Mechanical models for the self-organization of tubular patterns

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Introduction

Life depends on the ability to control size and shape at different scales. At the molecular levels, chemical signaling relies on the conformational changes of molecules: mutations leading to abnormal changes of conformations often cause diseases such as tumors, tissue abnormality and malfunction. At the cellular level, the ability of cells to form various shapes has a vital importance.1,2 Such ability appears to control the fate of cells, including cell apoptosis,3 direct cell migration,4 and cell differentiation.5 At the tissue levels, the ability of cells to self-organize into various structures with a long-range coordination of their morphologies and phenotypes raises the possibility that an understanding of such processes could lead to a synthetic approach to engineer and/or regenerate tissue-scale structures and even organs without the aid of scaffolds.

The most abundant tissue structures that exhibit long-range coordination of cell morphologies and phenotypes are epithelial tubules, which appear in the respiratory, reproductive, digestive, and renal systems, and can be up to several centimeters long.6-9 In the past decades, theoretical studies have suggested that long-range morphological patterns can be created by chemical reaction-diffusion, and extensive experimental studies have been performed to investigate how chemical signals are involved in tubule formation in vivo.9,13 Nevertheless, attempts to create tissue-scale tubular structures by chemical stimulations in vitro have seen limited success. Consequently, current engineering of long tubules and other tissue-scale structures still relies on scaffolds,14,15 which typically require complicated fabrication and must remain with the engineered tissue, limiting their clinical applications.

Besides chemical stimulation, evidence increasingly suggests that extracellular matrix (ECM) plays an important role in tubular pattern formation.9,12,16 Type I collagen (COL), for example, an ECM molecule that can form long linear fibers, often appears around long tubules in vivo.17 Cells have also been shown to create long-range linear morphological patterns (up to 600 μm) through mechanical interactions with collagen molecules in vitro.4 Such interactions require cell surface receptors integrins that link ECM with various intracellular cytoskeletons.18 In particular, when the matrix environment is changed from laminin-rich (the major ECM component in basement membrane19) to collagen-rich, cells can switch integrin-linked cytoskeleton from intermediate filaments to actomyosin, thereby switching their phenotypes from stable epithelial structures to motile cells.20

These experimental observations raise the possibility that cells might use mechanical interactions, along with their chemical signals, to create tissue-scale tubular patterns. Indeed, a growing interest has been developed to investigate the roles of mechanical force in tissue development, remodeling, regeneration, and tumorigenesis.21-25 Understanding how cell mechanics are involved in tubular pattern formation requires familiarity with engineering principles of mechanics, the viscoelastic properties of biomaterials, and the integration of force and stress within tissue-scale structures as morphogenesis progresses. Here, we review experimental evidence and propose quantitative mechanical models by which cells can create tubular patterns.
organs including lungs, blood vessels, salivary glands, mammary glands, and renal ducts involves an iterative branching of cells from pre-existing cell sheets to the surrounding ECM. Similar processes can be found in embryo gastrulation, where a group of cells from ectoderm bend inward to form endoderm.

To create tubular patterns, the two important parameters are the spatial scale and the geometry of the tubules. One appealing mechanism to create repetitive tubular pattern is the chemical reaction-diffusion scheme proposed by Alan Turing (lateral inhibition). Lateral inhibition relies on the interplay of short-range activation and long-range inhibition (Fig. 1). Such inhibition can result from the consumption of precursors or the creation of inhibitors. Lateral inhibition requires that the activation self-amplifies, while creating inhibition to suppress other activations. As a result, individual activations mutually repel each other, leading to a regular spacing, L, between neighboring activations. In turn, position cues can spontaneously emerge to pattern cells into regular/periodic structures.

While chemical patterning has been observed in simple chemical systems and developmental processes, there is no successful example to create tubular patterns and other tissue-scale patterns by chemical stimulations in vitro. The main difficulty arises from the extensive remodeling of ECM and the substantial movements of cells during tissue self-organization and morphogenesis, which could significantly interfere with the chemical patterning processes. Further, in the presence of other cells such as mesenchymal cells, the environment surrounding epithelial cells becomes more complex, and the secretion, degradation, and distribution of diffusible chemical factors become less predictable.

