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Mechanical Engineering and Material Science

Geometric Characterization of Wild-Type and AngII-Treated Mouse Intestines under Applied Loading

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Abstract

The purpose of this study was to apply bench-top imaging (optical coherence tomography or OCT) to small intestinal tissues from wild-type (normotensive) and angiotensin II (AngII)-treated (hypertensive) mice, focusing on the visualization of different anatomical structures and geometric characterization of the intestinal wall under applied loading. Quantifying such properties of the intestinal wall will allow for further understanding of the functionality of the gastrointestinal tract (GI), the effect of hypertension on intestinal mechanical properties, and the relationship between chronic GI inflammation and tissue remodeling on the progression to colorectal cancer. To supplement the images gathered from OCT imaging (loaded and unloaded samples from wild-type and AngII models), microstructural composition and organization was assessed by histological and immunohistochemical analysis.

Introduction

According to the American Cancer Society, "…excluding skin cancers, colorectal cancer is the third most common cancer diagnosed in the United States" [1]. This, coupled with the myriad of other diseases (IBS, Crohn's disease, and diverticulitis) that can affect the GI tract, it makes further exploration into the mechanical properties and structure of these tissues in health and disease so pertinent. GI remodeling is driven by persistent inflammation which can be elevated as a consequence of conditions such as hypertension. To further understand the effect of hypertension on the properties of the GI tract, mechanical tests were performed on wild-type and angiotensin-II (AngII)- treated mice which revealed clear structural differences between the two. Angiotensin-II is a drug that causes increased blood pressure by constricting smooth muscle cells within the walls of arteries [2]. In the current study, there were three main methods employed to investigate the structure and properties of the intestinal wall- optical coherence tomography (OCT) imaging, histology (and immunostaining), and pressure cannulation. Combined, these methods allowed us to visualize changes in the intestinal wall following AngII treatment and helped identify possible studies that could be performed to further investigate the structures of the intestine and its mechanical properties in health and disease.

Methodology

Male C57BL/6J mice at 10 weeks of age were given a dose of either saline (control) or AngII (at $490\ \frac{(\frac{ng}{kg})}{min}$) via a osmotic mini pump implanted subcutaneously on the back of the mouse. After 14 days, the upper GI tract (small intestine) from the control and AngII-treated mice was extracted and stored in phosphate buffered saline solution (PBS) at 4 degrees Celsius until prepared for downstream analysis (e.g., imaging, histology, or pressure canulation).

To prepare the tissue for OCT imaging, a section of the small intestine (approximately 1.5 inches) was sectioned off. It was first flushed with PBS solution to remove the digestive enzymes secreted by intestinal mucosal cells during digestion. Then the outer connective tissue (microvasculature) was surgically removed using a scalpel prior to placement of the intestinal sample under the OCT scanner. The OCT imaging system utilizes reflected light to produce an image, in which "the echo time delay of the reflected light" is measured, "using low-coherence interferometry" [3]. Both processing steps ensured better light penetration into the intestine walls and produced clearer OCT images. During initial exploratory studies, OCT images were taken throughout different regions along the GI tract. However, in the current study, a special emphasis was placed on the structural differences between control and AngII-treated (diseased) segments of small intestine tissue.

To examine the microstructure of the small intestine by histology, the tissue was flushed, and connective tissue was removed, as described previously. It was then soaked in a 20% sucrose solution until the intestine sank to the bottom of the vial (approximately ten minutes) indicating that water in the tissue was replaced by sucrose solution. It was then left in a 10% formalin solution for 24 hours to preserve the tissue structure throughout the histology process. The tissue was then placed in OCT gel (an embedding medium used for freezing tissue), frozen at -80

degrees Celsius, and then cut into 12 μm thick slices with a cryostat. The tissue slices were then placed on slides and hematoxylin and eosin (H&E) staining was carried out to visualize tissue structure and cell nuclei. While using this stain, the hematoxylin appears dark purple (nuclei) and the Eosin stains pink (extracellular matrix). Once the slides were properly stained, a coverslip was placed on the slide to protect the tissue prior to examination under an optical microscope (Olympus). One other type of staining performed in this study was Masson's Trichrome staining (or trichrome) which is "used to visualize connective tissues." After successful staining, fibrillar collagen appears as blue, the muscle tissue appears as red, and "nuclei are dark brown spots" [4].

