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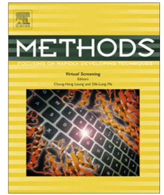

Monitoring developmental force distributions in reconstituted embryonic epithelia

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ABSTRACT

The way cells are organized within a tissue dictates how they sense and respond to extracellular signals, as cues are received and interpreted based on expression and organization of receptors, downstream signaling proteins, and transcription factors. Part of this microenvironmental context is the result of forces acting on the cell, including forces from other cells or from the cellular substrate or basement membrane. However, measuring forces exerted on and by cells is difficult, particularly in an in vivo context, and interpreting how forces affect downstream cellular processes poses an even greater challenge. Here, we present a simple method for monitoring and analyzing forces generated from cell collectives. We demonstrate the ability to generate traction force data from human embryonic stem cells grown in large organized epithelial sheets to determine the magnitude and organization of cell–ECM and cell–cell forces within a self-renewing colony. We show that this method can be used to measure forces in a dynamic hESC system and demonstrate the ability to map intracolony protein localization to force organization.

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1. Introduction

Coordinated cell movements are required in physiological processes important to development, growth, and disease states, including embryogenesis, adult stem cell differentiation, wound repair, and metastasis. The ability of a cell to determine when and how to move in the context of a tissue is dependent on its ability to sense and respond to extracellular cues, which include the forces supplied by neighboring cells and the rigidity and composition of the extracellular matrix. An extrinsic force can cause dramatic changes in cell state, for example, shear stress caused by blood flow can affect endothelial cell proliferation, apoptosis, migration, and gene expression [1]. But merely altering the properties of the substrate to which cells are adhered also causes significant changes to the cell, as cells apply forces to their substrates and respond to the resistance that they sense [2]. The resulting changes include reorganization of the cytoskeleton and cellular adhesions, including those at cell–cell and cell–ECM boundaries. These changes can feedback to stabilize a morphological transition, as modulation of ECM properties that affect cell–ECM forces directly alters cell–cell tension [3]. As adhesions form and break down, their associated proteins are stabilized or degraded, which can affect downstream signaling pathways and lead to a transcriptional response. Though we know much about how cells acting alone sense, process, and transmit mechanical signals, less is understood about the forces that regulate cells working as collectives in the physiological context of a tissue.

Cell reorganization in the context of a tissue is a primary component of embryonic development, as cells must be precisely localized within the complex embryo as their fate is specified. Work in model organisms has defined specific examples of forces driving the cellular rearrangements of embryogenesis, for example, mechanical stimuli are required for epidermal elongation in *Caenorhabditis elegans* [4], mechanical stretching of *Drosophila* wing discs promotes cell proliferation during development [5], and mechanotransduction through cell–cell adhesion is a driver of *Xenopus* gastrulation [6]. In all these cases, actomyosin organization and rearrangement play a role, particularly in terms of coordinating the cell–cell versus cell–ECM adhesion forces that are coupled to actomyosin networks [7,8]. Actomyosin contractility

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also plays an instructive role in gastrulation [9–11], when cells within the epiblastic epithelium undergo an EMT to migrate into the primitive streak and form the endoderm and mesoderm germ layers [12,13]. While much is known about the soluble signals that emanate proximal and distal to the streak to drive this process, the extent to which the forces elaborated during maturation of the epiblastic epithelium contribute to gastrulation is unknown. Thus, the way cells are spatiotemporally organized in terms of cell–cell and cell–ECM protein localization and the resulting force maturation in the developing embryo is a relatively unexplored but potentially crucially important component of developmental signaling.

What we do know about the forces present in the developing embryo is largely drawn from experiments in model organisms that use techniques such as compression tests on whole embryos or explanted embryonic tissue to assess deformability [14], laser ablation to locally cut a tissue and measure the resulting tension release [15,16], or FRET-based approaches to track the activation of mechanosignaling proteins such as Rac and Rho during development [17]. A recent novel method to study endogenous forces in living and developing tissues using fluorescently labeled microdroplets has enabled measurements of the cell-generated stresses in the dental mesenchyme of live mice, and is promising for future developmental studies [18]. However, such studies are difficult to perform in the presence of external manipulations in a highly controlled environment.