**Mechanics-Based Non-Turing Models for Tubular Pattern Formation**

General features of mechanical forces in pattern formation. In chemical-based lateral inhibition, the formation of spatial patterns requires that the activation and the inhibition possess different spatial scales; this is achieved by having activators and inhibitors with different diffusion constants. Further, to form robust spatial patterns in complex and fluctuating environments, it is necessary that the spatial scales of activators and inhibitors possess a certain degree of flexibility. The diffusion constants of bio-molecules, however, are usually not tunable, limiting the dynamic range of tissue pattern formation by chemical-based processes.

Compared with chemical-based patterning processes, several features of mechanical forces make them easier to create spatial patterns. One feature is the tunability of cell motility, which makes it possible to create a broad range of spatial scales. Consider two types of cells that interact with each other. These cells can create different motilities by tuning their cytoskeletal mechanics and/or the expression level of surface receptors that mediate cell-cell or cell-ECM adhesions. Assume that the slow-moving cells act as the activator and the fast-moving cells act as the inhibitor. We can then see how their interactions lead to lateral inhibition. Examples can be found in the patterning of skin appendages, such as hair follicles and feather buds, where mesenchymal cells interact with epithelium and change motility by expressing different amounts of receptor N-CAM. Such cell motion-based lateral inhibition is also applicable to the cases where a single type of cells switch their phenotypes in response to an inhibitor released by the cells (such as transforming growth factor). In these cases, the difference of spatial scales is established by the diffusion of the inhibitor and the tunability of the cell motility.

Another feature that makes mechanical force useful in patterning processes is its vectorial nature. Such feature allows information of mechanical forces to be delivered in the same direction over a long spatial range in a short amount of time. This effect reduces the dispersion and/or dissipation of information, a common consequence in chemical diffusion. Further, the vectorial nature of mechanical forces creates spatial anisotropy. Consider a mechanical process that involves two forces transmitted along two orthogonal axes. These forces can be generated by moving cells along the ECM fibers. In contrast to isotropic molecular diffusion, the magnitude of each force lasts for a long spatial range along its own principle axis, but limited in the other. This effect leads to a difference of spatial scales for the distribution/dispersion of forces between orthogonal axes, a feature that cannot be achieved by simple chemical diffusion.

Perhaps the most important feature of mechanical forces is that they can continuously propagate between and across cells through cytoskeletons, intercellular adhesions, and ECM. This feature provides long-range and instant communication within the tissue-scale structures. The instant propagation of forces over multiple cells can be used to mimic the long-range effect required for chemical-based lateral inhibition. Further, patterning cues mediated by mechanical force can propagate among...
If cells can use mechanical forces to program tubular patterns, one immediate question is how far cells can develop mechanical interactions with one another. Certainly, the spatial scales of such interactions are parameterized by the architecture of the environments. Measuring these scales requires a quantitative platform. Owing to the advanced technologies of micro-patterning, several groups have engineered platforms to study how mechanical stress within the tissues depends on the geometry of tissue boundaries, how far cells can mechanically sense each other through ECM, and how cells change their phenotypes in response to the change of ECM.4,47,48 On the boundaries of tissue, for example, it has been shown that the effects of mechanical forces between cells are determined by the curvature of the boundaries.46 Likewise, it was observed that cells can mechanically sense each other at a sub-millimeter scale, and through mechanical interactions, cells can collectively change their phenotypes by translocating molecules such as Yes-associated protein (YAP), a signaling molecule for mechano-transduction,49 into the nuclei.4

