Mechanics of Tissues

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Final Report

M. L. Manning (Syracuse University)
H. López-Schier (Helmholtz Zentrum München)
P. Ziherl (University of Ljubljana & Jožef Stefan Institute)

Summary

Organized within the ESF Research Networking Programme *Quantitative Models of Cellular and Developmental Biology*, the workshop "Mechanics of Tissues" held at the Jožef Stefan Institute in Ljubljana, Slovenia, October 29-30 2012 was a focused event dedicated to models of simple biological tissues. Over the past decade the interest in the mechanics of tissues has steadily grown, and the physical forces and processes involved are increasingly more appreciated. The diversity of theories proposed, developed, and tested has increased considerably as well. The aim of the workshop was to establish some degree of correspondence between the various models, thereby singling out the dominant factors that govern tissue shape and structure.

The workshop brought together a group of expert biologists, bioengineers, and physicists, most of whom are theorists. Also present were experimentalists who provided an insight into the modern *in vitro* and *in vivo* techniques. The scientific program consisted of 16 invited talks and of a round table. The majority of the talks revolved around embryonic tissues and epithelia, covering their behavior at various levels ranging from genes and signaling to cell packing and topological structure of tissues. At the round table, we discussed the apparent and factual disagreements as well as the complementarity and the common features of the different models. Also identified were several outstanding unresolved questions.

Despite the short duration the workshop provided an environment for intense discussions between the participants and the questions addressed will undoubtedly help steering the future development of the field. There were a total of 22 participants at the event. As most of them are from EU, we are hopeful that the workshop will lead to new intra-European research collaborations in theoretical developmental biophysics.

Description of Scientific Content

During the past decade, the understanding of mechanics of many biological structures and processes at the level of tissues has progressed steadily. Supported by the insight provided by advanced experimental techniques, the theories and the numerical models proposed have become increasingly more elaborate so as to accurately capture the observations. Some of the complexity built into the physical models is undoubtedly needed to describe the coupled workings of the mechanical, biochemical, and genetic factors. Yet certain theoretical features presently deemed necessary may prove subdominant as some aspects of the behavior of tissues may emerge as a collective effect often rooted in general considerations related, e.g., to geometry. At the same time, these advances are ever more appreciated by the experimentalists.

The time is ripe for a review of the state-of-the-art in the field and for an assessment of outstanding challenges. As a part of activities of the ESF Research Networking Programme *Quantitative Models of Cellular and Developmental Biology* (QuanTissue; `http://www.quantissue.eu`), the two-day workshop "Mechanics of Tissues" brought together a small group of biologists, bioengineers, and physicists who discussed recent developments in the mechanical properties of cells and tissues, cell migration, interaction of cells with substrate, structure of tissues, artificial tissues, and pattern formation and morphogenesis of tissues and related cellular structures. The workshop consisted of 16 invited talks and of a round table, and it provided a stimulating atmosphere for exchange of ideas.

The workshop was opened by **H. López-Schier**, the chair of the QuanTissue Steering Committee. He introduced the network and outlined the aim of the meeting. In the first talk, **E. Farge** (Institut Curie) discussed mechanogenetic coupling in embryonic development in Drosophila. This presentation touched upon a poorly understood phenomenon of mechanotransduction in evolution. The data presented suggest that mechanical strain caused by morphogenetic movements may induce mesoderm differentiation, and that beta-catenin may be involved in the process. Farge proposed that the conserved role of the beta-catenin mechanosensitive pathway in the establishment of mesoderm identity at such large evolutionary distances may imply mesoderm speciation as mechanically induced by gastrulation morphogenetic movements back to the last common Protostome-Deuterostome ancestor.

D. Cuvelier described the *in vitro* biomechanics of multicellular aggregates done in the group of F. Brochard-Wyart at Institut Curie. In their pursuit of viscoelastic properties of the aggregates, they carried out a series of pipette aspiration experiments which show that the aggregates are reinforced as the pressure is increased. This may be due to a mechanosensitive active response of the cell cortex. The spreading experiments showed that the aggregate produces a precursor film which may be in either dense monolayer state or consist of individual cells. This behavior is reminiscent of the noninvasive-metastatic transition seen in tumors.

