



This is a repository copy of *Mechanocellular models of epithelial morphogenesis*.

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/122834/>

Version: Accepted Version

Article:

Fletcher, A.G. orcid.org/0000-0003-0525-4336, Cooper, F. and Baker, R.E. (2017) Mechanocellular models of epithelial morphogenesis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372 (1720). 20150519. ISSN 0962-8436

<https://doi.org/10.1098/rstb.2015.0519>

This is an author produced version of: Fletcher AG, Cooper F, Baker RE. 2017 Mechanocellular models of epithelial morphogenesis. *Phil. Trans. R. Soc. B* 372:20150519. <http://dx.doi.org/10.1098/rstb.2015.0519>

Reuse

Unless indicated otherwise, fulltext items are protected by copyright with all rights reserved. The copyright exception in section 29 of the Copyright, Designs and Patents Act 1988 allows the making of a single copy solely for the purpose of non-commercial research or private study within the limits of fair dealing. The publisher or other rights-holder may allow further reproduction and re-use of this version - refer to the White Rose Research Online record for this item. Where records identify the publisher as the copyright holder, users can verify any specific terms of use on the publisher's website.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk
<https://eprints.whiterose.ac.uk/>

Review article: Mechanocellular models of epithelial morphogenesis

Alexander G. Fletcher^{1,2,*}, Fergus Cooper³, Ruth E. Baker³

¹ School of Mathematics and Statistics, University of Sheffield, Hicks Building, Hounsfield Road, Sheffield, S3 7RH, UK

² Bateson Centre, University of Sheffield, Sheffield, S10 2TN, UK

³ Mathematical Institute, University of Oxford, Andrew Wiles Building, Radcliffe Observatory Quarter, Woodstock Road, Oxford, OX2 6GG, UK

* Author for correspondence (a.g.fletcher@sheffield.ac.uk).

Keywords

Epithelial morphogenesis, computational modelling, finite element model, immersed boundary method, subcellular element model

Abstract

Embryonic epithelia achieve complex morphogenetic movements, including in-plane reshaping, bending and folding, through the coordinated action and rearrangement of individual cells. Technical advances in molecular and live-imaging studies of epithelial dynamics provide a very real opportunity to understand how cell-level processes facilitate these large-scale tissue rearrangements. However, the large datasets that we are now able to generate require careful interpretation. In combination with experimental approaches, computational modelling allows us to challenge and refine our current understanding of epithelial morphogenesis and to explore experimentally intractable questions. To this end a variety of cell-based modelling approaches have been developed to describe cell–cell mechanical interactions, ranging from vertex and ‘finite element’ models that approximate each cell geometrically by a polygon representing the cell’s membrane, to immersed boundary and subcellular element models that allow for more arbitrary cell shapes. Here we review how these models have been used to provide insight into epithelial morphogenesis and describe how such models could help future efforts to decipher the forces and mechanical and biochemical feedbacks that guide cell and tissue-level behaviour. In addition, we discuss current challenges associated with using computational models of morphogenetic processes in a quantitative and predictive way.

1. Introduction

The past decade has witnessed remarkable progress in quantitative studies of morphogenesis fuelled by advances in microscopy, image analysis, and fluorescent reporter methods (1). The resulting toolkit has enabled processes to be quantified and correlated across multiple scales: from the spatio-temporal dynamics of specific molecules within cells (2,3); to individual cell shape changes and movement; to tissue-scale growth and deformation (4). This has led to an increasing recognition that morphogenesis involves a complex interplay between cell signalling and mechanical forces (5). Gene expression and protein activity modulate the cellular generation of, and response to, forces. In turn, mechanical cues may have a direct role in regulating these biochemical processes, and affecting cell behaviour, for example by controlling growth (6) or triggering apoptosis (7). Improving our understanding of such feedbacks enables a more holistic view of development and may have future implications for improved tissue engineering and repair strategies.

Morphogenesis is frequently driven by the growth and deformation of epithelial tissues, which form polarized sheets of cells with distinct apical and basal surfaces, and tight lateral attachments located nearer their apical surface. The coordinated movement, shape change and intercalation of cells in an epithelial sheet facilitate complex morphogenetic processes, from tissue elongation through convergent extension (8) to bending and invagination (3) and tube formation (9). The mechanical forces driving these processes are multiscale in nature (10) and include the action of molecular motors, membrane-bound adhesion components, and extrinsic forces from underlying tissues (11,12). Until recently, such forces were not experimentally measurable, and thus the role of mechanics in morphogenetic processes not well characterized. This has changed, however, with recent advances in measurement techniques, in particular in vivo (13,14).

The resulting force measurements, combined with cell- and tissue-level summary statistics on geometry and morphology that can be extracted from long-term live imaging, constitute an incredibly rich amount of data on morphogenetic processes. Computational modelling offers a useful framework for integrating such data and disentangling the roles of mechanics and signalling (15). The iterative development of models and experiments allows us to refine our mechanistic understanding of biological observations and test competing hypotheses (16). In particular, quantitative measurements enable us to constrain models, for example through parameter estimation, increasing the ability-potential of such models to be used in a predictive way.

A variety of approaches have been developed to model how processes at the cell level determine tissue size, shape and function during morphogenesis (17).

A large class of these models neglect cytoplasm and cell junctions, treating an epithelial tissue as a continuum (for example, viscoelastic) material (18–21) and employing finite elements or similar methods to discretize the tissue for simulation purposes. In essence, the continuum approximation averages over length scales much larger than the typical diameter of a cell. It can thus be difficult to incorporate heterogeneity between cells within a population. Accelerated in part by the reduction in cost of computing power, a number of discrete or 'cell-based' approaches have also been developed that treat cells as discrete entities. They provide natural candidates for studying the regulation of cell-level processes but are less amenable to mathematical analysis than their continuum counterparts. We restrict our focus to this burgeoning class of models in the present review.

These Cell-based models vary in complexity, from those that consider the movement of cells on a fixed lattice (22,23), to models that account for continuous cell movements and consider cell shape to be fixed (24) or varying. The latter include models that track the centre of each cell as a point, determining cell neighbours through an 'overlapping spheres' or Delaunay triangulation approach (25–27), and vertex models that include an explicit description of cell shape. Such models are well suited to investigating the 'passive' mechanics of autonomous epithelial monolayer deformations. However, few existing models properly integrate descriptions of cell mechanics with models of biochemical signalling or genetic programming, or allow for the complex cell shapes that arise due to localized adhesion, constriction and protrusion.