Figure 2A shows an example of such platforms, by which one can measure how groups of cells change their patterns of mechanical interactions in response to the changes of ECM and how far they can interact with one another. Remarkably, it was found that cells can mechanically interact with each other through collagen fibers over a distance ~600 μm, which is far beyond the persistence length of collagen fibers and the typical diffusion length created by molecular diffusion (~10–20 μm).4 The tension created by the cells along the collagen fibers then facilitates the formation of long-range linear cellular patterns (Fig. 2B and C).4 In particular, the traction forces created by individual cells help the alignment of collagen fibers, which then
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Figure 3. Branching patterning due to a buckling effect. (A) The schematics of the buckling effect. (B) The schematics of the coordinates for the modeling of tubular surface topology. (C) Predictions of the model. The spacing between branched sites, $L$, decreases with the increment of cell number, $S - S_0$, and (ii) increases with the bending stiffness, $k$. Results were obtained using Equation 6 with $S_0 = 100$ and $\sigma = 1$. In (i), $k = 100$. In (ii), $S = 120$.

Branching patterns emerging from mechanical processes. We now discuss how cells could use tension on the cell-matrix interface, i.e., the boundaries of tissues (Fig. 2B), and other chemical-based cell regulations (such as cell proliferation, switch of phenotypes, and tuning of cell motility) to program spatial patterns. We propose and discuss the following models.

Formation of branching patterns by a buckling effect. One possible effect of the tension on the cell-ECM interface is to create a bending stiffness that helps cells align linearly with the collagen fibers. This effect can suppress the surface roughness of the tubules. In the presence of growth factors such as epidermal growth factor which stimulates cell proliferation, however, the tubule cannot carry excessive cells without changing its surface topology or its length. As a result, the cells will be bended into the lumen or the ECM space (i.e., buckling) if the length is confined. A spatial pattern then emerges by the mechanical balance between the bending stiffness and the proliferation pressure (Fig. 3A). Using a Helfrich energy function, Mark et al. have introduced a bending stiffness-based mechanical model to address dynamic instability in an expanding cell sheet. Here, we discuss how cell proliferation and the bending stiffness at the cell-ECM interfaces can give rise to branching patterns.

Consider a flat cell-ECM interface on the boundary of a linear tubule (Fig. 3B). For simplicity, we use a 1-D topology to represent the surface of the tubule. The surface area is then the integral of arc length of the tubule. The length of the tubule is confined as $S_0$ by the surrounding matrix environment. After a certain amount of time, the number of cells increases and the proliferation pressure pushes the tubule to reach a new equilibrium length $S$, which is not allowed due to the confinement, leading to the formation of buckles (i.e., bending cells into the matrix, Fig. 3A).

To analyze branching pattern formation, let’s use coordinate $\vec{r} = (x, y)$ to describe the surface of the tubule (initial $y = 0$ everywhere on the tubule surface). Adapting the equations from reference 52 the simplest 2-D free energy function $F$ that takes into account both the bending energy and the confinement of tubule length is:

$$F = \frac{\kappa}{2} \int_{x=0}^{x=x_0} ds \left(\frac{d^2 r}{ds^2}\right)^2 + \frac{\sigma}{2} \int_{x=0}^{x=x_0} ds - S.$$

(1)

Here, $\kappa$ is the bending stiffness, $\sigma$ is the intracellular pressure to push the extension of the tubule, and $ds = (dx^2 + dy^2)^{1/2}$ is the arc length along the tubule surface. Following the variation procedure introduced in reference 52, we can perform a variation of $F$ with respect to the coordinates $\vec{r}$, which gives the equation of motion of $\vec{r}$:

$$\eta \frac{d\vec{r}}{dt} = -\frac{\delta F}{\delta \vec{r}}.$$

(2)

Here, $\eta$ is the viscosity of the tubule surface when cells move into the ECM space. To investigate how branching patterns emerge on the tubule surface, consider a small perturbation of $y$ forming a wavy surface, i.e., $y = \varepsilon \exp[\omega(x)t]$, where $\varepsilon$ is the wavenumber, we have a dispersion relation:

$$\eta \frac{d\varepsilon}{dt} = -\kappa \varepsilon^3 \omega - \sigma \left[S - S_0\right] \varepsilon^2 \omega + O(\varepsilon^4).$$

(3)

Using mode analysis with $y(k) = y_0(k) \exp[\omega(k)t]$ where $k$ is the wavenumber, we have a dispersion relation:

$$\eta \omega(k) = -\kappa k^4 + \sigma \left[S - S_0\right] k^2.$$

(4)