Immunohistochemistry was also utilized in the current exploration of healthy and diseased small intestine tissue. 4',6-diamidino-2-phylindole (DAPI), elastin (Alexa Fluor 633 hydrazide), and cytokeratin 14 (CK14) primary antibody were the three types of fluorescent staining included in the study. DAPI is useful for visualizing the cell nuclei and CK14 is an epithelial cell marker which would stain the mucosal lining of the intestine; elastin is a structural protein expected to be present in the intestinal extracellular matrix. Healthy mouse small intestine was prepped in a similar way as it was for the H&E staining and the slides were stained as follows:

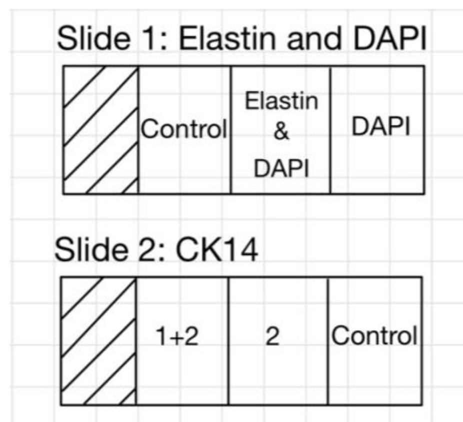

Figure 1: Diagram of the immunostaining sections for Elastin, DAPI, and CK14

The two slides were divided into thirds and in each section, there was a different combination of stains. On slide one (left to right), the first section was the control, the second was stained with elastin and DAPI and the third was just DAPI. For the second slide (left to right), it was both primary and secondary, the second section was secondaries only, and the last section was the control.

The final method used to investigate tissue properties was pressure cannulation of both healthy and diseased mouse intestinal tissue. The tissue was prepped in a similar manner as it was for the OCT imaging, prior to mounting on metal rods (14 gauge). To mount the tissue, each side of the intestine was tied down with 6-0 silk suture and then the intraluminal pressure was increased in increments of 0.1 PSI (approximately 5 mmHg). Prior to cannulation, the mounted intestine was flushed with PBS to minimize air bubbles during loading and unloading. The pressure was increased by 0.1 PSI then lowered back down to 0 PSI and OCT images were taken after each pressure change (at maximum loading and unloading).

Results

The use of OCT imaging revealed distinct differences in mucosal villi patterns between the healthy and diseased mouse intestinal tissue. Sample images are included below.

