11 Quantifying Ciliary-Generated Traction Forces During Locomotion in a Simple Marine Animal's Epithelial Tissues

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Abstract

In animals, epithelial tissues mainly provide a protective barrier function, but these tissues are also subjected to mechanical forces during physiological activities such as locomotion. Studying the response of tissues subjected to dynamic forces is an important area of research. Here, we investigate the mechanics of epithelial tissues in a marine animal, the *Trichoplax adhaerens*, that lacks both muscles and neurons. It has a simple body plan consisting of two epithelial layers with ciliated cells enclosing a layer of fiber cells and no organ system. These asexually reproducing organisms do not possess a fixed shape and the cells are in contact with each other by means of adherens junctions. Recently, it has been demonstrated that mechanical forces due to their own motility give rise to localized ventral and dorsal fractures at fast loading time scales. These tissue fractures lead to formation of holes, some of which heal over time while others break into long strings that eventually rupture giving rise to daughter organisms. In silico models of these tissues have revealed a transition from ductile to brittle properties. The mechanical driving forces underlying these phenomena are thought to originate from the interaction of the animal's lower epithelium with the substrate. The lower epithelium consists of cells with cilia that exert a transient adhesion, which allows the organism to walk over a substrate. However, the traction forces generated by the ciliary motion have not yet been measured. Here, we fill in this gap by utilizing the traction force microscopy (TFM) technique. TFM has been extensively used to study traction forces of single cells and during collective cell migration. The TFM technique involves microscopic timelapse imaging to quantify the displacement field due to deformation of a soft elastic substrate by the ciliary adhesions to compute traction forces. Here, we show that TFM can successfully measure traction forces in this animal, and we also demonstrate the application of monolayer stress microscopy to calculate the principal stresses.

Keywords

Epithelial tissue mechanics · Traction force microscopy · Biomechanics · Non-bilaterians · Marine biology

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11.1 Introduction

Epithelial tissues in animals are typically rigid structures that provide support and protection to organs and embryos. However, these tissues are subjected to various types of dynamic forces and extreme strain during the animal's development or adulthood [1]. Therefore, the tissues need to be able to withstand and adapt to these mechanical forces in order to maintain their integrity. The mechanics of tissues hence plays a prominent role in biological processes such as physiology [1–6] and development [7–11] and determine an organism's survival and success.

An important area of focus in the rapidly growing field of tissue mechanics is the question of how the properties of individual cells and their collective interactions result in the emergent tissue-scale rheological properties. Epithelial tissues have been found to be able to provide adaptive responses to mechanical forces by their ability to undergo both elastic and plastic deformation [1]. Elastic tissue responses typically include reversible, solid-like deformation. The cellular mechanisms that help maintain elastic tissue deformation under external loading include super-elastic responses [12], active contractions [13], and local mechanical reinforcement [14, 15]. Plastic tissue deformations are commonly associated with liquid-like "cellular flows" during various physiological processes [1, 6] and during development [9]. Here, the cells when subjected to force loading may dynamically rearrange or reconfigure giving rise to localized "cellular flows". The local cellular flows can potentially give rise to the property of tissue ductility, *i.e.*, the tendency of tissues to become thin due to tensile loading. Another possible extreme mode of plastic deformation involves the cells losing contact with each other, leading to tissue brittleness, *i.e.*, localized tissue fracture and loss of tissue integrity [5, 16]. Here, we measure and quantify the traction forces involved in the deformation of tissues under dynamic loading. Our long-term goal is to quantify traction forces to understand what determines whether a tissue flows or fractures, *i.e.*, what determines the ductile–brittle transition.

Here, we study epithelial tissue mechanics in the *Trichoplax adhaerens*, an animal capable of rapid and highly adaptive plastic deformation [6, 16]. The *Trichoplax* is a simple marine animal that lacks both muscles and neurons. It is an early-divergent, non-bilaterian belonging to the basal animal phylum of Placozoa. It has a simple and flat pancake-like body plan (Fig. 11.1c), and the thickness of these animals is constant at 25 μm, but they exhibit a wide variation in size (width) (~50 μm to ~ 5 mm). The flat body plan consists of three tissue layers, an upper dorsal epithelium and a lower ventral epithelium that sandwich a layer of fiber cells. These animals do not have organs and lack both a basement membrane and an extracellular matrix. The cells in the epithelial tissues are connected to each other solely by adherens junctions. The cells in the epithelial tissues are monociliated, and the cilia in the ventral epithelial cells have the unique ability to adhere to a bottom substrate. These ventral cilia enable the animals to exert sufficient traction forces to walk or crawl. Unlike other animals, the *Trichoplax* does not have a fixed stereotypical shape. Instead, the animals continuously change their shapes in a random manner driven by the ciliary traction forces. These animals exhibit a wide and extreme shape morphospace, ranging from circular to long narrow thread-like shapes (Fig. 11.1).

