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A toolbox to explore the mechanics of living embryonic tissues

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ABSTRACT

The sculpting of embryonic tissues and organs into their functional morphologies involves the spatial and temporal regulation of mechanics at cell and tissue scales. Decades of *in vitro* work, complemented by some *in vivo* studies, have shown the relevance of mechanical cues in the control of cell behaviors that are central to developmental processes, but the lack of methodologies enabling precise, quantitative measurements of mechanical cues *in vivo* have hindered our understanding of the role of mechanics in embryonic development. Several methodologies are starting to enable quantitative studies of mechanics *in vivo* and *in situ*, opening new avenues to explore how mechanics contributes to shaping embryonic tissues and how it affects cell behavior within developing embryos. Here we review the present methodologies to study the role of mechanics in living embryonic tissues, considering their strengths and drawbacks as well as the conditions in which they are most suitable.

Contents

1. Introduction

It is a constant in science that the appearance of new measurement tools enables numerous discoveries and often steers science in new directions. In addition to one of the most celebrated techniques, the light microscope, examples of techniques that have pushed forward the limits of developmental biology abound. These include advances in imaging, such as the development of confocal [1,2], two-photon [3,4] or light-sheet microscopy [5,6], but also tools to reveal the molecular mechanisms underlying embryonic development, such as the discovery of Green Fluorescence Protein [7] or the recently developed CRISPR/Cas system for gene editing [8]. These and many other techniques have helped reveal numerous biochemical signals that are essential to orchestrate cell behavior during development [9].

While biochemical signals are known to play a fundamental role in the control of tissue morphogenesis [9], it is now clear that mechanics also critically affects the vast majority of cell behaviors required to properly sculpt tissues and organs [10–35]. A large number of *in vitro* experiments have shown that mechanical cues affect the coordination of cellular movements in absence of instructive biochemical signals [36–41], the rate of cell proliferation [42–46], the orientation of the cell division axis [47–49], and even cell differentiation [50–54]. Tissue-like 3D cell culture experiments have also shown the relevance of mechanics in guiding cell proliferation [55], branching morphogenesis [56–59] and tumor progression [60–62]. These discoveries were possible thanks to a vast array of *in vitro* biophysical techniques to apply controlled forces on cells, quantitatively measure cellular forces, and also tune the mechanical properties of the cellular microenvironment, both

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in 2D and 3D geometries [63–70]. Given the relevance of the findings obtained by *in vitro* experiments, it is apt to ask if mechanics does affect cell behavior *in vivo*, within developing tissues and organs, to the extent it does *in vitro*.

There exist many important works, detailed below in the context of different measurement techniques, that highlight the relevance of mechanics in embryonic development. However, compared to our knowledge of the biochemical signals involved in tissue morphogenesis [9], our understanding of mechanical signals *in vivo* is still in its infancy. This is mainly because of specific limitations in the current techniques to measure mechanics within developing 3D tissues. Recent efforts to create new tools and adapt *in vitro* techniques to measure cell and tissue mechanics *in vivo* and *in situ* (i.e., locally within developing embryos) promise to reveal how mechanical cues affect morphogenetic processes and individual cell behaviors within living embryos.

In this review we aim at providing a comprehensive overview of the techniques used today to measure and/or perturb mechanics in living embryonic tissues of animal species. Here, the terms *in vitro*, *ex vivo* and *in vivo* are defined as follows: *in vitro* refers to studies of cells in culture conditions, such as standard 2D cell culture, 3D cell culture using hydrogels as scaffolds, as well as multicellular aggregates; *ex vivo* refers to dissected portions of tissue that keep, at least partially, the original tissue architecture; *in vivo* refers to the intact developing embryo. The discussion presented below on the strengths and limitations of the different techniques needs to be understood within the framework of *in vivo* (and *ex vivo*) measurements. Indeed, many of the techniques discussed below have a long and successful history related to *in vitro* experiments, where some of the limitations mentioned below associated specifically to their use *in vivo* do not exist. Before describing the existing techniques, we first discuss the cellular structures that control cell mechanics within living embryonic tissues, and also the different (and independent) mechanical quantities that can potentially affect cell behavior *in vivo*.

2. Mechanics at cell and tissue scales

Whenever a movement is observed in a cell or an embryonic tissue, there is necessarily an underlying force generating it. This is because inertia is irrelevant in these processes and no movement can exist in the absence of a force generating it, highlighting the essential role of the distribution of forces in a tissue to drive morphogenetic flows and tissue deformations. These forces have their origin in the cell [12,13,16,25,27,29,71], where a relatively small number of cellular structures contribute to their generation (Fig. 1). Typically, cells within tissues generate forces via actomyosin contractility at the cell cortex and also through traction forces [13,16].