J.-L. Maître (Institute of Science and Technology Austria) presented new results which demonstrate that the functions of cell adhesion and cortex tension in cell-cell contact formation are quite different. In some coarse-grained mechanical models, these two effects are combined into a single quantity referred to as the effective tension. Maître's experimental evidence from zebrafish gastrulation suggests that this view may not be correct: Cortex tension controls cell-cell contact expansion by modulating interfacial tension at the contact whereas adhesion has little direct function in contact expansion. Instead it is needed to mechanically couple the cortices of adhering cells at their contacts, allowing cortex tension to control contact expansion.

G. Salbreux (Max Planck Institute für Physik komplexer Systeme) presented the results of a model of cone mosaic formation in teleost fish retina. This model is based on the coupling of the planar cell polarity and anisotropic mechanical stresses at the level of tissue. In particular, the distribution of planar polarity proteins is assumed to affect the adhesive properties of cell-cell interfaces, whereas large scale stresses deform the cell and influence the polarity. Using numerical simulations, he showed that the global mechanical tension in the retina affects the rearrangement of cells and leads to cone cell ordering.

H. López-Schier (Helmholtz Zentrum München) described the high-resolution videomicroscopy studies of planar cell inversion in immature hair cells within the plane-polarized sensory epithelium of the zebrafish lateral line, which suggest that this process is biased by the orientation of the mitotic division that generates the inverting cell pair. These findings show that this carefully choreographed cellular rearrangement in a confined space occurs through a combination of strong homotypic cell adhesion and fast heterotypic junction remodeling.

F. C. MacKintosh (Vrije Universiteit Amsterdam) discussed the role of active stresses in intra- and extracellular networks. He described recent advances both in theoretical modeling of such networks as well as in experiments on reconstituted *in vitro* acto-myosin networks and living cells. He showed how such internal force generation by motors can lead to dramatic mechanical effects, including strong mechanical stiffening, and how the collective activity of myosin motors generically organizes actin filaments into contractile structures. This can be understood in terms of the highly asymmetric load response of actin filaments: They can support large tensions, but they buckle easily under piconewton compressive loads.

J. J. Muñoz (Universitat Politècnica de Catalunya) proposed that the separation of the cell activity and its rheological behavior often assumed in mechanical models is probably too strong a simplification. He presented a simple evolution law for the remodeling process which strongly affects the viscoelastic properties of the tissue and directly relates the classical viscoelastic constants of the material to the internal remodeling rate of the cell. He used this model to interpret the experimental tests of tissues undergoing reversible softening and fluidization.

W. Supatto (Ecole Polytechnique) reported on recent advances in multiphoton microscopy to perform multicolor and fast imaging deep inside embryos. He presented the implementation and application of two-photon light-sheet microscopy, combining two-photon excited fluorescence with orthogonal illumination, and he demonstrated its high spatial resolution deep inside biological tissues, high acquisition speed and low phototoxicity. These improvements were illustrated with live imaging of embryonic tissues.

P. Silberzan (Institut Curie) described recent experiments of the evolution of a physically confined proliferating epithelium. The boundary conditions are set by a very robust surface treatment preventing cell adhesion. Using different geometries, this experiment can re-create cell movements similar to those seen in morphogenesis and the evolution of a 2D monolayer toward 3D structures. Also discussed was the importance of a pluricellular peripheral acto-myosin "cable" in these processes.

S. Svetina (University of Ljubljana & Jožef Stefan Institute) offered a bilayer-couple interpretation of epithelial invagination in sea urchin. The support for this analogy lies in the general energy functional for the mechanical behavior of closed multilamellar membranes. The sea urchin blastula wall can be modeled as a closed laminar membranous system of three layers – the monocellular sheet and the extracellular apical lamina and hyaline layers. This theory can reproduce the continuous shape transition from a sphere to an invaginated gastrula quite similar to that seen in sea urchin embryo, which suggests that the process may be in part due to collective mechanics of the embryonic epithelium.

A.-L. Routier-Kierzkowska (University of Bern) was the only speaker specialized in plant rather than animal tissues. She described the Cellular Force Microscopy (CFM), a micro-indentation technique for *in-vivo* stiffness measurements of individual cells. Depending on experimental conditions, the stiffness measured by CFM can provide an insight into cell turgor, local wall elasticity as well as cell wall strength. By comparing the measurements to simulations, the different contributions to the measured stiffness can be identified. Another approach to quantify cell wall elasticity is to track deformation in response to changes in turgor pressure. These experiments show that the shoot meristem is divided into zones of distinct elastic properties.

