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Quantitative microscopy and imaging tools for the mechanical analysis of morphogenesis

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Abstract

The importance of mechanical signals during embryogenesis and development, through both intercellular and extracellular signals, are coming into focus. It is widely hypothesized that physical forces help to guide the shape, cellular differentiation and the patterning of tissues. To test these ideas many classical engineering principles and imaging technologies are being adapted. Recent advances in microscopy, mechanical testing and genetic and pharmacological techniques, alongside computational models are helping to dissect the activity of mechanical signals in development at the cellular and molecular level. These inroads are permitting the study of mechanical changes in tissue structure and stiffness, and will provide deeper insights into the role of mechanics in both developmental biology and disease.

Keywords

deformation; cell shape change; tissue and cellular mechanics; stress; traction; modulus; elasticity; viscoelasticity

Introduction

Quantitative descriptions of motion and deformation are the foundation for any biomechanical analysis of morphogenesis. These descriptions of structures and their deformations provide a framework to understand the physics of biological structures as they react to force and mechanical stress. Optical microscopy and other imaging techniques are the core elements of devices that probe mechanical properties of materials, providing researchers with the ability to apply defined forces or deformations to biological samples and to investigate the transmission of mechanical strain and stress throughout a tissue. Imaging can provide precise descriptions of variations in tissue structure but can also serve as a tool to both interrogate gene expression and manipulate protein function. The use of light-based tools to manipulate signaling pathways and the use of fluorescence-based biosensors are revolutionizing the field of biomechanics providing for the first time the ability to manipulate mechanics and stimulate molecular signaling pathways while simultaneously measuring the response of embryonic tissues.

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Whereas simple physical models can be used to describe forces and deformation acting on simple materials and structures (see Box), it is more challenging to formulate and test predictive models that include the heterogeneous and dynamic environments of cells, tissues and developing embryos. The mechanics of these complex biological structures can include both viscous and elastic properties [2]. Multidisciplinary efforts from a number of groups have developed new techniques to study these complex mechanical structures. These efforts have driven improvements in understanding regulation and consequences of single cell mechanics [3, 4], the role of tissue-level mechanics in disease [5, 6], the role of mechanics during morphogenesis [7-9] and the influence of cell and tissue mechanics on embryonic patterning [10-13]. A combination of modeling and experimental measurements of bulk properties, along with high-resolution analyses using confocal imaging and mechanical micromanipulation are helping to elucidate the role of mechanics during development. Ultimately, efforts to understand the evolution of embryonic shape from the elementary principles of mechanics will need to consider dynamic developmental programs that include heterogeneous mechanical properties of cells and tissues and how those developmental programs are influenced by differentiation and feedback signals.

In this review we present the role of optical techniques in biomechanics, survey less familiar imaging modalities and highlight recent advances in manipulating mechanics and controlling signaling pathways relevant to studies of morphogenesis and development.

Mechanical measurements of tissue and cellular components

Optical techniques are key elements of biomechanical measurements and are critically important to interpreting experimental results. One of the first direct mechanical tests to be scaled and modified to tissue studies uses a simple deflecting beam to compress isolated embryonic tissues [14]. The deflecting beam consists of an optical fiber whose lateral displacement, measured by a sensitive quadrant detector, acts as the spring to apply a defined force to the face of regularly shaped tissue explants (figure 1a). This biomechanical test, an example of uniaxial compression, is one of the simplest to interpret. Spring-and-dashpot physical models permit the description of mechanical and viscoelastic attributes of embryonic tissues in terms of a time-dependent Young's modulus. Viscoelastic properties of tissue can be correlated with their tissue-scale anatomy and cellular microstructure to provide clues on how the genome controls the mechanics of morphogenesis.

Another technique uses tissue indentation to map spatial heterogeneity in developing tissues. For this technique, the position of a blunt capillary tip is moved linearly with a piezoelectric crystal. Movement of the tip occurs when a voltage is placed across the crystal. As the tip pushes into the tissue with a constant force the depth of the indentation can be resolved by tracking the capillary to provide a local measurement of the compression modulus (Figure 1b). Spatial or temporal heterogeneity within the tissue can be mapped by recording the modulus at multiple positions or times. One example of indentation demonstrated a change in relative stiffness as the primitive chick heart tube is shaped [15]. These indentation-based techniques provide many advantages for the non-destructive measurement and mapping of mechanical properties over complex tissue topologies.