Recent in vitro approaches permit analysis of how forces are organized in cell collectives, which provides relevant context to the cells in terms of cell–cell and cell–matrix forces. It is becoming increasingly evident that cells in collectives behave differently than single cells in terms of junction assembly and mechanotransduction events [19], so in vitro methods for careful study of specific collective cell properties will allow for tractable systems in which to better understand these emergent phenomena. In one approach known as traction force microscopy, adherent cells are grown on hydrogels containing fluorescent microbeads, whose displacement indicates the force applied by the cell to its substrate. Such work has provided insights into collective cell migration [20,21], heterogenous distribution of physical forces in colonies [22], and epithelial tissue dynamics [23]. FRET-based approaches have also been applied to epithelial collectives to assess transduction of mechanical forces [24] and intercellular tension distribution [25]. Applying these approaches to other cell types of epithelial origin, particularly those that are relevant to developmental processes, has the potential to uncover previously unknown requirements for the elaboration of forces in dictating cell fate and driving differentiation.

Human embryonic stem cells (hESCs) are isolated from the inner cell mass of a blastocyst, and are thought to be the in vitro equivalent of the pluripotent epiblast [26]. We previously showed that hESCs cultured on mechanically deformable polyacrylamide substrates of an appropriate stiffness can recapitulate the structural and morphological organization of an in vivo epiblast, including a columnar epithelium with basally displaced nuclei and well-developed E-cadherin-based adherens junctions with cortical F-actin fibers [27]. Because hESCs cultured in this manner represent an epithelial sheet formed in a context that is relevant to embryonic development, understanding the mechanical properties exerted by these cells as they organize into colonies reminiscent of epiblastic organization could provide insight into the contribution of mechanical forces to embryo formation.

To build on our previously demonstrated ability to establish viable hESC colonies on soft hydrogels [27], we sought to leverage the recently developed techniques described above [20,22,23] to quantify the forces exerted by hESCs grown in colonies as they organized into epithelial sheets. To this end, we recently developed methods for hESC patterning coupled with measurements designed to analyze development and maturation of cell–ECM and cell–cell forces. We describe a method for preparation of polyacrylamide gels that can be used for force measurements and are robustly compatible with adherence and long-term culture of hESCs. We demonstrate how to plate hESCs in pre-gastrulation-stage embryo-sized colonies of defined geometries onto traction force hydrogels of defined compliance in a way that allows the cells to unrestrictedly adhere and organize, with the ability to model the embryo in terms of size, shape, and composition. With this system, we show how to apply traction force microscopy to measure cell–ECM forces in real time as the colonies mature, and also assess how cell–cell forces develop with a technique called monolayer stress microscopy [20], allowing us to generate a comprehensive picture of how forces develop in a developmentally relevant epithelial model system. Many differentiation protocols that begin with human pluripotent stem cells use monolayer culture as a starting point for differentiation [28–32], and it has been shown that both extrinsic and intrinsic cell–matrix forces affect differentiation in other contexts [33,34], so the ability to quantify and manipulate forces could enhance our understanding of how mechanics is involved in many aspects of development.

This method enables us to observe and measure the cell mechanics that underlie embryonic processes. Embryonic stem cells have distinct mechanical properties, being softer and more sensitive to stress than their differentiated counterparts [35] and presumably than other model epithelial lines such as MDCK or MCF10A cells, systems in which most work has been done to establish principles for collective cell cohesion and organization. We thus describe a tool for understanding the relationship between tissue-scale self-organization and force in a simplified system that bridges the gap between pure tissue culture studies and those performed in embryos. Recent work has indicated that self-organized patterning of hESCs occurs when colony geometry is controlled [36], a result that was attributed to paracrine gradients but may also include a contribution from adhesion forces in these developmental analogs. With our system, we can examine not only how protein and signaling gradients are set up within epiblastic epithelium-like colonies, but also track the cell-intrinsic forces involved in differentiation and developmental processes. Our technique also allows for retroactive superimposition of relevant biomarker expression with traction force maps, enabling the establishment of links between endogenous forces and embryonic epithelial organization in the context of tissue geometries that are widely applicable to live-cell temporal studies of development, differentiation, and migration.

2. Methods

The methods we present here build on our previously published method for preparing and culturing hESCs on ligand modified polyacrylamide gels [27], which was used to study the effects of substrate compliance on hESC differentiation at a population level in which hESCs were plated as single cells or small randomly plated colonies. However, as described above, the organization of cells within tissues underlies collective cell behaviors such as germ layer differentiation during development. This modified method provides improvements in gel casting and the chemistry of ligand conjugation but, more significantly, allows for control of colony size, shape, and position and integrates with current matrix traction force methods to quantify cell matrix and intercellular forces. Importantly, position control provides a means of non-destructively acquiring these forces, allowing end point analysis for correlation of molecular markers and underlying forces. This is accomplished by a set of unique approaches involving custom designed 3D printed culture wells and microscope stage mounts

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