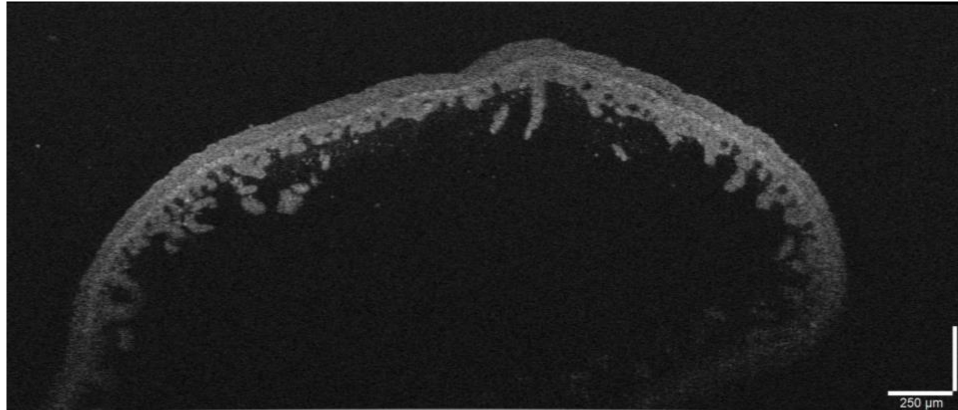

Figure 2: OCT image of healthy mouse intestine

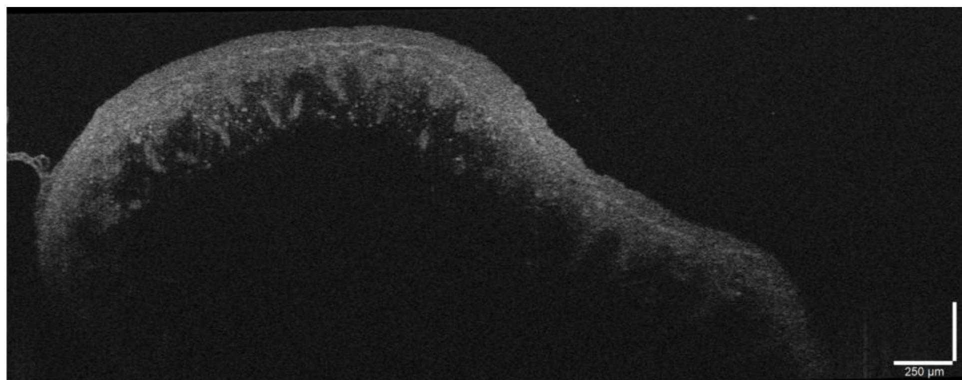

Figure 3: OCT image of diseased mouse intestine

In the OCT image of the healthy mouse intestine, there are clear villi projections whereas in the OCT image of the diseased mouse intestine the villi projections are less defined. Not only that, but the approximate thickness of the intestinal wall in the disease model appears to be larger than in the healthy model. There is also a well-defined boundary between inner and outer layers in healthy tissue that is less apparent after AngII treatment. In general, there appears to be visual differences between the diseased and healthy tissue that is captured by the OCT images.

The use of H&E staining provided further insight into the structure of healthy and diseased mouse intestinal tissue. Images of the slides are shown below at different magnifications.

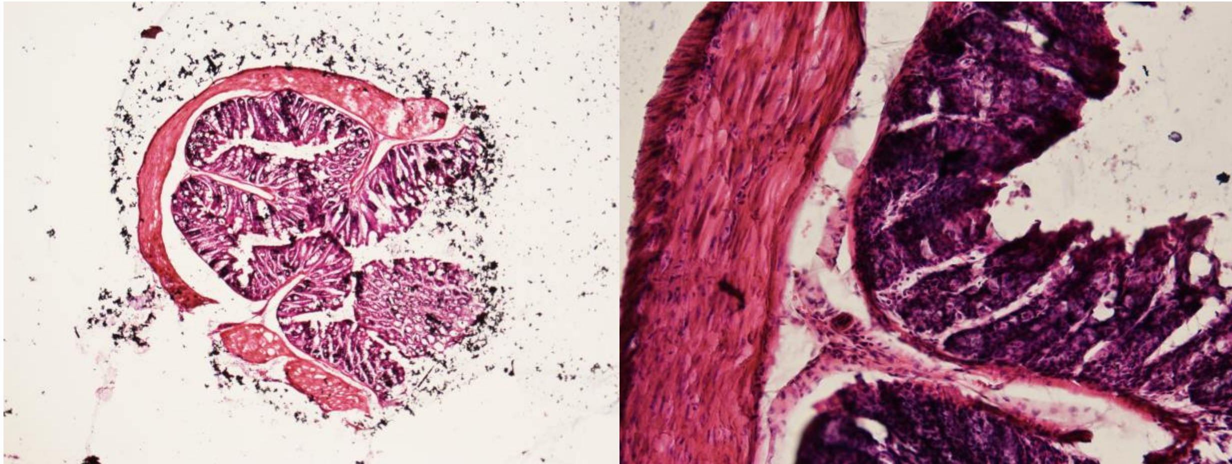

Figure 4: Healthy mouse intestine H&E stain, 4x (left) and 20x (right)