Using indentation on a much finer scale, atomic force microscopy (AFM) can be used to map topology and stiffness-like properties [16] and can also probe chemical composition with functionalized indenting tips. Functionally derivatized tips are capable of spatially probing the surface mechanics, molecular organization and chemical activity of multicellular tissues. These tips have been useful in studying the binding energy of specific proteins [17] and cellular adhesion forces [18]. Such tips can also mechanically stimulate cells on a very fine scale[19].

Biological measurements of tensile and shear stress are possible using uniaxial tension or micro-aspiration techniques (Figure 1c). Simple tensile tests have been conducted whereby a tissue fragment is stretched between two parallel wires [20]. Alternative biophysical descriptions for the regulation of surface tension by cell-cell adhesions have been theorized for many years. The differential adhesion hypothesis has found support in microscopically observed phenomena such as cell sorting, aggregation and engulfment studies. Such interfacial phenomena have been observed in the forming *Drosophila* eye [21] but can also be recreated by a number of biophysical and biomechanical processes such as differential contractility [18, 22, 23] and compound mechanical structures [24, 25]. Novel biomechanical approaches will be needed to visualize cells movements as shear stresses are controllably applied to resolve the roles of adhesion and contraction in shaping embryonic tissues.

Microscopy and imaging

Central to any biomechanical analysis of morphogenesis is the description of embryonic anatomy including the size and position of various tissues and cells and their composition. Microscopy techniques of varied resolving powers, contrast mechanisms and stress-inducing methods have been essential to studies of embryo mechanics. Stereoscopes offer simplicity in optics and a resolving power that is sufficient to many studies of gross tissue deformation [26] and mechanical anisotropy [27]. The compound microscope increases resolving power to offer sub cellular resolutions, but lacks broad field of view and depth of focus, although technological efforts are underway to address these shortcomings. Both stereo and compound designs are compatible with two of the most versatile contrast methods in biological research: white light and fluorescence. White light is robust and powerful at resolving changes in pigmentation and refractive index. The contrast that these tools provide can be complemented with fluorescent probes that allow the localization of genetic markers, cellular structures and individual proteins, and can provide physiological information about the chemical microenvironment.

Fluorescent probes can provide considerably more information than basic localization studies. Förster resonance energy transfer [28, 29] and polarization anisotropy [30] permit direct observation of changes in protein structure or higher order complex formation under mechanical perturbation. The emission characteristics of endogenous fluorophores [31] can be used to reveal metabolic states [32]. Structural proteins such as tubulin, collagen and myosin may exhibit scattering effects and elicit second harmonic generation [33-35]. Combined with genetic and pharmacologic studies, optical techniques are now being directed at mapping stress-strain fields and protein dynamics involved in tissue-level and sub cellular phenomena.

Localized tissue and cellular mechanics

Dynamic cell- and sub-cellular measurements of stress and force production are being realized through high-resolution microscopy approaches. Traction forces directed onto deformable substrates are obtained from tracking the strain fields of fluorescent beads imbedded within a thin gel of known stiffness (Figure 1d) [36]. Traction forces analyzed from explanted *Xenopus* tissues reveal higher contractility related to the depolymerization of microtubules and activation of a Rho-GEF, which also translates to a stiffening of the bulk tissue properties [37]. Traction forces adding to the assembly of a fibronectin matrix have been noted to increase in the presence of increased tension [38]. Measurements from force generation at the edges of bulk tissue have extrapolated the theory of traction force to tissue growth in three dimensions [39]. In addition to the traction forces, intrinsic or exogenous

contrast may be analyzed together to map internal molecular, physiological, or metabolic heterogeneities from cellular to molecular scales.