Here we summarize how several recent cell-based models have sought to overcome these limitations, and discuss how these models could help future efforts to study the interplay between chemical and mechanical signals in epithelial morphogenesis. Our aim is to provide a biologically accessible overview of the models' underlying assumptions, strengths and weaknesses, and the computational challenges associated with their further development, rather than an exhaustive comparison of the constitutive laws and material behaviour of the different models; for more detailed physical descriptions of recent approaches, see, for example, (28). We present these models, broadly speaking, in order of increasing computational complexity, starting with vertex models that contain the simplest explicit, dynamic description of cell shape. An overview of the strength, limitations and example applications of each class of model is presented in Table 1.

2. Vertex models

We take as our starting point two-dimensional vertex models, a popular example of off-lattice cell-based models that approximate cell apical surfaces geometrically by polygons defined through the interfaces between adjacent cells (29). In these models, the movement of junctional vertices is assumed to be governed by the strength of cell-cell adhesion, actomyosin cortical contractility and cell elasticity. Originating from models of inorganic structures such as soap bubbles, vertex models have been widely used to investigate the deformations of homogeneous and patterned epithelial tissues.

A highly cited example of the utility of vertex models is the work of Farhadifar et al. (30), who performed a systematic analysis of the equilibrium cell packing geometries and their dependence on cell mechanical and proliferative parameters with application to the *Drosophila* wing epithelium. By comparing simulations with experimental results on laser ablation of individual cell-cell interfaces, the authors arrived at a set of parameter values for which their model accounts for the observed vertex movements induced by laser ablation, epithelial packing geometries, and area variations. This work demonstrates how such models may be parameterised, and their predictions tested, ~~quantitatively~~ against experimental data.

3. Incorporating mechanical complexity

While successful in recapitulating much of the gross behaviour of planar epithelial sheets, vertex models typically ignore contributions such as cell-matrix adhesion (31) and medial actomyosin contractility (32). These models also ~~tend to neglect~~~~lack resistance to shear deformation through~~ active remodelling of cytoskeletal components. One approach to including cytoskeletal remodelling is to introduce viscoelastic elements representing the cell membrane and cytoplasm (figure 1a). This approach was first adopted by Odell et al. (33,34), who modelled a cross section of an embryo as a ring of cells with interconnected vertices subject to a viscoelastic force. The authors assumed that apical edges actively contract in response to stretch. With additional system-specific assumptions, this model recapitulated patterns of deformation as observed in, for example, sea urchin gastrulation or *Drosophila* ventral furrow formation (figure 1b). Several more recent studies have focused on the different patterns of cell mechanical properties that can generate observed tissue deformations. For example, models of *Drosophila* ventral furrow formation have suggested a possible role for pushing by cells neighbouring the furrow, or buckling due to uniform tissue-wide changes in apical tension (35).

An alternative extension of the vertex model has been developed by Brodland and colleagues (36), who decompose each polygonal cell into triangular 'finite elements', joined at the centroid of the polygon. This approach treats the cytoplasm as a continuous viscous material and assumes that cell-cell interfaces experience a constant force. As in vertex models, the motion of cell vertices is driven by interfacial tension between cells of the same and different types; however, here the volume of each cell is held constant. This model has, for example, been successfully used to test the roles of differential adhesion (37) and differential surface contraction to cell sorting and engulfment (38), to assess the mechanical efficiencies of different tissue reshaping mechanisms (39), and study the contributions of applied stress and edge-tension anisotropies to germ band retraction in the *Drosophila* embryo (40).

4. Incorporating additional geometric complexity

The models discussed in Sections 2 and 3 share the assumption that cell shape is well approximated by a polygon of specified degree. In recent work by Tamulonis et al. (41), the cross-section of each cell is modelled by a polygon comprising a large number of vertices, allowing for more complex cell shapes (figure 1c). Membrane elasticity is modelled by associating a linear spring with each cell edge, whose stiffness and equilibrium length varies according to whether the edge is apical, basal or lateral. The apical (and, in some simulations, basal) corners of neighbouring cells are also connected by very stiff springs, representing adherens junctions. Apical constriction is implemented via an intracellular spring between each endodermal cell's apical corners. The authors do not impose a constant cell volume, instead assuming the cytoplasm to be linearly elastic, resulting in an additional force acting at each vertex. This model was applied to study gastrulation of the starlet sea anemone *Nematostella vectensis*, which culminates some cells adopting a characteristic 'bottle' shape. The model successfully reproduces several key features of gastrulation and suggests that bottle cell formation may emerge from the balance of spatially patterned mechanical forces: strong apico-basal contractility, reduced cell-cell adhesion and a lateral constraint (figure 1d). It will be interesting to see how widely conserved [this](#) combination of apical constriction and reduced cell-cell adhesion is as a mechanism for generating complex cell shapes in embryonic epithelia.

While the model by Tamulonis et al. (41) assumes a fixed number of nodes per cell, a recent development of the finite element model by Brodland and colleagues replaces straight edges by polylines with an arbitrary number of segments, allowing for curved cell boundaries (42).

The authors allow the number of nodes per cell to vary dynamically according to some threshold on segment length, and also replace the triangular decomposition with an orthogonal dashpot system. By comparing simulations of annealing, engulfment and cell sorting, the authors show that cells with polyline boundaries exhibit a more fluid, biologically realistic behaviour than those with straight edges, which experience shape constraints limiting their movement and deformation.

In other work, Ishimoto & Morishita developed a 'bubbly' vertex model (43), motivated by observations of curved cell boundaries within a range of epithelia and 'two-vertex' cells within the mouse olfactory epithelium. The framework uses a generalised form of the tissue potential energy that is a function of the curvatures and vertex positions, where the Young-Laplace law represents the force balance along the cell boundary. This significantly increases the computational cost of simulation, but provides an interesting extension to the standard vertex model that may be applicable to a variety of morphogenetic processes.