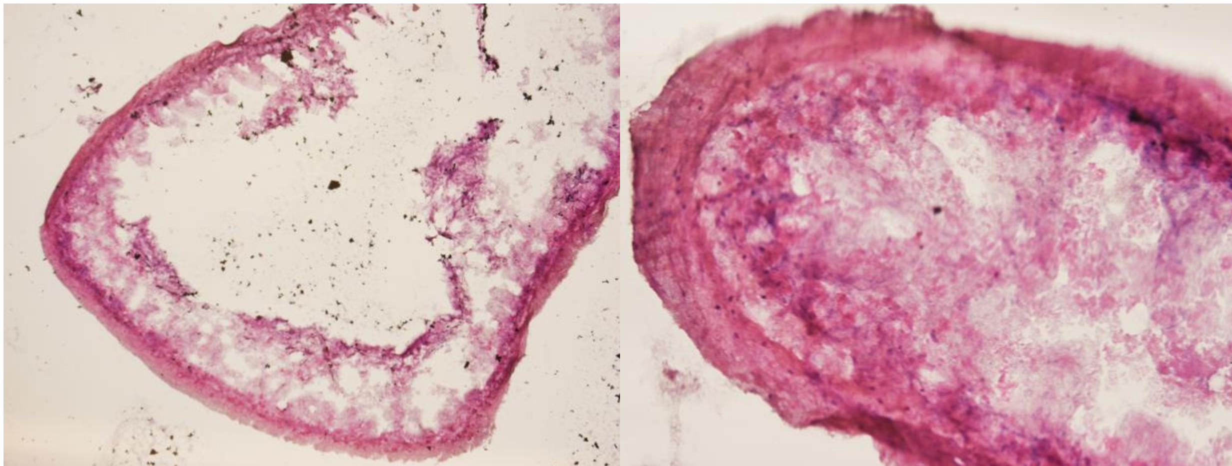

Figure 5: Diseased mouse intestine H&E stain, 4x (left) and 20x (right)

As shown in the H&E sections of the diseased and healthy mouse intestinal tissue, the healthy intestine contains a well-defined undulated villi pattern (dark purple), which is to be expected of a short-axis cut across the intestinal wall. Conversely, the AngII-treated diseased mouse intestine showed no distinct villi pattern, and the intestine had no well-defined pattern. This is similar to the loss of definition between the inner and outer layer observed in the OCT images. However, it

is important to note that the healthy and diseased H&E stains were not performed in the same batch therefore this difference in structure could be due to inconsistencies between H&E staining rounds. Further H&E stains could be performed on the healthy and diseased tissue at the same time to eliminate batch effects and unaccounted variance.

The final type of histological staining performed in the current study was Trichrome staining (only performed on the healthy tissue).

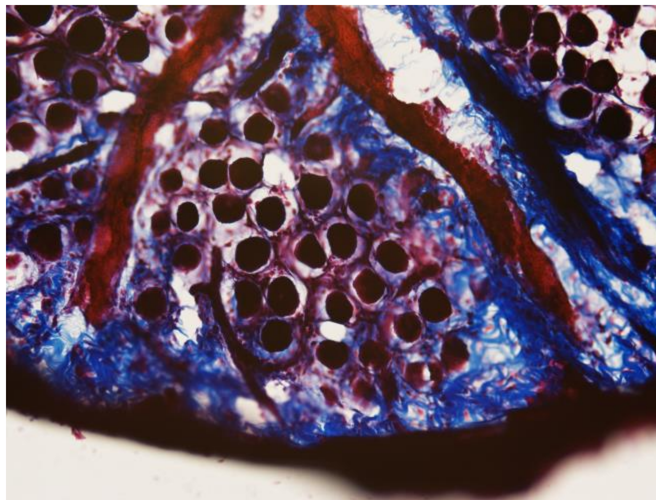

Figure 6: 20x Trichrome stain of healthy mouse intestine

From this image of the Trichrome stain, the nuclei (dark spots), collagen (blue), and muscle tissue (red) are all evident. In this specific section, the tissue was sectioned en-face in order to visualize the structure around the mucosal villi structures. This is another way to visualize the healthy tissue and better understand its structure in relation to mechanical properties. Future work will include similar en-face preparation to visualize changes in mucosal structure following AngII treatment.

Immunostaining was also only performed on the healthy tissue and, based off the images collected, it was concluded that there may be more effective methods to visualize the intestinal structure.

