Tensile Forces in Tissues during Morphogenesis and Wound Healing

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KEYWORDS. Morphogenesis, wound healing, tensile force, laser ablation

BRIEF. Laser ablation is used to determine how embryonic wounds affect the morphogenesis of *Drosophila*.

ABSTRACT. Morphogenesis, the biological process by which tissue is formed and defined as an organism develops, include both genetic and mechanic processes. The genetic processes have been extensively studied; however, the mechanic processes have only recently been explored thanks to advances in microscopy. This experiment is intended to better understand the mechanic forces that are at work during morphogenesis and wound healing in *Drosophila,* fruit fly, embryos. To accomplish this, each *Drosophila* embryo was ablated with a laser to create a rupture in the tissue, a process called laser ablation. The embryos were recorded under the microscope while they continued developing. This data was then analyzed to determine the forces present during development. It was found that there is a dramatic increase in the displacement of the tissue in *Drosophila* embryos immediately following laser ablation. As time progressed, the wounded tissue healed, and the velocity of the tissue decreased as well as the distance the tissue traveled decreased. After the wound has healed, the tissue in the embryo returns to its normal state of movement as it continues development which is determined through calculations. This illustrates how an embryonic wound affects the morphogenesis of *Drosophila*. In order to determine if these results are typical of all *Drosophila*, different strains could be compared to one another in future studies.

INTRODUCTION.

Morphogenesis, the formation and differentiation of tissues as an organism develops, is a fundamental aspect of developmental biology. This process includes both genetic and mechanical aspects [1]. The genetic facets have been extensively studied throughout the years. However, the mechanical aspects, including the processes that can be observed during morphogenesis, have received little attention [4].

One study that investigated the embryonic mechanics in *Drosophila melanogaster* embryos looked specifically at dorsal closure, an essential stage of embryo development [3]. In the beginning of dorsal closure, the dorsal surface of the embryo is covered by amnioserosa cells. As dorsal closure progresses, the two ends of the amnioserosa, called canthi, and the lateral epidermis are pulled together covering the amnioserosa cells [5]. This process takes ~2 to 3 hours [2]. It was determined that the amnioserosa cells more closely resemble a continuous sheet. Kinetics, after a wound is made, follow power-law behavior, and dorsal closure is accompanied by redistribution of tensile stress [3].

Using the methods of such studies, the morphogenesis of *Drosophila melanogaster* was investigated with specific concentration on germ band retraction, a stage of embryonic development. At the beginning of germ band retraction, stages 12-13 of embryonic development, the germ band is curled around the epithelial amnioserosa cells on the dorsal side of the embryo, see Movie S1 in Supporting Information. As germ band retraction continues, the germ band cells uncurl from around the amioserosa cells [5]. It is also important to investigate what effect a wound that occurs in an embryo during morphogenesis has on the development of said embryo. Such information adds to the study of how wounds effect embryonic development.

This experiment is intended to determine the tensile forces during morphogenesis and wound healing in *Drosophila* embryonic tissue during germ band retraction. Laser ablation was used to wound the live embryos during this particular stage of embryonic development. It is important these forces are studied closely in order to better understand tissue development and healing.

Learning more about morphogenesis and wound healing can further add to the knowledge of embryonic development. Research such as this can help to answer some of the larger questions about morphogenesis such as the forces causing morphogenesis. It can also aid in answering questions about wound healing such as the forces causing wound healing, where the stresses lie, and physics backing how a wound heals.

METHODS.

Drosophila embryo preparation.

The strain sGMCA of *Drosophila melanogaster* which expresses GFP-moesin was used for this research. *Drosphila* embryos were collected the night before each experiment was performed. All embryos collected were stored at 14.6°C, so they would be in germ band retraction for experiments the following morning. These embryos were then dechorionated in a 50% bleach solution to remove the outer membrane [3]. The samples were then immersed in halocarbon oil, and placed between cover glass and an oxygen-permeable membrane to be imaged with a laser-scanning confocal microscope [3].