The status of signaling pathways and the composition of large multi-protein complexes such as focal adhesions [40] are critical to understanding the cellular and molecular response to mechanical stimulation and the capacity of multi-cellular tissues to transmit force. Biosensors have been used for many years to directly study metabolism, pH and calcium dynamics within developing embryos. Novel biosensors developed for cultured cell studies may be used to probe the activity of signal transduction pathways [41], intramolecular strain [42, 43] and protein complex formation [44] *in vivo*. In addition to the development of tools for laser ablation or "drilling" (discussed below), a variety of optically-triggered reagents have been developed to control or perturb intracellular signaling pathways, including: optically activated proteins [45-47], photoconvertable fluorophores [48] and ablation [49, 50](Figure 2a). New options to control molecular signaling pathways and interrogate their status in live cells will allow studies into the coupling between physiological and biomechanical processes.

Ablation studies allow further control over the mechanical microenvironment through disruption of multicellular arrays, down to the cell membrane and underlying cytoskeleton, while permitting researchers to monitor the effects on fluorescently tagged proteins and structures. Laser induced injuries have recently been used to study the recruitment of proteins and dynamic remodeling of the cytoskeleton in single cell wounds [51] and to study the tissue-mechanical responses to whole cell or cell-boundary ablation [52](Figure 2b). In a multicellular embryo, stresses transmitted along cell-cell junctions have been studied by ablation of a membrane region and monitoring morphology of the surrounding response [53-57]. In mammalian cells, single actin stress fibers have been ablated to study the unstressed fiber relaxation and subsequent mechanical response at the substrate [58]. Likewise, magnetic tweezers have shown promise as a local modulator of the tissue environment [59] (Figure 2b). The cross correlation of signals from fluorescence channels with local manipulation provides a rich set of inputs to test and refine mechanical models of epithelial morphogenesis.

Mechanics, computation and modeling

Computational approaches lend a unique set of tools for biomechanical analysis, as they provide the means to understand experimental observations and to direct new mechanical studies. For example, the coordinated movements of mesodermal and ectodermal cells during *Drosophila* gastrulation could only be observed through large scale cell tracking and computational analysis [60]. Given the challenge of direct biomechanical analyses, computational studies are attempting to infer forces from image data representing estimates of mechanical strains alone. Large-scale cell tracking has been use to discern between shape changes and intercalation, demonstrating tissue specific differences in the modes of convergence and extension [61]. In a related approach, tissue deformation patterns during invagination of the ventral furrow in *Drosophila* have been investigated in the context of local cell strain maps determined from cell boundaries collected using time-lapse confocal microscopy. This technique, referred to as video force microscopy, reveals that spatiotemporally complex forces are needed to drive invagination; high forces must act within the apical surface of the mesoderm and lower forces must be present within the basal ectoderm. However, the assumption of homogeneous and static mechanical properties are essential elements of the core model fit by the algorithm [62]. Such hybrid analyses combining computer-simulated mechanical models with image data are helping to decipher specific protein activities and the feedback nature of select signaling pathways [63-67].

Is there a role for mechanotransduction *in vivo*?

Cellular studies suggest a role for mechanotransduction during progression of diseases such as cancer [68] and heart disease [69] and during development. These studies suggest that morphogenesis may integrate a wide repertoire of responses to mechanical stimulation. However, clean experimental tests of mechanotransduction in the embryo and tissue isolates have been difficult. Such tests require both precisely controlled mechanical stimulation and simultaneous observation of signaling and cell mechanical responses. Several recent studies have applied sophisticated image based segmentation and quantitative analysis and have begun to dissect the relative contribution of signaling and mechanics. For instance, contractile pulses of myosin activity and adherens junction remodeling coordinate furrow formation during *Drosophila* gastrulation [70, 71]. Another study in *Xenopus* found that extracellular fibronectin matrix assembly is governed by tension via a complex pathway involving both cadherin and Wnt mediated signals [38]. It is believed that interplay between fibronectin, cadherins, and cytoskeleton elements help to establish long distance force transduction and sensing [37, 72], but the relationships and associated forces have been difficult to accurately decipher.