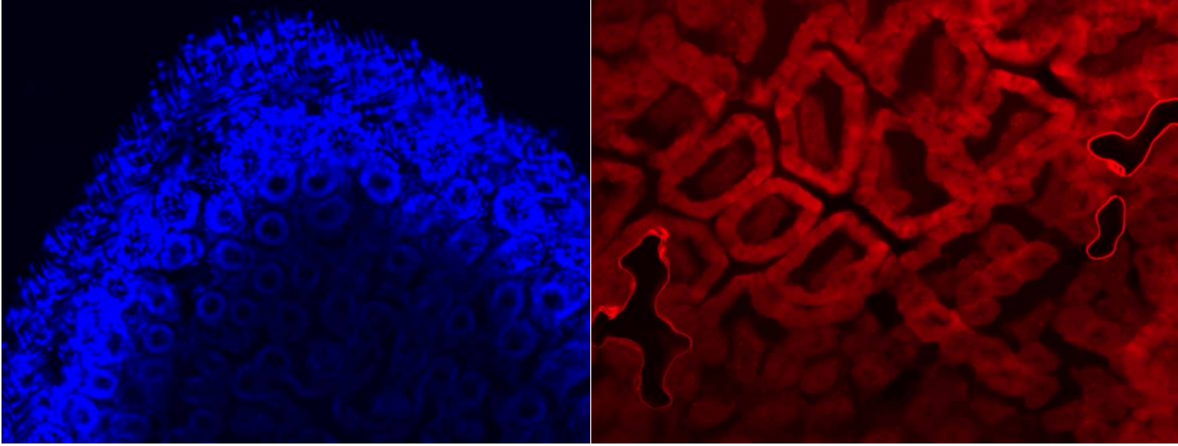

Figure 8: 20x DAPI stain (left) and 20x Elastin stain (right)

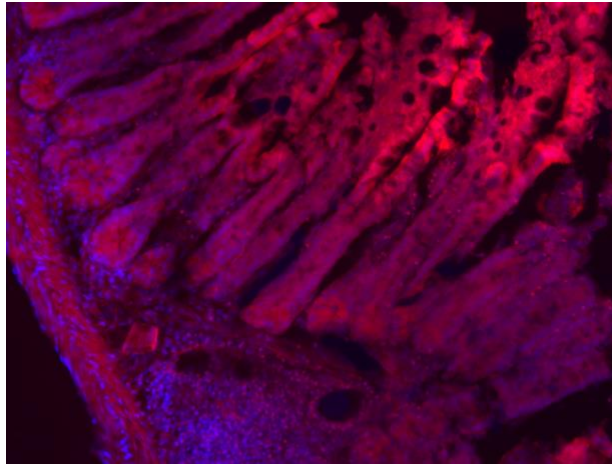

Figure 9: 20x DAPI and Elastin (images are overlapped)

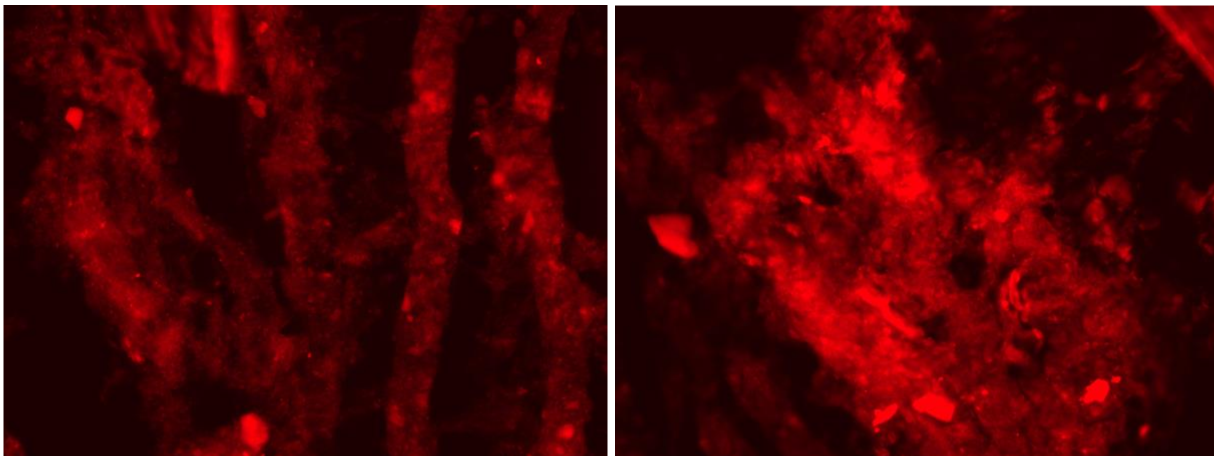

Figure 10: 20x Primary and secondary CK14 (left) and 20x Secondary only CK14 (right)