We conclude our discussion of models that allow complex cell morphologies by considering the immersed boundary method (IBM) (44–49). Originally developed to study the flow of blood around heart valves (50), the IBM considers the dynamics of one or more elastic membranes, that represent cell boundaries, immersed in a viscous incompressible fluid (figure 2a), which represents the cytoplasm, ~~and~~ extracellular matrix ~~and fluid~~ (46). The IBM has been applied to three-dimensional problems, such as the deformation of leukocytes and (51) and red blood cells (52), but for simplicity we restrict our focus to two dimensions here. We emphasize that the fluid does not interact directly with the immersed boundaries, and the boundaries do not directly partition the fluid. The fluid obeys the Navier-Stokes equations with an imposed body force acting due to the elastic interactions of each cell. The precise functional form of this body force may be formulated rigorously as a strain relation (49), or else by decomposing it into inter- and intra-cellular interactions contributions (figure 2a) (53). The immersed boundaries move due to the fluid flow without slipping. The numerical solution of this model involves discretizing the fluid onto a regular square grid, while the immersed boundaries are represented by a finite number of points along their length.

The first application of the IBM to collective cell dynamics, by Rejniak and colleagues, focused on the growth of solid tumours under differing geometric configurations, initial conditions, and progression models (46,47). Although not yet used extensively to model epithelial morphogenesis, the IBM has been applied extensively in biology and elsewhere (45,48,54). The flexibility of the IBM is exemplified by an application by Dillon and colleagues

to vertebrate limb bud morphogenesis, where an immersed boundary now represents a tissue domain rather than a cell (55).

Although still primarily used in other settings, the IBM has several features that make it well suited to modelling epithelial morphogenesis, as argued by Tanaka et al. (44). First, the IBM allows cell shapes to be an emergent property rather than a constraint of the model. In particular, in contrast to the vertex and finite element models, the IBM does not require cells to be in confluent tissues, and thus appears well suited for detailed modelling of problems involving cell-cell interface dynamics such as intercalation, shear, loss of epithelial organization and delamination, with less need for explicit rules for processes such as T1 and T2 transitions. Second, unlike most vertex models, cells maintain a constant area in the absence of fluid sources or sinks, and thus the IBM enables detailed modelling of regulated growth and death processes. Third, the IBM also lends itself well to efficient numerical solution on periodic domains (54), which may be a sensible choice for considering a small snapshot of a larger tissue. Fourth, it is straightforward to explicitly incorporate subcellular structures such as the nucleus within the IBM through the use of additional immersed boundaries, for example to investigate the role of intracellular mechanical heterogeneity in morphogenetic processes where significant cell bending or deformation occurs (56). Finally, as already briefly mentioned, the extension to ~~3D~~three dimensions is conceptually straightforward without the need to specify large numbers of different types of vertex rearrangements (57).

We illustrate the utility of the IBM in figure 2b, which shows a simulation of epithelial cell packing where neighbouring cell shapes evolve in response to a central cell shrinking (for example, in preparation for extrusion from the sheet).

5. Three-dimensional models

Models of embryonic epithelia can reduce complexity by adopting a two-dimensional approximation (either in plane, as in convergent extension (32); or cross section, as in ventral furrow formation (34)). However, some morphogenetic events require a three-dimensional model. A number of studies have extended vertex models to three dimensions. These include models of systems where apical patterns of myosin appear to control morphogenesis that allow a two-dimensional sheet of cells to buckle out of the plane, as in the case of dorsal appendage formation (58), as well as models that represent cells as three-dimensional prisms (59–61). Examples of three-dimensional finite-element models include studies of neurulation ~~studies of~~ by Brodland and colleagues (62). Another relevant

model in this context was proposed by Savin et al. (63) to describe the development of gut looping. The IBM has not yet been applied three-dimensional cell populations, and existing software implementations are not straightforward to generalise to three dimensions (44).

We conclude by considering the sub-cellular element model (SEM), where now discrete elements are used to represent both the cell membrane and cytoplasm. The SEM was initially developed by Newman (64) as a flexible framework for simulating the detailed dynamics of emergent cell shape changes in response to mechanical stimuli. In the SEM, each cell is composed of a large, and possibly varying, number of small volumes of cytoplasm (or other organelles) called sub-cellular elements. Each sub-cellular element of a cell is modelled as a single point at its centre of mass, which changes position over time subject to three processes: (i) weak random fluctuations; (ii) elastic interaction with elements of the same cell; and (iii) elastic interaction with elements of other cells (figure 3a).

The motion of each sub-cellular element is subject to a strong viscous drag due to the surrounding cytoplasm. As with most cell-based models, it is assumed that viscous terms dominate inertial terms. The biomechanical properties of cells are encoded in elastic interactions between elements that are defined using phenomenological potential functions encoding close-range repulsion and medium-range attraction (65) for elements of the same or different cells. It is difficult to associate such functions directly with particular cytoskeletal components or other structural protein systems. However, computational studies of bulk properties at the tissue scale suggest that the precise functional form of the potential has little impact on the system dynamics (25,66).

By carrying out *in silico* bulk rheology experiments on a single cell over a time scale of around 10 seconds, it is possible to scale the parameters of the SEM such that its passive biomechanical properties are independent of the number of elements that make up each cell (66). These experiments follow a creep-stress protocol in which a constant extensile force is applied to a cell's surface while the opposite surface is fixed, before the extensile force is released and the strain is measured as the extension of the cell in the direction of the force, relative to its initial linear size (figure 3b). The SEM agrees qualitatively with *in vitro* rheology measurements (67), exhibiting a finite strain that plateaus after around one second, with complete recovery of the original cell shape after the force is released (66). Over longer timescales (100 seconds) cells instead respond actively to external stresses, for example by undergoing cytoskeletal remodelling, making them more fluid-like. This can be incorporated into the SEM by inserting and removing subcellular elements of a cell in regions of high and low stress, respectively (68).

To date, the SEM has not been widely used to study biological processes outside the area of epithelial morphogenesis. Christley et al. (69) developed a model of epidermal growth on a basal membrane that incorporates a simple algorithm for cell growth (through the incremental addition of sub-cellular elements) and division (through the redistribution of sub-cellular elements to two daughter cells) ~~that~~ coupled to a subcellular gene network representing intercellular Notch signalling. The SEM has also been coupled to a fluid flow model to simulate the attachment of platelets to damaged blood vessel walls in thrombus development (70). By using a detailed mechanical model of the platelets, the authors ~~determine the relationship between uncovered a dependence on the~~ platelet stiffness ~~to how~~ ~~and movement moves and rotates~~ in the fluid and, consequently, how ~~it~~ platelets attaches to injured sites on the vessel wall. In the context of developmental biology, the primary application of the SEM to date has been a computational study of primitive streak formation by Sandersius et al. (71). Like the IBM, the SEM enables straightforward inclusion of subcellular structures such as nuclei for the study of processes such as (pseudo)stratification; and cell rearrangements are emergent rather than imposed as constraints.