Eqn. 4 indicates that the tubule surface is marginally stable. It also predicts that instability occurs (i.e., $\omega(k) > 0$) when $S > S_0$. The wavenumber with the maximal growth rate, $k_{max}$, and the corresponding spacing between branched sites, $L$, are:

$$k_{max} = \frac{\sigma \left[S - S_0\right]}{2\kappa},$$

(5)

$$L = \frac{2\pi}{k_{max}} = \frac{2\pi \sqrt{2\kappa}}{\sqrt{\sigma \left[S - S_0\right]}}.$$

(6)

This model predicts that the spacing between branched sites along the tubule surface, $L$, decreases with the increment of cell number [Fig. 3C (ii)], which is controlled by the dosage of growth factors, while $L$ increases with the bending stiffness (i.e., the tension at cell-ECM interface) [Fig. 3C (ii)].

Branching pattern formation by surface tension and cell scattering. Besides bending stiffness, another possible effect due to the tension on the cell-ECM interface is to minimize the
surface area of epithelial tubules and to confine the motion of cells on the surface. To investigate how such effect can lead to branching pattern formation, consider a group of cells that escape or scatter from a preexisting tubule surface into the surrounding ECM (Fig. 4A). In vivo and In vitro, such scattering can be induced by stimulating cells with scattering factors such as hepatocyte growth factor. As more cells scatter into the ECM, the local curvature and the local surface area of the tubule increase (Fig. 4A). These scattering cells can interact with the ECM and create force to “pull” even more cells to scatter outward. Further, the pulling force is in a direction orthogonal to the tension (i.e., spatial anisotropy due to the vectorial nature of mechanical force, Fig. 4B), leading to a “local” effect to convert more non-scattering cells into the scattering cells. Localization of such conversion effect can be destroyed if the scattering cells move along the tubule surface (i.e., lateral diffusion).

Based on these arguments, we assume that the rate at which the scattering cells are created is enhanced by the local convexity of the tubule surface and suppressed by the lateral movement of scattering cells along the tubule surface. In the absence of chemical stimulations, branching patterns might spontaneously emerge due to a mechanical counterbalance between the scattering and the tension: while the tension globally minimizes the surface area and suppresses the surface convexity, the scattering effect locally amplifies the surface convexity.

To investigate whether branching patterns can spontaneously emerge in the absence of chemical stimulations, consider the cross-section of a cylindrical tubule along its tubule axis (Fig. 4C). For simplicity, we again use a 1-D contour to represent the surface of the tubule with $\vec{F} = (x, y)$ as the surface coordinate. The surface area is the integral of arc length of the tubule. Following Eqn. 1, we write a simple free energy function $F$ to describe the minimization of tubule surface area:

$$F = T \int_{x=0}^{x=S_0} ds.$$  

(7)

Here, $S_0$ is the initial length (i.e., surface area in 1-D topology) of the tubule, $T$ is the tension that minimizes the surface area, and $ds = (dx^2 + dy^2)^{1/2}$ is the arc length along the tubule surface. Variation of $F$ with respect to the coordinates $\vec{F}$ gives the equation of motion of $\vec{F}$:

$$\eta \frac{d\vec{F}}{dt} = -\frac{\delta F}{\delta \vec{F}} + \vec{f}(c_{sc}).$$  

(8)

Here, $\eta$ is the viscosity of the tubule surface when cells move into the ECM. We add a new term $\vec{f}$, which is the traction force created by the scattering cells to pull the surface outward. This traction force is a function of the local density of scattering cells, $c_{sc}$, which we assume to increase at a rate proportional to the local convexity of the surface:

$$\frac{dc_{sc}}{dt} = G(-\frac{d^2\vec{F}}{ds^2}, \hat{y}) - gc_{cs} + Dc_{cs} \nabla^2 c_{cs}. $$  

(9)

Here, the first term describes the local curvature-dependent increment rate of scattering cells. To ensure that the rate is positive when the surface forms a local convex curvature, we multiply the curvature term with the unit vector of $\hat{y}$. The second term describes the conversion of scattering cells back to non-scattering cells and/or the escape of scattering cells into the ECM. The third term describes the diffusion of scattering cells along the tubule surface. Comparing Eqns. 8 and 9, the characteristic time scale for scattering cell formation is $g^{-1}$, while the characteristic time scale for the morphological change of tubule surface (i.e., branching formation) is $\eta$.