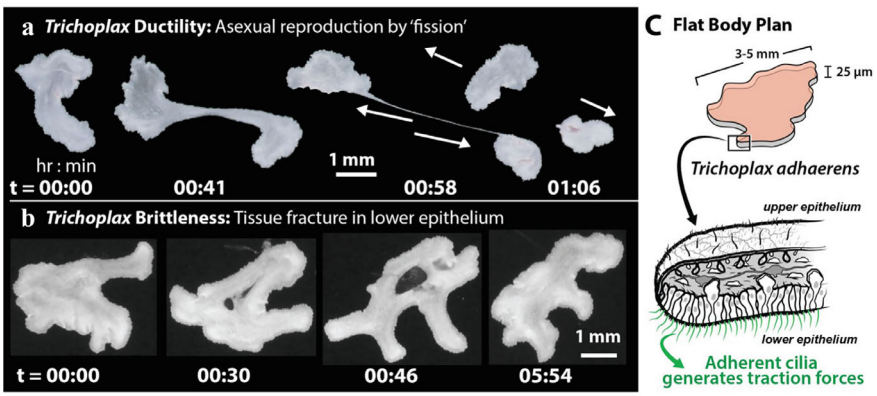

Fig. 11.1 Ductile and brittle tissue deformations accompany extreme plastic shape changes in the Trichoplax adhaerens: **a**. Ductility: time-lapse images show rapid tissue thinning and breaking in the central region of the animal, giving rise to two daughter animals—the process of asexual/vegetative reproduction. **b**. Brittleness: time-lapse images show formation of a tissue fracture hole in the ventral epithelium. **c**. Body plan: cartoon illustrates the animal's flat body plan and cut section shows the upper/dorsal and lower/ventral epithelium. The ventral cilia are adherent and generate traction forces on substrates. Images in this figure are adapted and reproduced, from Refs. [6, 16]

The *Trichoplax* reproduces by a process of vegetative or asexual fission. An individual animal forms two regions that pull apart and result in a ductile tissue deformation. The thinning tissue in the middle becomes a small thread that eventually breaks and completes the division process [16] (Fig. 11.1a). These animals are also able to sustain tissue fractures in both the ventral and the dorsal epithelia. The tissue fractures arise due to a brittle tissue deformation at first in the lower epithelium (Fig. 11.1b). In most cases, this ventral fracture can heal itself in about an hour. But sometimes, when the animal is sufficiently large, the ventral hole expands and can also lead to a dorsal tissue fracture [16].

These unique animals can exhibit both ductile and brittle deformations for their physiological purposes of asexual reproduction and shape change [16]. Hence, the *Trichoplax adhaerens* is an excellent model animal system that can be used to gain a fundamental understanding of ductile–brittle transitions in epithelial tissues subjected to rapid and dynamic force landscapes [6, 16–19]. Here, we carry out quantitative experimental measurements of the traction forces exerted by the cilia in the epithelial cells of the lower epithelium on the substrate. Measurement of the traction forces can help unravel the cellular mechanisms that determine whether the tissues will flow or fracture.