6. Incorporating signalling

While some morphogenetic processes can be modelled by assuming a specified patterning of cell mechanical properties (73,74), in the case of mechano-transduction the mechanism underlying such patterning may need to be treated explicitly. An appealing property of cell-based models is the ease with which they may be modified to incorporate such feedback. Vertex and cellular Potts models now frequently couple descriptions of morphogen transport and signalling to cell behaviour (61,75–78), while a recent vertex model of active cell intercalation during *Drosophila* germband extension incorporates an explicit description of planar cell polarity and medial Myosin II dynamics (79). A similar approach has been taken to describe the role of Myosin II patterning in driving intercalation during germband extension (80). Such biological detail is rarer in the more complex mechanocellular models described above; though recent examples include (44,69). Further development of increasingly detailed mechanocellular models will require the careful derivation of key relationships between the fluorescence intensity of relevant proteins and mechanical properties from live-imaging datasets.

7. Outlook

We conclude by highlighting some of the technical developments required to increase the utility of the cell-based models discussed above as computational tools for the study of epithelial morphogenesis.

Model choice and implementation

An increasingly important consideration is availability of software. At present Chaste (81) is the only open source simulation tool publically available for off-lattice models of cell populations, including vertex and finite-element models. Implementations of the IBM and SEM also exist within this framework. The more widespread availability of such tools and, in particular, the use of industrial-grade software engineering approaches to ensure robust, extensible code and reproducible results, are crucial as computational modelling evolves from a qualitative to a quantitative tool in cell biology. A technical requirement for this is the development and use of stable, accurate and efficient numerical algorithms for solving models.

Related to this problem is the choice of particular cell-based model for a given problem. The decision as to which is the best model to interrogate a specific research question is subjective, and often based on the experience of the modeller and the software they have access to obtaining and extending. Moreover, the issue is often exacerbated by the fact that it is difficult to accurately compare ~~between~~ different modelling approaches because the modeller cannot generally distinguish between differences that are due to model assumptions and those that arise from the specific details of the numerical implementation of the model (82). A systematic comparison of the relative strengths and weaknesses of each model, and the underlying biophysical assumptions, remains lacking; this would go a long way towards addressing the above problem. A rare example of such a comparison is the simulation study by Pathmanathan et al (25) ~~(CITE)~~; this type of analysis could be extended to other cell-based approaches.

Key challenges in future model development

The majority of existing models of epithelial morphogenesis neglect interactions with neighbouring tissues, yet there is increasing evidence for their importance. While a small number of theoretical studies have included an explicit representation of basement lamina or stromal tissue (83,84), further work is required to make progress in this area. In particular, the development of methodologies to interface models that include descriptions of cell shape, mechanics and biochemical signalling in different ways and on different scales, will

be crucial. As mentioned above, the extension of cell-based models to three dimensions is both an urgent requirement and technically challenging. An overarching question in this context is what physics needs to be included in a cell-based model, and how to implement this across different frameworks, as we continue to add greater amounts of biological detail. This requires balancing mechanical realism with computational tractability.

Integrating models and quantitative data

Recent years has witnessed dramatic changes in our ability to extract multiplex, quantitative data, on a range of spatio-temporal scales, from actomyosin dynamics within single cells, to tissue-level morphogenetic changes, including folding, bending and within plane reorganisation. A major remaining challenge for the modelling community is to understand how to best integrate and interpret these data ~~into~~ with cell-based modelling frameworks. A goal for the future should be a concrete pipeline that includes: data acquisition, analysis and fusion; model development, reduction and parameterisation; model validation / selection and the guidance of future experimental directions. Key challenges in this regard involve developing efficient methods for computational inference and experimental design, and designing standardised approaches to report uncertainty.

Summary

Here we have sought to provide some representative examples (in order of increasing complexity and geometric realism) that give a clear picture of developments to date, rather than an exhaustive list of models. These models are most suited for situations where we are particularly interested in capturing irregular cell shapes ~~that because they~~ are important for the system-level behaviour, such as bottle cells in gastrulating embryos (41). The development of such models, in combination with recent advances in the live imaging of embryogenesis and image analysis, means that the field is now in a position to develop and validate biologically realistic models in a quantitative manner. Having the ability to extract geometric and mechanical summary statistics from data and parameterise models in an integrated manner will be crucial if we are to exploit the full potential of combined experiment-modelling efforts.

Authors' contributions

A.G.F., F.C. and R.E.B. contributed to the development of the ideas presented in the paper and wrote the paper.

Competing interests

We have no competing interests.

Funding

F.C. is supported by an EPSRC-funded Systems Biology Doctoral Training Centre Studentship (EP/G03706X/1).

References

1. Oates AC, Gorfinkiel N, González-Gaitán M, Heisenberg C-P. Quantitative approaches in developmental biology. *Nat Rev Genet.* 2009;10(8):517–30.
2. Gorfinkiel N, Blanchard GB. Dynamics of actomyosin contractile activity during epithelial morphogenesis. *Curr Opin Cell Biol.* 2011;23(5):531–9.
3. Martin AC, Kaschube M, Wieschaus EF. Pulsed contractions of an actin–myosin network drive apical constriction. *Nature.* 2009;457(7228):495–9.
4. Blanchard GB, Kabla AJ, Schultz NL, Butler LC, Sanson B, Gorfinkiel N, et al. Tissue tectonics: morphogenetic strain rates, cell shape change and intercalation. *Nat Methods.* 2009;6(6):458–64.
5. Miller CJ, Davidson LA. The interplay between cell signalling and mechanics in developmental processes. *Nat Rev Genet.* 2013;14(10):733–44.
6. Hufnagel L, Teleman AA, Rouault H, Cohen SM, Shraiman BI. On the mechanism of wing size determination in fly development. *Proc Natl Acad Sci.* 2007;104(10):3835–40.
7. Vincent J-P, Fletcher AG, Baena-Lopez LAI. Mechanisms and mechanics of cell competition in epithelia. *Nat Rev Mol Cell Biol.* 2013;14(9):581–91.
8. Lecuit T, Lenne P-F. Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. *Nat Rev Mol Cell Biol.* 2007;8(8):633–44.
9. Lubarsky B, Krasnow MA. Tube morphogenesis. *Cell.* 2003;112(1):19–28.
10. Davidson L, von Dassow M, Zhou J. Multi-scale mechanics from molecules to morphogenesis. *Int J Biochem Cell Biol.* 2009;41(11):2147–62.
11. Kiehart DP, Galbraith CG, Edwards KA, Rickoll WL, Montague RA. Multiple forces contribute to cell sheet morphogenesis for dorsal closure in *Drosophila*. *J Cell Biol.* 2000;149(2):471–90.
12. Butler LC, Blanchard GB, Kabla AJ, Lawrence NJ, Welchman DP, Mahadevan L, et al. Cell shape changes indicate a role for extrinsic tensile forces in *Drosophila* germ-band extension. *Nat Cell Biol.* 2009;11(7):859–64.

