Engineering control over 3D morphogenesis by tissue origami

Abstract:

Controlled folding of tissues occurs in development and would enable advances in tissue engineering. In this issue of Developmental Cell, Hughes et al. (2018) use *in vivo*, *in vitro*, and *in silico* approaches to uncover microscale mesenchymal cell distribution blueprints that robustly drives macroscopic 3D folding of tissues.

Main text:

During morphogenesis, complex tissue shapes and patterns arise from cellular mechanical forces that cause invagination or folding events (Davies 2013). Our ability to recapitulate tissue self-organization *in vitro* has previously been limited, and complexity in engineered tissues has instead often been achieved through controlled assembly or additive manufacturing methods (Khademhosseini, Langer 2016). Major challenges to bringing tissue folding under engineering control arise from the multiscale nature of these dynamic processes, which involve microscale cell forces and highly localized mechanical deformations that sum up to macroscale folding events. The folding trajectory and final outcome are highly dependent on the spatial location of contractile cells, mechanical characteristics of the tissues, and cell types involved. Spatiotemporal control over growth factor presentation in dynamically changing three-dimensional tissues has also posed a major challenge. Elegant work from Hughes et al. (2018), published in this issue of *Developmental Cell*, presents a new experimental approach accompanied by a modeling framework to spatially pattern tissue folding with a high degree of control.

Acknowledging that folding or buckling of tissues requires a strain mismatch between two adjacent layers, Hughes et al. (2018) began by drawing inspiration from developmental examples of morphogenetic folding. Although strain mismatches can occur through a variety of mechanisms, they focused on mesenchymal condensation or compaction, a driving force in the formation of mouse gut villi and chick feather buds (Walton 2016, Shyer 2017). They show that the presence of aggregates of contractile fibroblasts underlying the basal surface of the epithelium presage locations where villi subsequently appear. Seeking to recapitulate this process in vitro, they adopt a previously established cell-patterning technique predicated on printing adhesive domains of DNA “velcro” to enable spatial patterning of multicellular clusters of fibroblasts in gels composed of type I collagen and Matrigel. Using this system of patterned tissue culture, the authors observed that cell-generated contractile forces induced local condensation and restructuring of collagen fibers paralleling changes observed in the mouse gut.

In their model system, cell clusters form condensates while compacting the surrounding ECM and imposing local strains at tissue interfaces. Regions of aligned collagen (termed “straps”) form preferentially between nearest neighbor clusters as they compact. Self-organization of mechanically active condensates provides a systematic way of patterning tension through controlling the initial position and density of cell clusters within the tissue. A two-parameter finite element model calibrated by experimental measurements qualitatively captures folding trajectories of reconstituted tissues from ‘blueprints’ of cell clusters. Empowered by this predictive computational model, the authors demonstrate the generality and robustness of their technique by showing a diverse set of 3D tissue architectures autonomously folded from rationally designed networks of mesenchymal condensates. These architectures include a combination of isotropic, anisotropic, compound curvature, and opposing

curvature motifs to generate coiled tissues, tubular structures, and corrugated objects inspired by the looping of the intestine, formation of ducts and vessels, and the periodic shape of the dermal-epidermal junction in the skin, respectively.

Morphogenesis occurs robustly at interfaces between distinct tissue layers despite the presence of heterogeneous cell populations. Furthermore, engineering complex and functional tissues requires the incorporation of multiple cell types, so Hughes et al. (2018) explored the possibility of using mesenchymal condensation to convey and organize non-fibroblastic cells. Hypothesizing that less contractile epithelial or endothelial cells would not interfere with fibroblast-driven folding events but instead behave as "passengers," they co-patterned gut epithelial or endothelial cells with fibroblasts and allowed tissue folding to proceed. With folding at locations programmed by cell patterning, they observed the formation of crypts of gut epithelial cells located at the base of tissue invaginations, morphologically similar to the architecture of developing small intestine. In co-culture gels containing endothelial cells, folding resulted in the formation of tracts of endothelial cells that subsequently formed lumens. Intriguingly, these endothelial cells were observed to directionally migrate due to local reorganization of collagen fibers, suggesting that these cells were not blind passengers simply along for the ride, but in fact sensed and responded to evolving changes in ECM topography, mechanics, and adhesive ligand density instigated by tissue folding. These exciting results suggest a generalizable approach to engineering complex and heterotypic multicellular structures by utilizing cell patterning and the varying contractile activity of different cell populations.

Hughes et al. (2018) establishes exciting groundwork for employing the actuation of soft tissues towards the in vitro assembly of organs. From a different perspective, the presented approach of reconstituted tissues of patterned mesenchymal condensates can be utilized as shape-programmable active materials for building autonomous biohybrid robots (Ricotti 2017). While this work focused on the use of collagen and Matrigel composite hydrogels, additional axes of morphogenetic control could be achieved through the use of biomaterials (Gjorevski 2016). Given the important role of local reorganization of ECM fibers within these gels, this work motivates the use of fibrous ECMs that are highly porous and deformable, yet conducive to the production of cell generated forces (Baker 2015). Additionally, although the approach here produced unidirectional folding trajectories, one can envision recent advances in synthetic ECM mimetics with controllable and reversible mechanical properties providing a means to dynamically reshape tissues (Rosales 2016). Aside from engineering the cell's microenvironment, optogenetic and inducible genetic constructs to directly modulate cellular contractility could also enable control over the degree as well as the sequential timing of folding events.

This work from Hughes et al. (2018) elegantly demonstrates the utility of integrating experiments with computational analysis for multiscale modeling of tissue mechanics. Although significant progress over the last decade has provided numerous means to measure and visualize cellular forces (Polacheck 2016), there is still much to be learned about ECM regulation of cell force generation, especially in complex 3D and fibrous settings such as those employed here. While the position-based simulation method taken by the authors does not allow quantitative analysis of internal stresses or mechanical properties, more accurate dynamic simulations could be developed to extract such information throughout the evolution of 3D morphogenesis. Using additional measurements of cellular forces to better inform mechanical models is a major next step that numerous groups are working towards. Incorporation of dynamic aspects such as cell migration, the production, remodeling, and degradation of ECM, and fluctuations in contractility due to growth factors and evolving ECM mechanics will yield a modeling framework that can

faithfully simulate tissue behavior. Such models in concert with robust mechanical characterization of developing *in vivo* tissues may yield deep insights into fundamental developmental processes that can in turn ultimately better inform tissue engineering.

References:

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