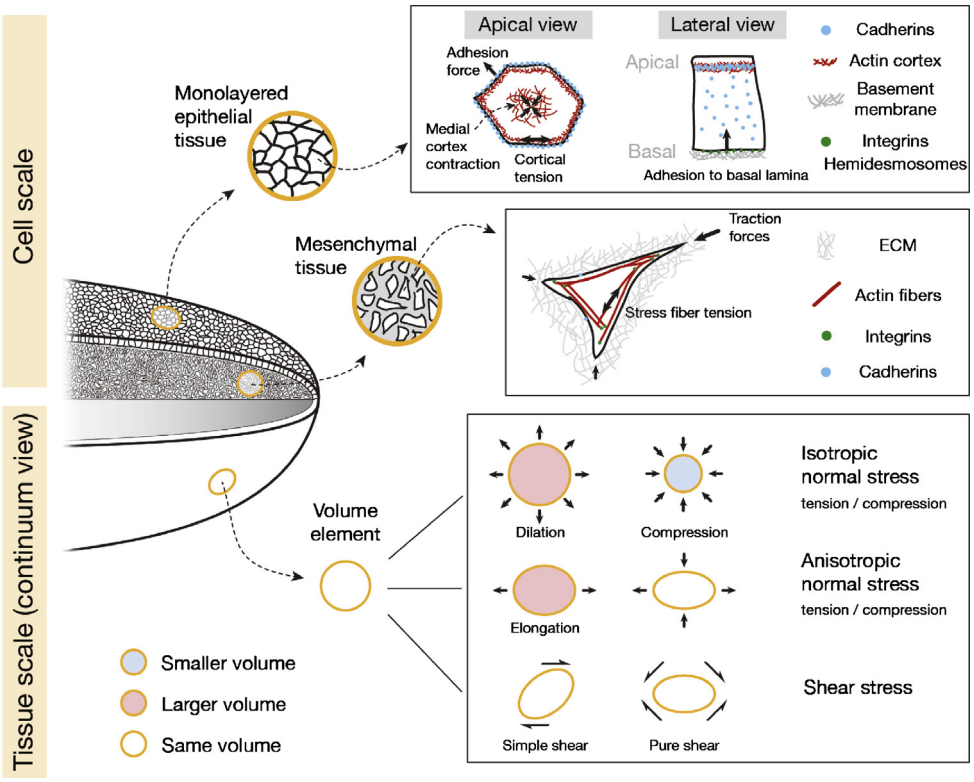

Fig. 1. Schematic representation of a developing embryonic tissue from cell and tissue scale (continuum) perspectives. From a perspective centered at the cell scale, there exist specific forces generated by several cellular structures that contribute to the shaping of tissues at larger scales [12–14,17,19,29]. In the case of monolayered epithelial tissues, forces generated in the medial apical cortex, as well as cortical tension and adhesion to neighboring cells, are known to contribute to morphogenetic processes [13,29]. Whenever epithelial cells are attached at their basal end to a basement membrane, substantial adhesion (and traction) forces may also be exerted there. In mesenchymal tissues, cells exert traction forces on the extracellular matrix (ECM) via focal adhesions, connecting the ECM polymers to integrin receptors at the cell surface [14,17,19,21]. Stress fibers inside mesenchymal cells generate tensile forces that are transmitted to focal adhesion sites and contribute to cell traction forces on the ECM. Cadherin molecules in mesenchymal cells mediate their adhesion and affect their collective movements [21,229,230]. From a tissue scale perspective, volume elements that contain several cells and average locally their properties constitute the basic unit [76,80,81]. In this view, which follows the language of continuum mechanics, deformations of these volume elements by mechanical stresses in the tissue describe quantitatively morphogenetic deformations and flows. In general, the stresses deforming the volume elements do not have a specific origin in a particular cellular structure, but rather reflect the local value of the mechanical stress arising both from local forces as well as forces transmitted from other portions of the tissue. Only a handful elementary deformations of a given volume element exist, and are generated by very specific mechanical stresses acting on the volume element. Uniform normal stresses on the volume element can lead to its dilation or contraction if the material is not strictly incompressible, whereas anisotropic normal stresses lead to elongations and contractions of the reference volume element along specific directions. Shear stresses can lead to elongations (pure shear), but also to combined elongations and rotations of the volume element (simple shear). The spatial variations in mechanical quantities (such as stress or mechanical properties) throughout the tissue, represented here by a graded gray scale, can be described as spatiotemporal maps in the continuum view.