Field Code Changed

Formatted: Font: (Default) Arial

13. Sugimura K, Lenne P-F, Graner F. Measuring forces and stresses in situ in living tissues. *Development*. 2016;143(2):186–96.
14. Davidson L, Keller R. Measuring mechanical properties of embryos and embryonic tissues. In: *Methods in Cell Biology*. Elsevier; 2007. p. 425–39.
15. Wyczalkowski MA, Chen Z, Filas BA, Varner VD, Taber LA. Computational models for mechanics of morphogenesis. *Birth Defects Res Part C Embryo Today Rev*. 2012;96(2):132–52.
16. Brodland GW. How computational models can help unlock biological systems. *Semin Cell Dev Biol*. 2015;47–48:62–73.
17. Tanaka S. Simulation frameworks for morphogenetic problems. *Computation*. 2015;3(2):197–221.
18. Budday S, Steinmann P, Goriely A, Kuhl E. Size and curvature regulate pattern selection in the mammalian brain. *Extreme Mech Lett*. 2015;4:193–8.
19. Allena R, Aubry D. An extensive numerical simulation of the cephalic furrow formation in *Drosophila* embryo. *Comput Methods Biomech Biomed Engin*. 2012;15(5):445–55.
20. Conte V, Munoz J, Miodownik M. A 3D finite element model of ventral furrow invagination in the *Drosophila melanogaster* embryo. *J Mech Behav Biomed Mater*. 2008;1(2):188–98.
21. Zamir EA, Srinivasan V, Perucchio R, Taber LA. Mechanical asymmetry in the embryonic chick heart during looping. *Ann Biomed Eng*. 2003;31(11):1327–36.
22. Hirashima T, Iwasa Y, Morishita Y. Dynamic modeling of branching morphogenesis of ureteric bud in early kidney development. *J Theor Biol*. 2009;259(1):58–66.
23. Longo D, Peirce SM, Skalak TC, Davidson L, Marsden M, Dzamba B, et al. Multicellular computer simulation of morphogenesis: blastocoel roof thinning and matrix assembly in *Xenopus laevis*. *Dev Biol*. 2004;271(1):210–22.
24. Honda H, Tanemura M, Yoshida A. Differentiation of wing epidermal scale cells in a butterfly under the lateral inhibition model - Appearance of large cells in a polygonal pattern. *Acta Biotheor*. 2000;48:121–36.
25. Pathmanathan P, Cooper J, Fletcher A, Mirams G, Murray P, Osborne J, et al. A computational study of discrete mechanical tissue models. *Phys Biol*. 2009;6(3):36001.
26. Smeets B, Odenthal T, Tijskens E, Ramon H, Van Oosterwyck H. Quantifying the mechanical micro-environment during three-dimensional cell expansion on microbeads by means of individual cell-based modelling. *Comput Methods Biomech Biomed Engin*. 2013;16(10):1071–84.
27. Mosaffa P, Asadipour N, Millán D, Rodríguez-Ferran A, J Muñoz J. Cell-centred model for the simulation of curved cellular monolayers. *Comput Part Mech*. 2015;2(4):359–70.
28. Davidson LA, Joshi SD, Kim HY, von Dassow M, Zhang L, Zhou J. Emergent morphogenesis: Elastic mechanics of a self-deforming tissue. *J Biomech*. 2010;43(1):63–70.

29. Fletcher AG, Osterfield M, Baker RE, Shvartsman SY. Vertex models of epithelial morphogenesis. *Biophys J*. 2014;106(11):2291–304.
30. Farhadifar R, Röper J-C, Aigouy B, Eaton S, Jülicher F. The Influence of Cell Mechanics, Cell-Cell Interactions, and Proliferation on Epithelial Packing. *Curr Biol*. 2007 Dec;17(24):2095–104.
31. Vogel V, Sheetz M. Local force and geometry sensing regulate cell functions. *Nat Rev Mol Cell Biol*. 2006;7(4):265–75.
32. Rauzi M, Lenne P-F, Lecuit T. Planar polarized actomyosin contractile flows control epithelial junction remodelling. *Nature*. 2010;468(7327):1110–4.
33. Odell G, Oster G, Burnside B, Alberch P. A mechanical model for epithelial morphogenesis. *J Math Biol*. 1980;9(3):291–5.
34. Odell GM, Oster G, Alberch P, Burnside B. The mechanical basis of morphogenesis. *Dev Biol*. 1981;85(2):446–62.
35. Rauzi M, Hočevár Brezavšček A, Zihnerl P, Leptin M. Physical models of mesoderm invagination in *Drosophila* embryo. *Biophys J*. 2013;105(1):3–10.
36. Brodland GW, Chen HH. The mechanics of heterotypic cell aggregates: insights from computer simulations. *J Biomech Eng*. 2000;122(4):402.
37. Steinberg MS. Reconstruction of tissues by dissociated cells. *Science*. 1963;141(3579):401–8.
38. Brodland WG, Chen HH. The mechanics of cell sorting and envelopment. *J Biomech*. 2000;33(7):845–51.
39. Brodland GW, Veldhuis JH. Assessing the mechanical energy costs of various tissue reshaping mechanisms. *Biomech Model Mechanobiol*. 2012;11(8):1137–47.
40. Lynch HE, Veldhuis J, Wayne Brodland G, Shane Hutson M. Modeling cell elongation during germ band retraction: cell autonomy versus applied anisotropic stress. *New J Phys*. 2014;16(5):55003.
41. Tamulonis C, Postma M, Marlow HQ, Magie CR, de Jong J, Kaandorp J. A cell-based model of *Nematostella vectensis* gastrulation including bottle cell formation, invagination and zippering. *Dev Biol*. 2011;351(1):217–28.
42. Perrone MC, Veldhuis JH, Brodland GW. Non-straight cell edges are important to invasion and engulfment as demonstrated by cell mechanics model. *Biomech Model Mechanobiol*. 2016;15(2):405–18.
43. Ishimoto Y, Morishita Y. Bubbly vertex dynamics: A dynamical and geometrical model for epithelial tissues with curved cell shapes. *Phys Rev E*. 2014;90(5).
44. Tanaka S, Sichau D, Iber D. LBIBCell: a cell-based simulation environment for morphogenetic problems. *Bioinformatics*. 2015;31(14):2340–7.
45. Rejniak K. A computational model of the mechanics of growth of the villous trophoblast bilayer. *Bull Math Biol*. 2004;66(2):199–232.