To investigate how branching patterns emerge on the tubule surface, consider a small perturbation of the tubule surface, i.e., $y << S_0$ and $c_{sc} << 1$. Expanding Eqn. 8 to the first order of $y$ and $c_{sc}$, we have:

$$\eta \frac{dy}{dt} = T \frac{d^2y}{dx^2} + f'(0)c_{cs} + O(y^2, c_{cs}^2).$$  

(10)

Here, $f'(0)$ is the derivative of $f$ with respect to $c_{sc}$ (at $c_{sc} = 0$), and we set $f(0) = 0$ since it is the force created by scattering cells. Likewise, expanding Eqn. 9 to the first order of $y$ and $c_{cs}$, we have:

$\textbf{Figure 4.}$ Branching pattern formation due to surface tension and cell scattering. (A) The schematics of cell scattering (cyan) from a tubule surface, by which the local curvature (i.e., convexity) of tubule surface increases. (B) The scattering cells create force (blue arrow) to drag or pull more cells outward, in a direction perpendicular to the tension. (C) The schematics of the coordinates for the modeling of tubular surface topology, $c_{cs}$ is the local density of scattering cells. (D) Numerical results for the approximated solution (Eqn. 15), black, and the exact solution (Eqn. 17), red, of the spacing $L$ in response to the change of tension $T$. The viscosity $\eta$ is set as 1 in (i), and 100 in (ii). To obtain the results, we set $g = 1, f' = 1, G' = 1$, and $D = 10$ in Eqns. 15 and 17.
\[
\frac{dc_{ss}}{dt} = -G'(0) \frac{d^2 y}{dx^2} - gc_{ss} + D_c \frac{d^2 c_{ss}}{dx^2} + O(y^2, c_{ss}^2).
\]

(11)

Here, \(G'(0)\) is the derivative of \(G\) with respect to the curvature (at zero curvature), and we set \(G(0) = 0\) (i.e., no spontaneous scattering on flat tubule surface in the absence of chemical stimulations).

**Branching pattern formation by separation of time scales.** We now consider two different conditions where cells can create branching patterns. In the first, we assume that the dynamics of scattering cell formation is much faster than the morphological change of the tubule surface, i.e., \(g > \eta^\gamma\), which is likely the case in a 3-D matrix environment that prohibits cell movement. Under this condition, we can solve Eqn. 11 adiabatically and express \(c_{ss}\) in terms of \(y\) using iterative substitution:

\[
c_{ss} = -\frac{G'(0) d^2 y}{g} \frac{D_c}{g} \frac{d^2 c_{ss}}{dx^2} - G'(0) \left[ \frac{d^2 y}{gdx^2} + D_c \frac{d^2 y}{g^2 dx^2} \right].
\]

(12)

Substituting Eqn. 12 into Eqn. 10, we have:

\[
\eta \frac{dy}{dt} \approx \left[ T - \frac{f' G'}{g} \right] \frac{d^2 y}{dx^2} - \frac{D_c f' G'}{g^2} \frac{d^2 y}{dx^2}.
\]

(13)

Using mode analysis with \(y(k) = y_0(k) \exp[\omega(k)t]\) where \(k\) is the wavenumber, we have a dispersion relation:

\[
\eta \omega(k^2) \approx - \left[ T - \frac{f' G'}{g} \right] k^2 - \frac{D_c f' G'}{g^2} k^4.
\]

(14)