Testing putative mechanotransduction pathways may be possible by harnessing the properties of laminar flow to control both the chemical and mechanical microenvironment of developing embryos. Microfluidic streams have been used to control temperature [73], study translocation of sub cellular components [74] and deliver signaling factors [75, 76](Figure 2c). Careful design of microfluidic culture systems may allow paracrine and autocrine signaling loops and gradients of exogenous factors to be differentially studied [77, 78]. Since microfluidic chambers can be mounted on conventional compound microscopes, a combination of precise fluidic control and light-based manipulations can simultaneously modulate the tissue environment. Combined molecular manipulation, micromechanical stimulation and image-based interrogation of signaling pathways now provide the essential tools for future biomechanical studies.

Conclusion

Imaging tools and techniques to study the biomechanics of morphogenesis and tissue assembly are poised to advance our understanding of the inside-out and outside-in signaling pathways active during development and disease. Advanced microscopy has been and will continue to be critically important for new discoveries. To dissect the coupling between biology and mechanics requires tools to measure and control mechanical attributes and sub cellular protein activities. As our understanding of the coupling between biology and mechanics advances, mechanical and computational models will become more descriptive and predictive allowing a clearer vision of the role of mechanical processes in biological signaling and development.

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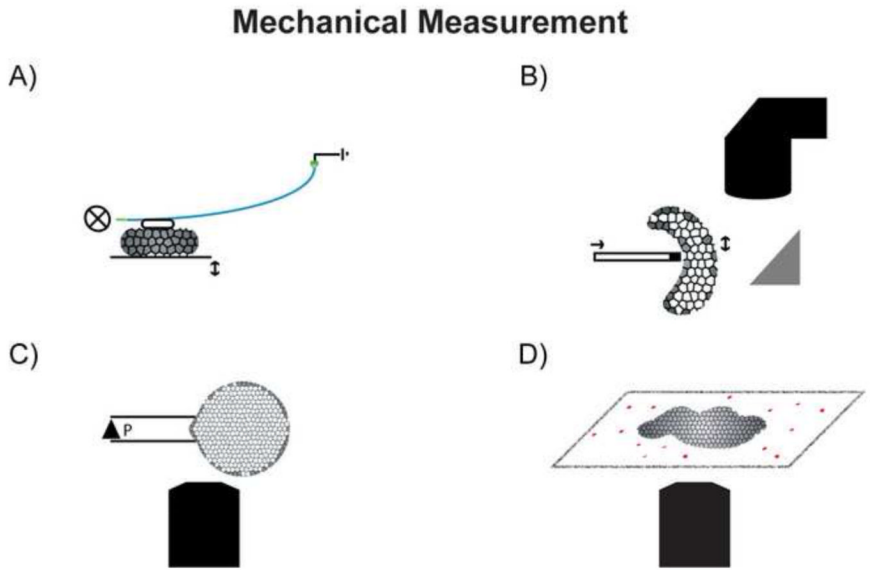


Figure 1.

Mechanical testing of biological samples requires sensitive measurement tools that are adaptable to physiological environments. A) An optical fiber can act as a spring in a simple compression test, with applied force recorded by a quadtrant detector as the fiber deflects. B) Another variant on a compression test relies on image tracking of local tissue deformation in response to an indenter. Measurements across the surface of the tissue are used to map spatial heterogeneity in mechanical properties. C) Surface tension or membrane stiffness can be measured by aspirating a portion of the embryo into a capillary and measuring the distance the tissue moves into the channel. D) Traction forces can be measured by tracking displacement of a compliant substrate, typically ecm coated polyacrylamide gels with imbedded microspheres.