46. Rejniak KA. An immersed boundary framework for modelling the growth of individual cells: An application to the early tumour development. *J Theor Biol.* 2007;247(1):186–204.
47. Rejniak KA. A single-cell approach in modeling the dynamics of tumor microregions. *Math Biosci Eng.* 2005;2(3):643–55.
48. Dillon R, Owen M, Painter K. A single-cell-based model of multicellular growth using the immersed boundary method. In: Khoo BC, Li Z, Lin P, editors. *Moving Interface Problems and Applications in Fluid Dynamics.* 2008.
49. Peskin CS. The immersed boundary method. *Acta Numer.* 2002;11:479–517.
50. Peskin CS. Flow patterns around heart valves: A numerical method. *J Comput Phys.* 1972;10(2):252–71.
51. Jadhav S, Eggleton CD, Konstantopoulos K. A 3-D Computational Model Predicts that Cell Deformation Affects Selectin-Mediated Leukocyte Rolling. *Biophys J.* 2005;88(1):96–104.
52. Le DV, White J, Peraire J, Lim KM, Khoo BC. An implicit immersed boundary method for three-dimensional fluid–membrane interactions. *J Comput Phys.* 2009;228(22):8427–45.
53. Rejniak KA. An immersed boundary framework for modelling the growth of individual cells: An application to the early tumour development. *J Theor Biol.* 2007;247(1):186–204.
54. Mittal R, Iaccarino G. Immersed boundary methods. *Annu Rev Fluid Mech.* 2005;37(1):239–61.
55. Dillon R, Othmer HG. A mathematical model for outgrowth and spatial patterning of the vertebrate limb bud. *J Theor Biol.* 1999;197(3):295–330.
56. Panousopoulou E, Green JBA. Invagination of ectodermal placodes is driven by cell intercalation-mediated contraction of the suprabasal tissue canopy. Nusse R, editor. *PLOS Biol.* 2016;14(3):e1002405.
57. Honda H, Tanemura M, Nagai T. A three-dimensional vertex dynamics cell model of space-filling polyhedra simulating cell behavior in a cell aggregate. *J Theor Biol.* 2004;226(4):439–53.
58. Osterfield M, Du X, Schüpbach T, Wieschaus E, Shvartsman SY. Three-dimensional epithelial morphogenesis in the developing *Drosophila* egg. *Dev Cell.* 2013;24(4):400–10.
59. Honda H, Motosugi N, Nagai T, Tanemura M, Hiiragi T. Computer simulation of emerging asymmetry in the mouse blastocyst. *Development.* 2008;135(8):1407–14.
60. Hannezo E, Prost J, Joanny J-F. Theory of epithelial sheet morphology in three dimensions. *Proc Natl Acad Sci.* 2014;111(1):27–32.
61. Okuda S, Inoue Y, Watanabe T, Adachi T. Coupling intercellular molecular signalling with multicellular deformation for simulating three-dimensional tissue morphogenesis. *Interface Focus.* 2015;5(2):20140095.

62. Brodland GW, Chen X, Lee P, Marsden M. From genes to neural tube defects (NTDs): Insights from multiscale computational modeling. *HFSP J.* 2010;4(3–4):142–52.
63. Savin T, Kurpios NA, Shyer AE, Florescu P, Liang H, Mahadevan L, et al. On the growth and form of the gut. *Nature.* 2011;476(7358):57–62.
64. Newman TJ. Modeling multicellular systems using subcellular elements. *Math Biosci Eng.* 2005;2(3):613–24.
65. Morse PM. Diatomic molecules according to the wave mechanics. II. Vibrational levels. *Phys Rev.* 1929;34(1):57–64.
66. Sandersius SA, Newman TJ. Modeling cell rheology with the Subcellular Element Model. *Phys Biol.* 2008;5(1):15002.
67. Wottawah F, Schinkinger S, Lincoln B, Ananthakrishnan R, Romeyke M, Guck J, et al. Optical rheology of biological cells. *Phys Rev Lett.* 2005;94(9).
68. Sandersius SA, Weijer CJ, Newman TJ. Emergent cell and tissue dynamics from subcellular modeling of active biomechanical processes. *Phys Biol.* 2011;8(4):45007.
69. Christley S, Lee B, Dai X, Nie Q. Integrative multicellular biological modeling: a case study of 3D epidermal development using GPU algorithms. *BMC Syst Biol.* 2010;4(1):107.
70. Sweet CR, Chatterjee S, Xu Z, Bisordi K, Rosen ED, Alber M. Modelling platelet-blood flow interaction using the subcellular element Langevin method. *J R Soc Interface.* 2011;8(65):1760–71.
71. Sandersius SA, Chuai M, Weijer CJ, Newman TJ. A ‘chemotactic dipole’ mechanism for large-scale vortex motion during primitive streak formation in the chick embryo. *Phys Biol.* 2011;8(4):45008.
72. Milde F, Tauriello G, Haberkern H, Koumoutsakos P. SEM++: A particle model of cellular growth, signaling and migration. *Comput Part Mech.* 2014;1(2):211–27.
73. Kursawe J, Brodskiy PA, Zartman JJ, Baker RE, Fletcher AG. Capabilities and limitations of tissue size control through passive mechanical forces. *PLOS Comput Biol.* 2015;11(12):e1004679.
74. Osterfield M, Du X, Schüpbach T, Wieschaus E, Shvartsman SY. Three-dimensional epithelial morphogenesis in the developing *Drosophila* egg. *Dev Cell.* 2013;24(4):400–10.
75. Aegerter-Wilmsen T, Smith AC, Christen AJ, Aegerter CM, Hafen E, Basler K. Exploring the effects of mechanical feedback on epithelial topology. *Development.* 2010;137(3):499–506.
76. Schilling S, Willecke M, Aegerter-Wilmsen T, Cirpka OA, Basler K, von Mering C. Cell-sorting at the A/P boundary in the *Drosophila* wing primordium: a computational model to consolidate observed non-local effects of Hh signaling. *PLoS Comput Biol.* 2011;7(4):e1002025.