Laser ablation and time-lapse image sequencing.

Each *Drosophila* embryo was wounded one time for an experiment. To create wounds, a single pulse laser ablation was administered to each embryo. No embryo was wounded more than once during experiments. A Q-switched Nd:YAG laser coupled with independent beam steering was used for all of the laser ablations. The laser was set at ~90 nJ; a strong enough fluence to slice the germ band cell without burning the cells around the wound, or cutting all the way through the embryo, see Movie S2 in Supporting Information. Images of embryo development, laser ablation, and wound healing were captured with a Zeiss LSM410 laser-scanning confocal microscope with a 40x, 1.3 NA oil-immersion objective and 488 nm excitation as time-lapse image sequences, see Figure S1 in Supporting Information.

Time-lapse image data analysis.

The time-lapse image sequences collected were processed with ImageJ and specific plugins [1]. First each image sequence was run through UnwarpJ, an ImageJ plugin [1]. This mapped the displacement fields of each image in image form. After this, the displacement of the image was calculated for each individual image using the following equation:

$$U_r = \vec{U} \cdot \hat{r} \qquad \textbf{Equation 1}$$

where U_r is radial and tangential strain, $\vec{U}$ is vector length, and $\hat{r}$ is vector displacement. The dot product of the vector length and the vector displacement equals displacement of the tissue in the radial direction. This is done by using ImageJ to calculate the x and y displacement of each image; then subtracting the wound in order to find the vector displacement. Next, the vector length of each image is multiplied by the vector displacement of each image to get the displacement of the tissue in each image. Since using Equation 1 in conjunction with ImageJ only gives results in image form, a modified version of Radial Profile, another ImageJ pluggin, was used to translate the images to numbers [1]. Using the center of the wound as the centroid of the calculations, it calculates the displacement of the tissue at increasing distances from the wound; see Figure S2 in Supporting Information for an illustration.

RESULTS.

It was found that the greatest velocity of tissue movement in the embryo occurred at the center of the wound and decreased the further away the tissue was from the center of the wound. The overall velocity decreased with time.

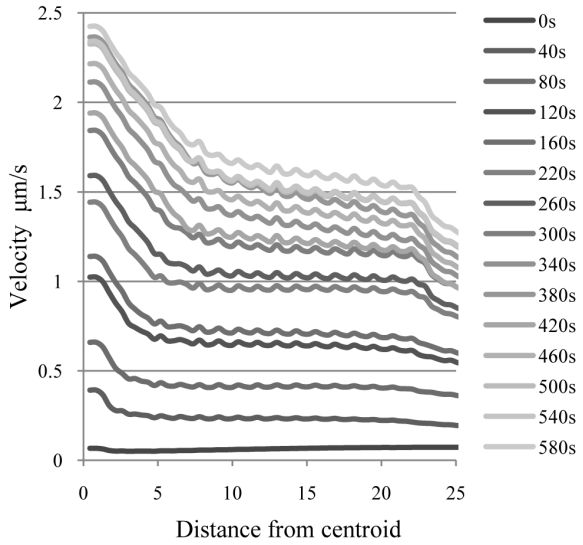

Figure 1. Displacement of embryonic tissue during germ band retraction in *Drosophila*. Each line corresponds to a single image of a time-lapse video; images were taken with a 20 second time delay. Laser ablation occurs in the germ band around line 4. Expansion of the wound ends around line 14, and wound healing occurs for the remainder of the graph.

In Figures 1 and 2, the x-axis displays the distance from the center of the wound the tissue was when its velocity was calculated. Each individual line represents a single image in a time-lapse image sequence from one laser ablation experiment. Each line in Figure 1 and 2 is building upon the previous line meaning each line displays its own displacement plus the previous images' displacement. This is so the increase in velocity or lack thereof is clearly visible from line to line, image to image, as time progresses.

