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The influence of geometry on the elastic properties of the Drosophila wing disc

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HIGHLIGHTS

- Concurrent 3D imaging and stretching of biological tissues.
- Finite element modeling of elastic properties of tissues.
- Description of elastic properties under direct consideration of the 3D structure.

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ABSTRACT

We consider the non-linear elastic behavior of the wing disc of the Drosophila larva. In stretching experiments, this epithelial tissue shows a highly non-linear force–displacement behavior. In order to understand the nature and origin of this non-linear reaction, we try to reproduce the deformation experiments using a two-dimensional finite element solution of the non-linear hyperelastic momentum balance equation. The results suggest that to a large extent the stress reaction of the tissue is due to purely geometric effects. Finally, we give a parameter estimate for a neo-Hookean constitutive model.

1. Introduction

The investigation of mechanical effects on developing tissues has recently gained increasing attention [1], ranging from the mechanisms of force generation in cell division [2], cell mobility [3], as well as tissue deformations on the scale of epithelia [4,5], and entire embryos [6–8], as well as the mechanical control of gene regulation [9,10] and growth [11–14].

In order to experimentally investigate the influence of forces in development, several types of force applications have been performed, most notably, laser ablation [4,8], but also theoretical inference of forces based on cell and/or tissue deformations [1,6,7]. Direct application of mechanical forces on tissues has also been performed, macroscopically [9,10,15] as well as microscopically by changing the activity of molecular motors [2,3]. However the extent of local force, which presumably is important in the biological effectiveness of control via mechanical forces, is difficult to quantify in these cases.

Here we study one such model system, namely the wing imaginal disc of Drosophila, a proto-organ inside the larva of Drosophila that will become the adult wing during pupariation. In this system, it has been shown theoretically [12–14] as well as experimentally [15,16] that mechanical forces are vital in the control of growth and size determination. In order to study molecular mechanisms at work in this control, as proposed in [14,16,17], forces need to be manipulated and quantified on the cellular level inside a live tissue. For this reason, we study the transmission of externally applied forces through the tissue,

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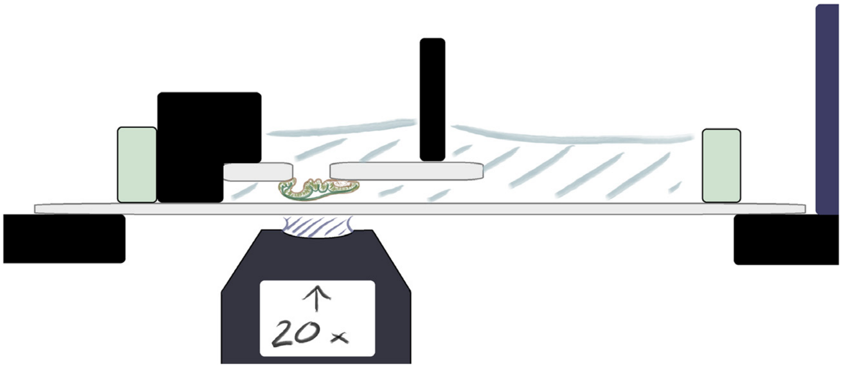

Fig. 1. Schematic of the stretching setup used in the experiments. Dissected wing imaginal discs are attached to cover slips using polylysine. One cover slip is moved with respect to the other while attached to a cantilever spring, whose deflection is measured in order to determine the applied force. The entire setup is mounted on a confocal microscope such that simultaneous three-dimensional microscopy and stretching are possible.

both experimentally and computationally. By determining the force extension curve of the tissue simultaneously with the three-dimensional structure deformation, we are able to disentangle the influence of geometry and the material on the stress inside the tissue at different applied forces. For this purpose, we use the structural information from the three-dimensional imaging and propose a material model, which is then solved using finite elements and compared with the experimental force extension curve as well as the experimental deformation. Using a linear hyperelastic model for the material, with a stiff layer surrounding the tissue corresponding to the extra-cellular matrix, we can describe the highly non-linear force extension curve to a reasonable approximation, while also capturing the structural changes in the overall folded tissue as a result of the stretching. With this we obtain a map of the stress-distribution inside the tissue for a given applied force. This information can be put back into studies of the influence of forces on development on the cellular level, since this allows the quantification of forces at specified positions given an external perturbation.

2. Experiments

2.1. Setup

The experimental setup used to apply a quantitatively determined force onto a wing disc is similar to that described in [18]. The wing discs are dissected and immediately inserted to a special stretching apparatus, where the wing disc is attached to two independently moving cover slips while submerged in a nourishing medium [19]. The stretching is applied by moving one of the cover slips, while keeping the other fixed, and the applied force being determined from the bending of a cantilever spring attached to the movable cover slip. This is shown schematically in Fig. 1. The dimensions of the cantilever as well as the accuracy in the positioning of the cover slip allow a controlled application of forces ranging between 1 μN and 300 μN. The stretching apparatus is mounted on top of an inverted Leica SP1 confocal microscope, allowing for the concurrent determination of the three-dimensional structure of the wing disc tissue, as well as in principle a concurrent determination of cell shapes using appropriate fluorescent markers [20]. With this setup, we can measure force–displacement curves, while simultaneously determining the three-dimensional structure of the tissue as well as the extra-cellular matrix, with a particular emphasis on the dynamics of the folds in the tissue during stretching.

2.2. Experimental results

Fig. 2 shows an example of a wing disc stretching experiment, where the shape of the wing disc in two perpendicular cross-sections is shown at different levels of stretching (0, 1, 14, 21 μN respectively). The wing discs investigated have been marked fluorescently by live markers of Lac:YFP (shown in green) and TROL:GFP (shown in red), thus showing the epithelial cells (Lac:YFP) as well as the extra-cellular matrix (TROL:GFP). Where the two channels overlap, the figure shows yellow. As can be seen, already a very small force of 1 μN leads to a considerable extension of the wing disc of 80 μm, indicating a very soft material. For further extensions however, considerably higher forces are necessary. This indicates a highly non-linear shape of the force extension curve, which is shown in Fig. 3. The geometry of the tissue shown in Fig. 2 shows a visible unfolding of the outer layer of the wing disc during the initial stages of the stretching, whereas at higher forces, the outer layer, consisting mainly of the extra-cellular matrix, is flat and stretched tight.

From these results, it seems that the highly non-linear elastic behavior of the wing disc tissue is rather not a material property, but merely an effect due to the unfolding of the geometry of the structure. In the following, we will investigate this question more closely with the help of a numerical model, which is fed with an assumption on the constitutive behavior, and calculates the deformation behavior and the stress distribution as a result. In consequence, it can be tested whether even