77. Salbreux G, Barthel LK, Raymond PA, Lubensky DK. Coupling mechanical deformations and planar cell polarity to create regular patterns in the zebrafish retina. *PLoS Comput Biol.* 2012;8(8):e1002618.
78. Hester SD, Belmonte JM, Gens JS, Clendenon SG, Glazier JA. A multi-cell, multi-scale model of vertebrate segmentation and somite formation. *PLoS Comput Biol.* 2011;7(10):e1002155.
79. Lan H, Wang Q, Fernandez-Gonzalez R, Feng JJ. A biomechanical model for cell polarization and intercalation during *Drosophila* germband extension. *Phys Biol.* 2015;12(5):56011.
80. Tetley RJ, Blanchard GB, Fletcher AG, Adams RJ, Sanson B. Unipolar distributions of junctional Myosin II identify cell stripe boundaries that drive cell intercalation throughout *Drosophila* axis extension. *eLife.* 2016;5.
81. Mirams GR, Arthurs CJ, Bernabeu MO, Bordas R, Cooper J, Corrias A, et al. Chaste: an open source C++ library for computational physiology and biology. Prlic A, editor. *PLoS Comput Biol.* 2013;9(3):e1002970.
82. Osborne JM, Fletcher AG, Pitt-Francis JM, Maini PK, Gavaghan DJ. Comparing individual-based approaches to modelling the self-organization of multicellular tissues [Internet]. 2016. (bioRxiv preprint). Available from: <http://dx.doi.org/10.1101/074351>
83. Marin-Riera M, Brun-Usan M, Zimm R, Välikangas T, Salazar-Ciudad I. Computational modeling of development by epithelia, mesenchyme and their interactions: a unified model. *Bioinformatics.* 2015;32(2):219–25.
84. Dunn S-J, Fletcher AG, Chapman SJ, Gavaghan DJ, Osborne JM. Modelling the role of the basement membrane beneath a growing epithelial monolayer. *J Theor Biol.* 2012;298:82–91.
85. Hirashima T, Iwasa Y, Morishita Y. Dynamic modeling of branching morphogenesis of ureteric bud in early kidney development. *J Theor Biol.* 2009;259(1):58–66.
86. Atwell K, Qin Z, Gavaghan D, Kugler H, Hubbard EJA, Osborne JM. Mechano-logical model of *C. elegans* germ line suggests feedback on the cell cycle. *Development.* 2015;142(22):3902–11.
87. Rauzi M, Verant P, Lecuit T, Lenne P-F. Nature and anisotropy of cortical forces orienting *Drosophila* tissue morphogenesis. *Nat Cell Biol.* 2008;10(12):1401–10.
88. Tamulonis C, Postma M, Marlow HQ, Magie CR, de Jong J, Kaandorp J. A cell-based model of *Nematostella vectensis* gastrulation including bottle cell formation, invagination and zippering. *Dev Biol.* 2011;351(1):217–28.

Figure captions

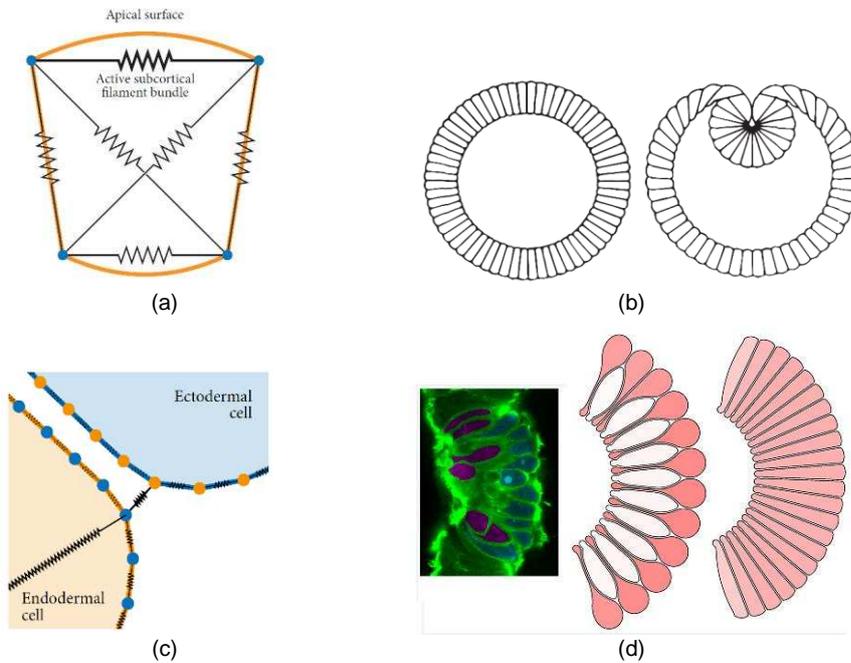


Figure 1. Polygon-based models of gastrulation. (a) Model of an epithelial cell cross-section, incorporating viscoelastic cytoskeletal elements and active apical contractility. Adapted from (34). (b) Simulating this model from a cylindrically symmetric configuration, while imposing a constant inner volume representing a yolk-filled lumen, leads to behaviour redolent of *Drosophila* ventral furrow formation. Reproduced from (34). (c) Model of endodermal (yellow) and ectodermal (blue) cells in the *Nematostella vectensis* blastula, showing contractile elements (black) within and between cells. Adapted from (41). (d) Simulation of bottle cell formation with this model. Left: In vivo image showing bottle (red) and squat (blue) cells. Middle/right: Two model configurations, where endodermal cells are bound apically only (middle) or also basally (right), resulting in distinct cell shapes during apical constriction. Reproduced from (41).

