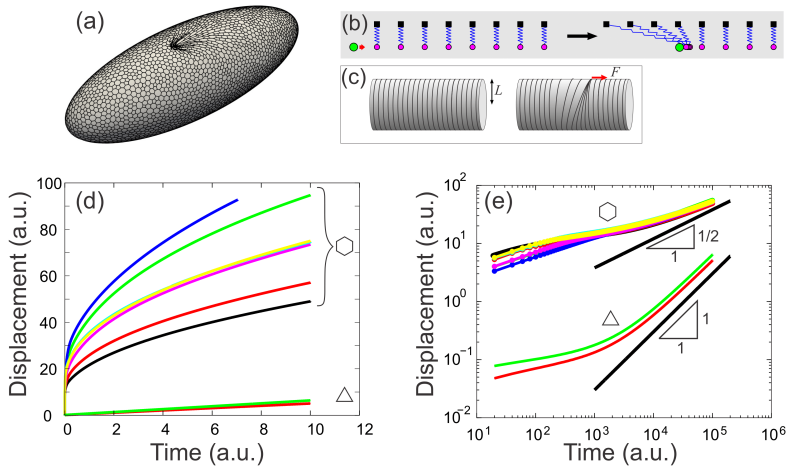

Fig. 2. Simulations of the minimal model reproducing the observed scaling. a). Simulated system is a network of hexagons, confined to a surface of an ellipsoid that is being stretched by concentrated force. b). Schematic of the 1D simplified model. A point (green circle) moves through a lattice of springs, engaging with one end of each spring as it moves past it. Note that springs are depicted as being vertical for illustration purpose: in our actual description forces only have a horizontal component. c). Schematic explaining the relation between the full model and the 1D approximation, see text. d). Simulations of the system in a). Curves show displacement of the point being pulled as a function of time. Hexagon and triangle label simulations of hexagonal and triangular meshes respectively. e). Same as d). on logarithmic plot. Curves corresponding to the case of hexagons are rescaled to collapse on the predicted master curve. Paremeters are: for the hexagonal cases, $k = 0.1$, $F = 0.01$, $\tau = 2$, $\eta = 0.001$, ellipsoid half-axes are 126 and 44 (black), 171 and 60 (red) 377 and 132 (green), 503 and 176 (blue), 251 and 88 (magenta, cyan, yellow), cell edge length δ is 4 (black, red, green, blue), 6 (magenta), 8 (cyan), and 10 (yellow). For the triangular cases, $k = 0.1$, $F = 0.01$, $\tau = 2$, $\eta = 1$ (red), $\eta = 0.1$ (green), ellipsoid half-axes are 251 and 88, $\delta = 4$. All simulation is publicly available at https://github.com/mcheikh1991/Results_Scaling_Law_For_Epithelial_Tissue_Rhelogy.git.

spaced elastic "rings" encircle the cylinder, as in Fig. 2c. These (fictitious) "rings" are meant to effectively capture the behavior of elastic elements comprising the floppy network in the original model and will correspond to individual springs in the simplified 1D description. The spring constant of such a ring is expected to scale like $k\Delta/L$, where k is the spring constant of a cellular edge, and L/Δ, the ratio of embryo radius to the length of a cellular edge, gives the number of edges comprising a ring. To relate this spring constant to $\bar{k}$ in Eq. 4, we need the density of springs (rings) per unit length, which scales inversely with the distance separating the springs, itself proportional to Δ. Hence, the final expression is $\bar{k} \sim k/L$, with cell edge length Δ cancelling from the final expression. Taken together, in simulations from Fig. 2d, we expect the displacement of the point being pulled to follow

$$X(t) = C\sqrt{\frac{FL}{k\tau}}t^{1/2} \quad . \qquad [7]$$

Eq. 7 predicts that loading curves from Fig. 2d can be collapsed on a master curve by choosing scaling factors according to Eq. 7, and that asymptotic dynamics are independent the length of a cellular edge Δ. These predictions were found to be in complete agreement with the scaling theory, Fig. 2e.

Our theoretical analysis together with measurement data from Fig. 1 allows us to estimate the spring constant of a cellular edge. This requires the knowledge of τ and dimensionless constant C in Eq. 7. Measurements from the lab of Dr. Anna Sokac showed that 80 % of F-actin comprising basal cell edges at the relevant developmental stage turns over on a time-scale of approx. $\tau = 20$ sec (6). Additionally, recent measurements from our lab showed that mechanical tissue response at this stage of development is entirely dominated by basal F-actin (2). Proportionality constant C is readily obtained from simulations in Fig. 2e and is found to have a value of approx. $C = 36$. Typical force in our measurements was approx. 20 nN, as previously measured in (1); radius of the embryo is approx. $L = 100\ \mu$m. Using these values, fitting a 1/2 power law to measurement data from Fig. 1, we obtain an estimate of $k \approx 23$ nN/μm, which is in excellent agreement with our independent measurements from (2). Note that our value for k_{basal} therein is relevant to long-time dynamics and therefore corresponds to the 20 % of total actin that does not turn over; for this reason, a correction factor of 5 is in order for a more direct (semi-)quantitative comparison.

ACKNOWLEDGMENTS. Please include your acknowledgments here, set in a single paragraph. Please do not include any acknowledgments in the Supporting Information, or anywhere else in the manuscript.

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