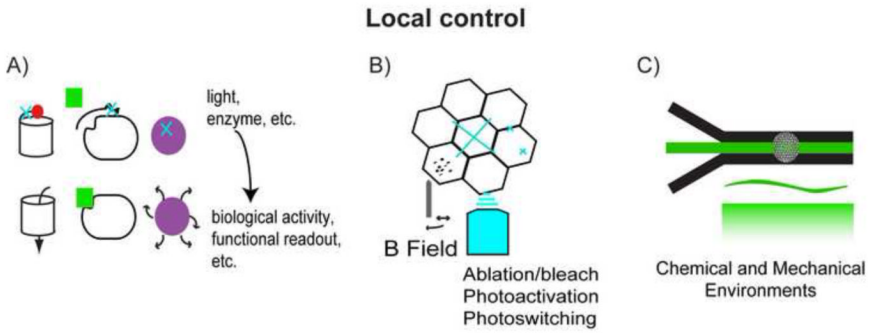
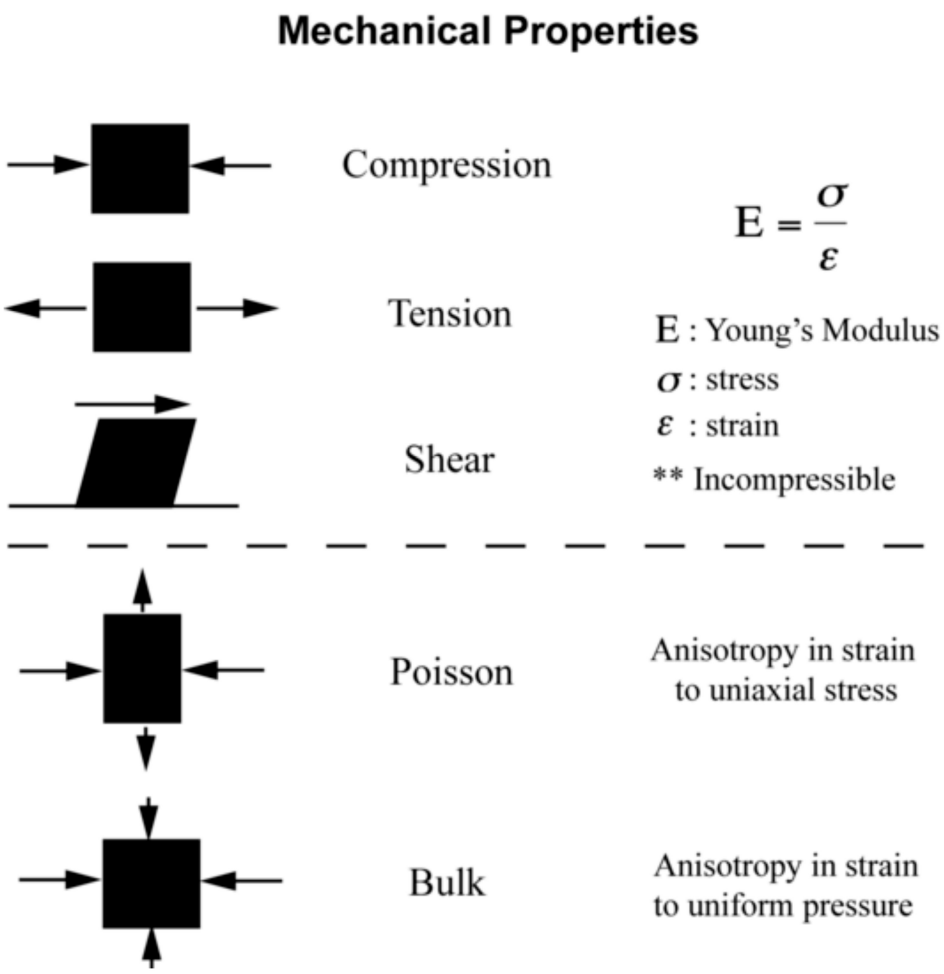


Figure 2.

A variety of techniques can control the local physiological environment at the tissue, cellular and molecular scales. A) Biosensors amenable to optical manipulation have been designed to open membrane channels, activate signaling pathways or release apoptotic signals. B) Localized laser ablation can induce changes in biosensors and can be used to cause local injury. Additionally, magnetic tweezers can be used to locally distort the tissue. C) The chemical and mechanical environment can be manipulated in a microfluidic culture system.



Box.

Mechanical properties are described by deformation in response to forces applied over a surface (stress (σ)). The descriptive moduli of compression, tension and shear are determined from the strains (ε) observed in response to applied stress. The poisson ratio and bulk modulus indicate the compressibility of materials under stress.