11.2 Background

The kinematics of epithelial tissues in the *Trichoplax* was quantified previously using a combination of cell-tagging, live imaging, and particle image velocimetry (PIV) techniques [16]. The animal was sprinkled with adhesive fluorescent microspheres resulting in a coarse-grained tagging of the entire upper tissue. The tagged animals were imaged in thin milli fluidic chambers (thickness 40 μm, width 13 mm $\times$ 13 mm square) on an inverted microscope, and live imaging was carried out simultaneously in bright-field and fluorescence. A high-speed camera connected to the microscope acquired images at 10 fps for several hours, at variable fields-of-view. These datasets were visualized using Flowtrace [20], a tool for visualizing coherent structures in biological fluid flows by generating pathlines (Fig. 11.2, upper panels). These dorsal tissue layer datasets were also analyzed using the particle image velocimetry (PIV) technique using the PIVlab package in MATLAB [21] to quantify the kinematics of the tissue flow fields. The animal was tracked manually for fixed time durations (several minutes), and the recording was stopped when the animal moves away from the field of view. The PIV analysis is carried out for segments where the field of view is fixed, and at short timescales (1 s). Our results showed that the motility of these animals induces spatiotemporally varying velocity fields and strain rates in the dorsal tissues [16] (Fig. 11.2, lower panels). Depending on the organism's motility, we found that the tissues can be subjected to both tensile and shear forces. Furthermore, we made the discovery that these mechanical forces can result in local brittle-like tissue fractures in the ventral epithelium [16] (Fig. 11.2b). This was the first discovery of in vivo tissue fractures in a living organism. Figure 11.2b shows an example of shear force-induced tissue fracture in the ventral epithelium, but we have also observed tensile force-induced tissue fractures in these animals [16].

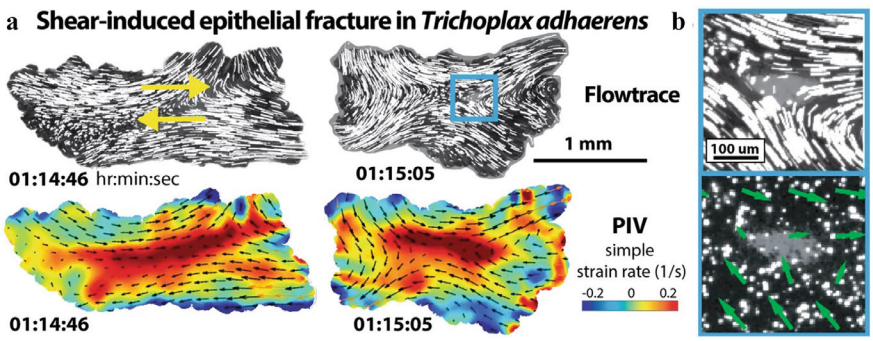

Fig. 11.2 Organismal motility induces shear forces that cause local tissue fractures in *Trichoplax adhaerens*. Bright-field and fluorescence microscopic imaging of the upper epithelium tagged with adhesive fluorescent microspheres reveals a shear fracture. **a**. Upper panel: flowtrace [20] visualizations show bead trajectories in a region of local shear-induced fracture (**b** shows zoomed-in insets). Lower panel: the internal strain rate contours from a PIV analysis [21] overlaid on the velocity vectors (**b** shows zoomed-in insets). The shear-induced fracture occurs at the central regions with peak strain rate values. Images in this figure are adapted and reproduced, from Ref. [16]

11.3 Methods and Results

Here, we performed the first-ever measurement of forces produced by the *Trichoplax adhaerens* using the traction force microscopy (TFM) technique [22–33]. In TFM, cell-induced substrate displacements are measured by applying digital image correlation to images of fiducial markers embedded in the substrate. Subsequently, an inverse problem is solved to compute the tractions which induce those displacements. Thus, the tractions are computed by applying the equations of force equilibrium to the substrate. *Trichoplax* were placed on polyacrylamide substrates having Young's modulus 1 kPa embedded with fluorescent particles located at the top of the substrate as described in Refs. [30–32]. Time-lapse images of the *Trichoplax* and fiducial markers were collected by optical microscopy. Substrate displacements were computed by applying digital image correlation [34] to images of the fluorescent particles, comparing time points of interest to a reference time point, wherein the reference was displacement free as the *Trichoplax* had migrated out of the field of view. Tractions were calculated using the Fourier transform-based approach [23] with corrections for a substrate of finite thickness [26, 27]. As shown in the results (Fig. 11.3a–c), the magnitude of traction is exceptionally small, in the range of a few Pa, which is at least an order of magnitude smaller than the tractions reported in most literature.

