Determining dynamics from statics in living tissue

Anisotropy links cell shapes to tissue flow during convergent extension
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There are numerous examples where living tissues reshape and remodel themselves, from the early stages of organism development to the onset of metastasis and tumor formation in cancer. A consensus is emerging that such processes are controlled not only by genetics, but also by inter- and intra-cellular stresses. Understanding how tissues generate and respond to stress is important, but difficult to study in bulk living tissue. Thus, identifying structural signatures of the mechanical properties of tissue would be extremely valuable to researchers, as one could analyze static images of tissue samples to extract cellular forces and mobility.

Recent theoretical studies of the vertex model for confluent tissues [1] suggest that the *asphericity* of cell shapes can be used to extract dynamics and mechanical properties of tissue from static images. Briefly, the vertex model treats a two-dimensional (2D) tissue of confluent cells as a tessellation of N polygons with perimeter p and area a with total energy:

$$E = \sum_{i=1}^{N} \left[(p_i - p_0)^2 + k_A (a_i - 1)^2 \right], \tag{1}$$

where p_0 is the preferred perimeter of each polygon, and k_A is a spring constant that constrains the polygon area to the preferred value $a_0 = 1$. Forces are distributed to the *vertices* of the tessellation such that the system remains confluent. For cells with the preferred area, p_0 acts as a dimensionless shape parameter; smaller values of p_0 yield polygons with more circular shapes, whereas larger values of p_0 allow cells to take on a variety of non-spherical shapes with the same perimeter-to-area ratio. The vertex model exhibits a novel density-independent rigidity transition at $p_0^* \approx 3.81$; when $p_0 > p_0^*$, the systems possess "floppy" vibrational modes, whereas when $p_0 < p_0^*$ the systems are rigid with no non-trivial zero-frequency vibrational modes. Experimental studies of confluent cell monolayers [2] and

dynamic simulations of the vertex and related models [3, 4] have suggested that p_0 controls tissue fluidity as well; tissues with average shape parameter $\overline{p} = p/\sqrt{a} < p_0^*$ are more solid-like with no cell rearrangements, whereas tissues with $\overline{p} > p_0^*$ are more liquid-like with frequent cell rearrangements. This prior work suggests that there are structural signatures of tissue mechanics and fluidity, which is an exciting prospect. However, the true test for this theoretical framework is its ability to describe the mechanics and dynamics in experimental studies of living tissue.

In their manuscript [5], Wang, *et al.* analyze cell shape during the convergent extension process in the developing *Drosophila* embryo to infer tissue mechanics and fluidity. Using sophisticated imaging techniques, the authors measured both tissue fluidity, via the instantaneous cell rearrangement rate, and cell shape over the ~ 40 minute convergent extension process. The authors find that there is no well-defined $\bar{p}$ value that signals the onset of tissue fluidity, even when they consider cell packing disorder (i.e. the percentage of pentagonal cells). These results are in contrast with the prior predictions of the vertex model [1, 6]. However, the *Drosophila* germband convergent-extension process involves highly anisotropic stresses; the authors therefore propose that cell shape anisotropy, as well as cell asphericity can predict tissue fluidity. The authors show that the shape alignment index Q introduced in previous work [7] and the coordination number z determine the onset of tissue fluidity at

$$\bar{p}_{\rm crit} = p_0^* + (z-3)/B + 4bQ^2, \tag{2}$$

where the coefficients $B = 3.85$ and $b = 0.43$ are determined from other numerical studies of the vertex model [6, 8]. Additional experimental studies on mutant *snail twist* embryos, where external anisotropic forces are absent, also show that Eq. 2 can be used to determine the onset of cell rearrangements. This work represents a significant step forward in understanding the interplay between cell shape, mechanics, and fluidity in living tissues.

However, as discussed in Ref. [5], Eq. 2 was not able to describe the onset of fluidity in *bnt* mutant embryos. In these mutants, cells exhibit fewer planar-polarized actomyosin networks, which drive the convergent-extension process in the wild type embryos. In the *bnt* mutant, very few cell rearrangements occur during convergent extension, which should indicate solid-like behavior. However, the authors report that $\overline{p}$ and Q take on values that suggest fluid-like behavior. The failure of the anisotropic vertex model to predict solid-like versus fluid-like behavior in the *bnt* mutants raises important questions. In particular, in what regimes can the cell shape parameter provide information on the fluidity of dense tissues? And are additional quantities that describe cell shape, such as shape correlations or fluctuations, necessary to infer tissue fluidity?

In the discussion of their results, the authors note that the near disappearance of cell rearrangements in the *bnt* mutants suggests that the tissue might be similar to a weak yield-stress solid. A yield-stress solid displays solid-like behavior in the limit of vanishing driving forces, but it flows when the applied stress exceeds the yield stress. The *bnt* mutation might therefore either raise the yield stress of the tissue or lower the activity of the cells such that the tissue remains below the yield stress and does not fluidize. However, the prediction of the anisotropic vertex model is that tissues with observed shape parameter $\overline{p} > \overline{p}_{\rm crit}$ (such as the *bnt* mutants) have a vanishing static shear modulus. These results are contradictory, since a yield stress solid has a non-zero static shear modulus. If the anisotropic vertex model

holds and the *bnt* mutation changed the driving force or barriers to cell rearrangements, cell shapes should reflect the solid-like behavior of the tissue.

An alternative explanation for the discrepancy between the anisotropic vertex model predictions and the results for the *bnt* mutant embryos is that the *bnt* cells are more rigid than those in the wild type embryos. In this case, cells in the *bnt* mutants can take on shape parameter values above $\overline{p}_{\text{crit}}$, yet the tissue can remain mechanically stable with a nonzero G. Additional bending constraints on cell shape, for example from increased cross-linking of the actomyosin cortex or microtubules, can rigidify individual cells, which causes solid-like behavior of the tissue as a whole [9]. Incorporating bending constraints in the anisotropic vertex model, and further investigating the mechanical response in the limit of zero driving, may help reveal how some tissues remain solid-like even though they possess cells with $\overline{p} > \overline{p}_{\text{crit}}$.

Regardless, the success of the anisotropic vertex model in extracting valuable dynamical information from the shapes of cells will invigorate new biophysical studies of living tissues. For example, we can now begin to interrogate the physical forces that determine tumor invasiveness through the analysis of tumor cell shapes [10]. While more work must be done to determine whether tissues are yield stress solids or weakly-driven fluids, the research presented in this commentary has provided the strongest evidence to date that there is an interesting link between the mechanical properties of tissues and the shapes of individual cells.

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