J. Galle (University of Leipzig) presented an individual cell-based computational model of the intestinal tissue. The model is capable of quantitatively reproducing a comprehensive set of experimental data on intestinal cell organization including intestinal organoid formation *in silico*. One of the main ingredients of this model is a flexible basal membrane which assigns a bending modulus to the organoid surface and which can be re-organized by cells attached to it depending on the cells' differentiation status, thereby determining the morphology of the epithelium. Based on the predictions of this model, Galle hypothesized that local tissue curvature is a key regulatory factor regarding stem cell organization in the intestinal tissue by controlling Paneth cell specification.

T. Risler (Institut Curie) discussed the stability of the interface between a multilayered epithelium and its adjacent stroma. By treating the epithelium as a viscous fluid with cell division, he found a novel hydrodynamic instability that leads to the formation of fingering protrusions of the epithelium into the stroma. This instability provides a physical insight into the undulation of the epithelium-stroma interface, which may potentially describe tissue dysplasia leading to cancerous invasion. One aspect of homeostasis is the regulation of tissue pressure, which can lead to a competition for space of purely mechanical origin and be an underlying mechanism for tumor growth. He showed that there exists a critical tumor size that must be overcome by metastases to nucleate macroscopic secondary tumors. This property qualitatively explains the observed size distributions of metastases.

S. Hilgenfeldt (University of Illinois at Urbana-Champaign) addressed the common structural and morphological features of biological tissues, foams, and other cellular materials. Many of them are universal and may be a consequence of mathematical necessity rather than due to specific physical interactions or biological processes. He explored these questions with cell shapes and structures of epithelial tissues to find that quantitative modeling is possible with only minimal reference to biological processes. He showed that the highly ordered epithelium of the Drosophila eye can be described in detail using concepts of energy functional minimization including cell elasticity and cell-cell adhesion. Conversely, the structure of the Drosophila wing is captured by a statistical mean-field approach derived from a local model, which is applicable to a much larger class of both living and inanimate systems.

M. L. Manning (Syracuse University) exploited analogies with foams and supercooled fluids to develop two models for the emergent mechanical behavior in zebrafish tissues. The first "dynamic" model treats cells as individual units and introduces interactions between cells to capture intracellular degrees of freedom. This minimal model shows that dynamics of cells in the interior of a tissue are similar to the dynamics of molecules in supercooled fluids near a glass transition. A second "steady-state" model studies ensembles of mechanically stable "jammed" cell packings and makes verifiable predictions about cell shape changes during organogenesis and at tissue boundaries. These results suggest that embryonic tissues are a strange viscoelastic "material": While the bulk properties are fairly generic, programmed cell shape changes can lead to interesting properties near surfaces.

P. Ziherl (University of Ljubljana & Jožef Stefan Institute) theoretically explored the flat and the corrugated epithelial morphologies in the model where the energy of epithelial cells is associated with the cell membrane alone and is based on cortex tension, interfacial tension, and adhesion. By numerically investigating the shapes of model 2D epithelia consisting of rectangular cells of fixed cross-section area, he studied the impact of the constriction of the cell side of largest tension on the formation of the ventral furrow in the embryonic *Drosophila* epithelium. Also discussed were the flat and corrugated periodic states of a free-standing epithelium.

Round Table

When planning the program of the workshop, we felt that the talks themselves will stimulate lively discussions. This was indeed the case and the debates continued during the breaks and meals, yet the round table at the end of the workshop did serve its purpose well. The questions raised included the establishment of length scales in tissue morphology, the problem of reference state in physics-based theories, the nature of (and the need for) bulk and surface elasticity of cells, the passive rheological properties of multicellular aggregates, the active contractility of single cells, the feedback between biochemical and mechanical signaling, the energetics of morphogenesis, the interactions between the extracellular matrix and cells, the statistical properties of cell shapes in epithelial layers etc. Notes taken were distributed to the participants. At the end of the round table, we shared the impression that in the future some of the complexity characteristic for the current theoretical insight of tissue mechanics may unfold, leading to simpler and more transparent models.

Assessment of Results and Impact

We are convinced that the workshop provided an intimate, top-level forum for discussions amongst theoretical and experimental researchers and helped provide new insight into tissue mechanics. This view is supported by the interest expressed by researchers contacted during the preliminary planning, by their willingness to participate and to present their recent results, and by their overall positive response during and after the workshop.