Here, we have simplified the notions of \(f'\) and \(G'\). Compared with Eqn. 4, Eqn. 14 also suggests that the tubule surface is marginally stable. However, in Eqn. 4 the dissipation effect is due to the bending stiffness \(k\), whereas in Eqn. 14 the dissipation results from the lateral movement of scattering cells along the tubule surface, \(D_c\). Instability occurs when \(Tg < f' G'\). Under such condition, the wavenumber with the maximal growth rate is obtained by solving \(d\omega(k^2)/dk^2 = 0\):

\[
k_{max} = \sqrt{\frac{g}{2D_c} \left( 1 - \frac{Tg}{f' G'} \right)}.
\]

(15)

This approximated result suggests that the spacing between branched sites along the tubule increases with the motility of scattering cells along the tubule surface and the tension at the cell-ECM interface, while it requires a critical collective effect to amplify the scattering cell density (parameterized by the term \(G'\)) and overcome the tension and the conversion (and/or escape) of scattering cells (parameterized by \(g\)).

**Branching pattern formation without separation of time scales.** In the second condition, we assume that the time scale in scattering cell formation is compatible with the time scale in morphological change of the tubule surface, i.e., \(g < \eta^\gamma\), which can occur if the scattering cells are actively secrete enzymes to degrade the surrounding matrix. Under this condition, we cannot use the adiabatic approximation (i.e., Eqn. 12). Instead, we have to perform mode analysis with \(c_{ss}(k) = c_{ss}(k) \exp[\omega(k)t]\) and \(y(k) = y_0(k) \exp[\omega(k)t]\) on Eqns. 10 and 11, to obtain the dispersion relation:

\[
\eta \omega(k^2) + \left[ \eta (g + D_c k^2) + Tg \right] \omega(k^2) + Tg \left( g + D_c k^2 \frac{G'}{f'} \right) = 0.
\]

(16)

Compared with Eqns. 4 and 14, Eqn. 16 also suggests that the tubule surface is marginally stable. Similar to Eqn. 14. Instability occurs when \(Tg < f' G'\). The wavenumber with the maximal growth rate is then obtained by solving \(d\omega(k^2)/dk^2 = 0\):

\[
k_{max} = \sqrt{\frac{g}{2D_c} \left( \sqrt{A_1^2 + A_2 A_3} - A_1 \right)},
\]

with (17)

\[
A_0 = \frac{f' G'}{g \eta D_c} + 1 \left( \frac{f' G'}{Tg} - 1 \right),
\]

\[
A_1 = \frac{2 f' G'}{g \eta D_c} + 1 - \frac{T}{\eta D_c},
\]

\[
A_2 = \left( 1 - \frac{T}{\eta D_c} \right)^2.
\]

Comparing the numerical results from Eqns. 15 and 17, we find that the approximated solutions (Eqn. 15) for the spacing asymptotically reach the exact solutions (Eqn. 17) in the high tension region, regardless of the viscosity \(\eta\) (Fig. 4D).

**Conclusion**

In this article, we discuss how mechanical forces propagating along biomaterials such as ECM can create tension to facilitate long-range coordination of cell morphology and phenotype, and propose quantitative models to address how branching patterns can spontaneously emerge by the counterbalance between tension and other mechano-chemical based processes. We also provide quantitative predictions that can be tested by experiments.
The effect of mechanical force on biological materials differs from that of chemical force in that it depends both on the force-molecular interactions and the structure of the underlying substrate. This opens a door for using biomaterials and cell mechanics to control and/or engineer tissue-scale structures by changing the topology and structure of the environment. Further, the difference of time scales in force propagation and chemical signaling enables future engineering and control of patterning cues by combining synthetic biology and the fabrication/manipulation of biomaterials.

There are several advantages of using physical vs. chemical forces to control the response of biological materials. For example, mechanical force is non-specific, which does not depend on the type of molecules, cells, and tissues involved. Thus, the effect and design principle is universal. Further, unlike specific chemical signaling, the non-specificity of mechanical forces allows them to be directly combined, providing a simple computation law for the programming of mechanics-based patterning processes. These features, along with the relatively simple processes required in generating mechanical processes, make mechanical force a promising tool to control and manipulate tissue morphologies.

Disclosure of Potential Conflicts of Interest

The authors declare that they have no competing interests.

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