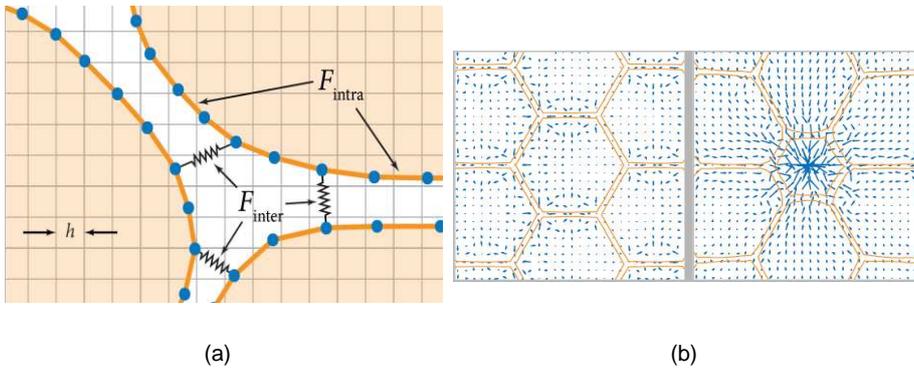


Figure 2. The immersed boundary method. (a) Model schematic, showing an off-lattice discretization (blue nodes) of the immersed boundaries representing individual cells (orange) and the regular grid use to discretize the fluid flow problem. Adhesion links exist between blue nodes within each immersed boundary, as well as between neighbouring boundaries. (b) A simulation viewed at two time points, showing the computed fluid velocity (blue arrows) and immersed boundary geometry (orange lines). Immersed boundaries are initially at rest in a honeycomb pattern before reacting to the central cell reducing its surface area.

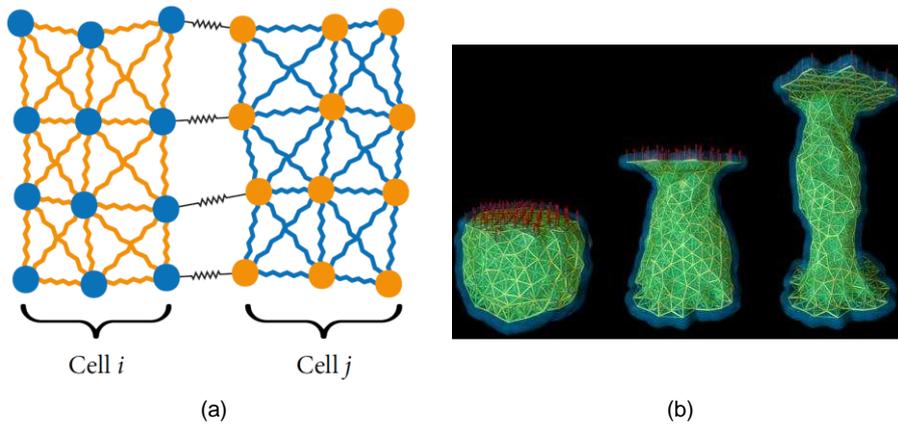


Figure 3. The sub-cellular element model. (a) Model schematic diagram showing two cells and a subset of the intra- and intercellular interactions between their elements. (b) SEM simulation under a creep-stress protocol. Reproduced from (68). © IOP Publishing. Reproduced with permission. All rights reserved.

<u>Modelling approach</u>	<u>Example applications</u>	<u>Strengths</u>	<u>Limitations</u>
<u>Continuum models</u>	<u>Brain cortical folding (18)</u> <u>Cephalic furrow formation (19)</u> <u>Ventral furrow formation (20)</u>	<u>Strong mathematical foundation; typically few parameters; well placed to study buckling and folding phenomena</u>	<u>Difficult to incorporate cell-level heterogeneity or subcellular processes</u>
<u>Lattice-based models (CA, CPM)</u>	<u>Epiboly (23)</u> <u>Branching morphogenesis (85)</u>	<u>Computationally cheap; straightforward to simulate many cells</u>	<u>Risk of lattice anisotropies and cell fragmentation; difficult to relate parameters to experimentally accessible quantities</u>
<u>Off-lattice cell-centre models</u>	<u>C. elegans germ line (86)</u>	<u>More physically motivated and easily parameterised than lattice-based models</u>	<u>More computationally costly than lattice-based models</u> <u>Lack explicit description of cell shape dynamics</u>
<u>Vertex models</u>	<u>Tissue size regulation (73,75)</u> <u>Germ-band extension (80,87)</u>	<u>Explicitly incorporate cell neighbour rearrangements; straightforward to generate experimentally testable summary statistics</u>	<u>Typically neglect cell-matrix adhesion, medial actomyosin contractility, active cytoskeletal remodelling</u>
<u>Viscoelastic models</u>	<u>Ventral furrow formation (33–35)</u> <u>Cell sorting (38)</u> <u>Germ-band retraction (40)</u>	<u>Include active cytoskeletal remodelling</u>	<u>Like vertex models, require cells to be in confluent tissues</u>
<u>'Multi-node' / curved edge models</u>	<u>Gastrulation (88)</u> <u>Cell sorting (42)</u>	<u>Detailed description of cell shape dynamics</u>	<u>More computationally costly than vertex and 'finite element' models</u>
<u>IBM</u>	<u>Limb bud morphogenesis (55)</u> <u>Turing patterns (44)</u>	<u>Do not require confluent tissues; allow detailed modelling of regulated growth and death processes; straightforward to incorporate subcellular structures</u>	<u>Unclear how to estimate 'fluid' properties from biological data; require sophisticated numerical solvers to avoid fluid 'leakage'</u>
<u>SEM</u>	<u>Primitive streak formation (71)</u>	<u>Allow detailed and emergent cell shape changes in response to mechanical stimuli</u>	<u>Computationally intensive; difficult to associate interactions functions directly with particular cytoskeletal components</u>

Table 1. Summary of applications to date of different modelling approaches for epithelial tissue morphogenesis. CA: Cellular automata. CPM: Cellular Potts model. IBM: Immersed boundary method. SEM: Subcellular element model.