This workshop was important for participants because it enhanced our awareness of the advantages (and disadvantages) of different models for the mechanics of tissues, provided an exciting synopsis of the progress possible with modern experimental techniques, and highlighted the important role of coupling between the biochemical and genetic factors and the mechanical behavior of tissues. The three subgroups of participants – experimentalists, theorists, and modelers – benefited from the uninhibited exchange of ideas and views, learning from each other about the details and the speculations usually not published in papers. Also important is that we now better appreciate the correspondence of the models operating at various scales and levels of refinement, recognizing the need for and the limitations of the effective parameters of these models. In particular, there was an ongoing useful discussion of how the rheology (mechanical response) of single cells is best incorporated into coarse-grained models for large numbers of cells in tissues.

The workshop contributed considerably towards the scientific goals of the QuanTissue. First, most of the participants have a background in physics or engineering rather than in biology. The workshop helped to introduce and integrate them into the various activities of QuanTissue, thereby making a stronger connection between the existing biologists in the networks and researchers at the forefront of physics and biophysics research. In particular, the workshop hosted a large number of researchers focused on biophysical aspects of developmental biology. Due to recent advances in experimental techniques (such as confocal and traction force microscopy, tissue rheometers, and genetic manipulation of mutants and knockdowns), a better understanding of forces in development is now an exciting possibility. The workshop highlighted the fact that a close and longstanding collaboration between developmental biologists is essential for understanding the interplay between forces and genetics that governs the highly complex pattern formation driving development. In addition, several talks and discussions at the workshop emphasized that similar techniques and models might be applied to the challenging problems of wound healing and morphogenesis.

Second, the workshop helped to establish connections which may well mature into research collaborations between the EU-based laboratories (either within QuanTissue or within some other formal framework) as well as between the EU and US groups. These connections will strengthen the activity in the field and make QuanTissue a more interdisciplinary and global effort.

The participants shared the impression that the environment provided by the workshop was rather unique compared to the other scientific events, and the organizers received several emails from participants after the workshop stating that it was productive for developing new collaborations and developing new directions for research. With its strong physics- and theory-oriented program, the workshop did fulfill its mission. We believe that in a few years, a similar meeting should be organized to promote the development of the field at a stage when rapid scientific communication is essential. In the future meeting, members of laboratories who could not be present at the Ljubljana workshop (especially researchers from EMBL Heidelberg and UK laboratories) should be invited. Also invited should be researchers from overseas (USA, Japan, Singapore…). We are convinced that the future workshop will be appreciated very much by the community of developmental biologists and biophysicists interested in tissues.

Final Programme

Monday October 29 2012

9.20- 9.30	Opening: H. López-Schier
9.30-10.00	E. Farge: *Mechanogenetic reciprocal coupling in embryonic development and evolution*
10.00-10.30	D. Cuvelier: *Mechanosensitivity and motility of cellular aggregates*
10.30-11.00	*coffee break*
11.00-11.30	J.-L. Maître: *Adhesion functions in cell sorting by mechanically coupling the cortices of adhering cells*
11.30-12.00	G. Salbreux: *Cone mosaic formation in the zebrafish retina*
12.00-12.30	H. López-Schier: *Planar cell inversion: The dance of two cells in a confined space*
12.30-14.00	*lunch*
14.00-14.30	F. C. MacKintosh: *Active stresses and self-organization in intra/extracellular networks*
14.30-15.00	J. J. Muñoz: *Rheological models for cell activity*
15.00-15.30	W. Supatto: *Advanced multiphoton microscopy for live imaging of embryonic morphogenesis: Multicolor and light-sheet illumination*
15.30-16.00	*coffee break*
16.00-16.30	P. Silberzan: *Growth of an epithelium under physical constraints*
16.30-17.00	S. Svetina: *Bilayer couple interpretation of epithelial invagination*
20.00-	*dinner*

Tuesday October 30 2012

9.00- 9.30	A.-L. Routier-Kierzkowska: *New methods for probing cell mechanics in growing plant tissues*
9.30-10.00	J. Galle: *On the biomechanics of stem cell niche formation in the gut: Modelling growing organoids*
10.00-10.30	T. Risler: *Undulation instability of epithelial tissues and metastases' nucleation*
10.30-11.00	*coffee break*
11.00-11.30	S. Hilgenfeldt: *Mechanics and statistics of morphology: Tissues, packings, and tilings*
11.30-12.00	M. L. Manning: *Emergent mechanical behavior in embryonic tissues*
12.00-12.30	P. Ziherl: *Buckling of model epithelia*

12.30-14.00 *lunch*

14.00-15.30 Round table

15.30 Closing