Next, we measured stresses within the *Trichoplax* using Monolayer Stress Microscopy [35, 36], which applies the principle of force equilibrium within the animal to compute the in-plane stress tensor from the distribution of cell-substrate tractions [31]. This method has been verified by a theoretical model [37] though a different model suggested that results of the method can in some cases be sensitive to assumptions made about the rheology of the cell monolayer [38]. Importantly, our implementation is independent of any assumed rheology (*i.e.*, the results are the same whether the animal is assumed to be elastic, viscous, or viscoelastic) [31]. The stress calculation depends on the out-of-plane thickness, and given that the lower tissue is not physically linked to the upper layer but only joined at the edge of the organism [13], we used 10 μm as the thickness of the lower epithelium [39]. To visualize the stress state, we first plotted the average of the principal stresses, which is primarily positive (tensile) with only some areas being negative (compressive) (Fig. 11.3d). Next, we plotted the max shear stress, which is larger in magnitude than the average of the principal stresses, indicating substantial shearing (shape-changing) stresses (Fig. 11.3e). Finally, as the stress tensor has three independent components, we also show the orientation of first principal stress, which tends to align along the long axis of the animal, albeit with notable variation over space (Fig. 11.3f).

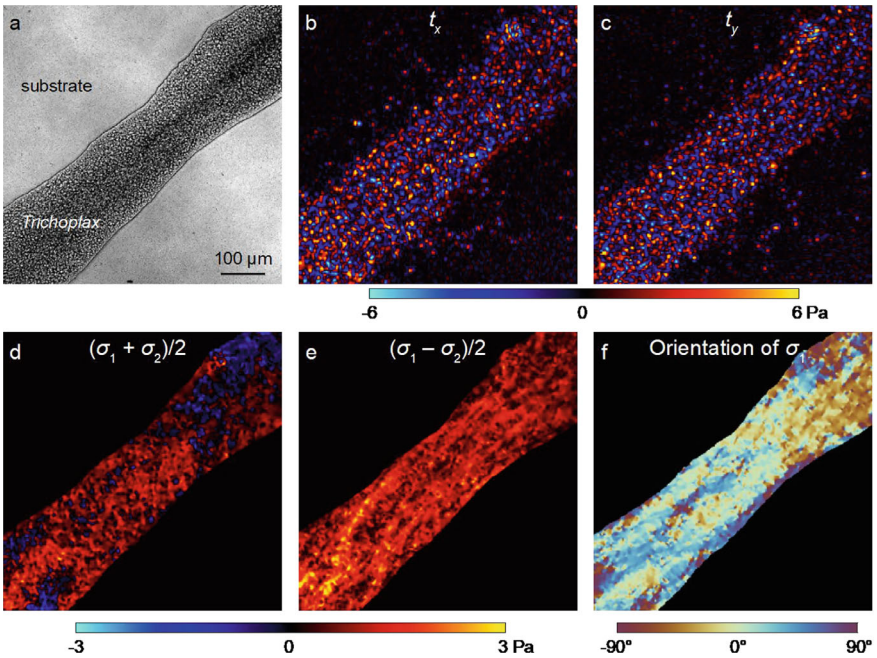

Fig. 11.3 Measurement of forces produced by *Trichoplax adhaerens*. **a** Phase contrast image of a representative *Trichoplax* crawling on a compliant substrate. In this example, the animal is larger than the field of view. **b**, **c** Tractions (x and y components) applied by the *Trichoplax* to the substrate. Data outside the *Trichoplax* indicate experimental noise; as can be seen, noise is substantially smaller than the traction measured. **d**, **e** Stresses within the animal, computed by monolayer stress microscopy. Shown are the average of the principal stresses (**d**) and the max shear stress (**e**). **f** Orientation of first principal stress

11.4 Conclusion

In this work, we have successfully demonstrated the application of traction force microscopy (TFM) to give the first-ever measurement of traction forces in the *Trichoplax adhaerens*, a simple, marine non-bilaterian. We have shown that the TFM technique works in this animal when soft polyacrylamide gels (1 kPa) are used. Our results show that the *T. adhaerens* can generate local tractions up to 10 Pa. We also demonstrate the application of monolayer stress microscopy to calculate the principal stresses within these animal tissues. Our long-term goal is to utilize TFM to quantify the forces that determine ductile–brittle transitions during the different physiological behaviors and to characterize the material properties of tissues in these animals.

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