Figure 1 illustrates the displacement of embryonic tissues over time. Each image was taken with a 20 second time delay. Laser ablation in this particular experiment occurs around line 4, the 4th line from the bottom of the graph, and expansion continues until line 14. After line 14, wound healing begins, and continues to the end of this experiment. As shown in Figure 1, the largest gap between lines occurs between lines 4 and 5. There is a great amount of displacement of embryonic tissue until expansion ends. Once wound healing begins, there is noticeably less displacement of embryonic tissue.

Figure 2 illustrates the displacement of embryonic tissues over time as well. Each image was taken with a 30 second time delay. The difference in these time delay of Figure 1 and 2 is due to the fact that the events in Figure 2 occur at a slower pace than Figure 1. In order to take good quality images throughout the entire experiment, it is necessary to increase the time delay between images when possible. When an image is taken of a specimen under a microscope, it is exposed to excess light. When this happens too often, photo bleaching of the images occurs which means it becomes very difficult to take a clear image of the specimen.

As shown in Figure 2, displacement of embryonic tissue decreases until wound healing ends. Once wound healing ends, displacement of embryonic tissue returns to a natural rate of displacement.

Figures 1 and 2 are both from the same experiment. These graphs are representative of the results from the other experiments that took place during the course of this study.

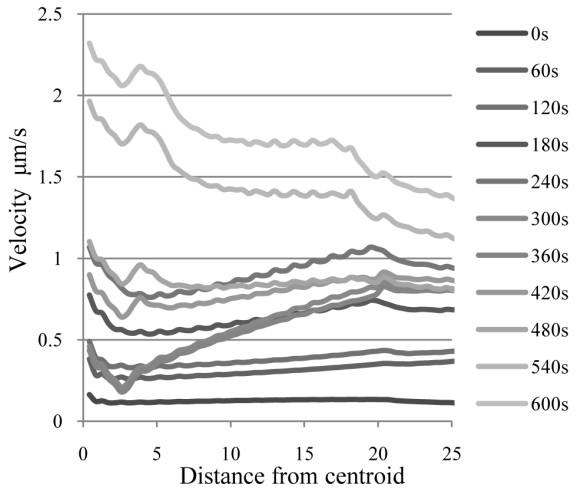

Figure 2. Displacement of embryonic tissue during germ band retraction in *Drosophila*. Each line corresponds to a single image of a time-lapse video; images were taken with a 30 second time delay. Wound healing is occurring when these images were taken.

DISCUSSION.

Immediately following laser ablation and as wound healing occurs, the greatest displacement of tissue is at the center of the wound. The velocity steadily decreases with distance from the center of the wound (Figure 1 and 2). The largest difference in displacement occurs between the first 20 seconds of laser ablation. Therefore, there is a sudden increase in displacement due to the laser ablation, or when an embryo is wounded during morphogenesis. The tissue in the embryo continues to have a greater displacement as the wound expands. As wound healing progresses, the displacement of the tissue dramatically decrease over all. Displacement returns to the normal movement of the tissue of this stage of development. During germ band retraction, the tissue is moving at a constant rate disassociating from the amnioserosa cells. This all illustrates what is physically happening as morphogenesis and wound healing occur.

These results were expected because the tissue movement during and immediately after laser ablation was similar to that observed in similar studies using laser ablation in *Drosophila* [2,3]. In further studies, different strains of *Drosophila* could be compared to determine if these results are typical of all *Drosophila* or just of the strain sGMCA which was used in this study. Since the strain of *Drosophila* used in this study is a mutant strain, there could potentially be slightly different results if this study was conducted on another strain of *Drosophila*.

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SUPPORTING INFORMATION.

Figure S1. Image sequence illustrating laser ablation and wound healing in a *Drosophila* embryo.
Figure S2. Illustration of Radial Profile.
Movie S1. Time-lapse video of normal *Drosophila* development.
Movie S2. Time-lapse video of micro-laser ablation and wound healing of *Drosophila*.

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