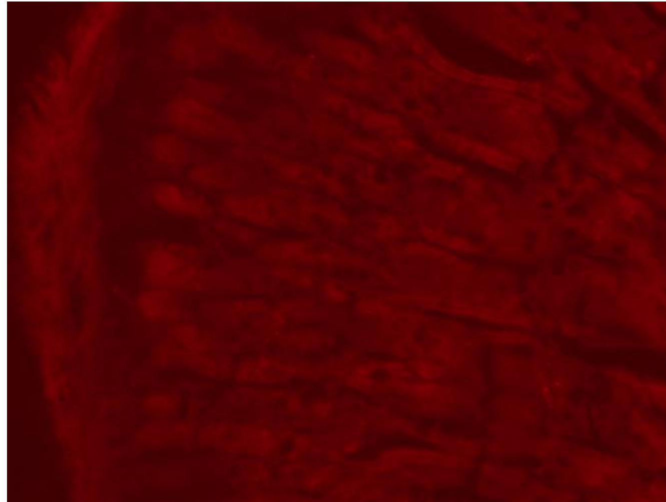

Figure 11: 20x Control CK14

Based on the fluorescent images that were collected, there were not strong conclusions to be drawn. The elastin stains in an en-face section did not reveal evidence of fibrillar elastin in the intestine, as there were no bright red lines. Instead, there was a more diffuse staining pattern that may indicate less concentrated elastin fibers in the area around the villi (Figure 8, right). Cross-sectional cuts showed punctate areas of elastin staining that tended to localize outside of the villi and within the intestinal wall (Figure 9). Similarly, with the CK14 stain in both the primary and secondary the images (Figure 10 and Figure 11) the areas of the intestinal tissue that were highlighted showed no apparent pattern and were sporadic. CK14 labels epithelial cells in various tissues but may not be the most appropriate marker for labeling intestinal mucosa.

The pressure cannulation was the last method performed in the current study. In this method, only OCT images of diseased tissue were successfully collected at different pressures. Further experiments could be conducted on healthy tissue in order to compare and contrast the mechanical capabilities between the two treatments. Given the baseline unpressurized images of healthy and diseased samples, one would expect that the AngII treatment will result in altered pressurization behavior.

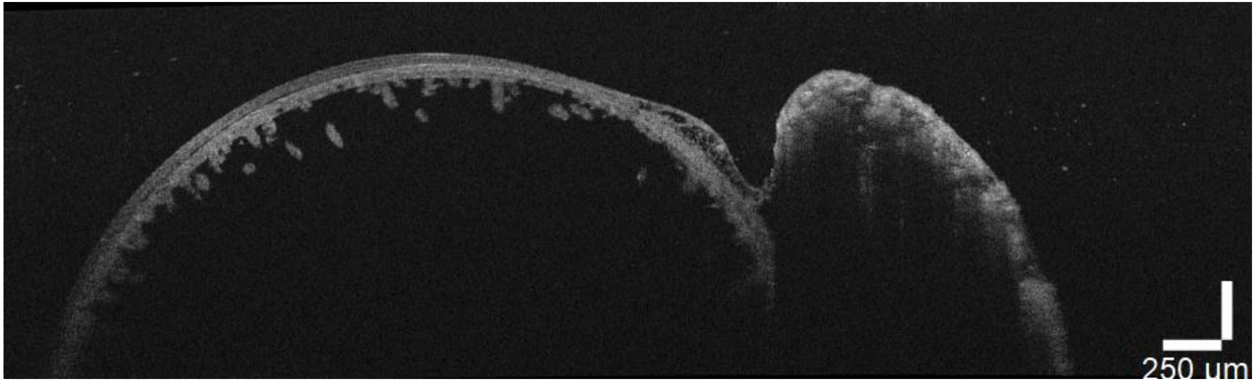

Figure 12: Diseased tissue at 0 PSI

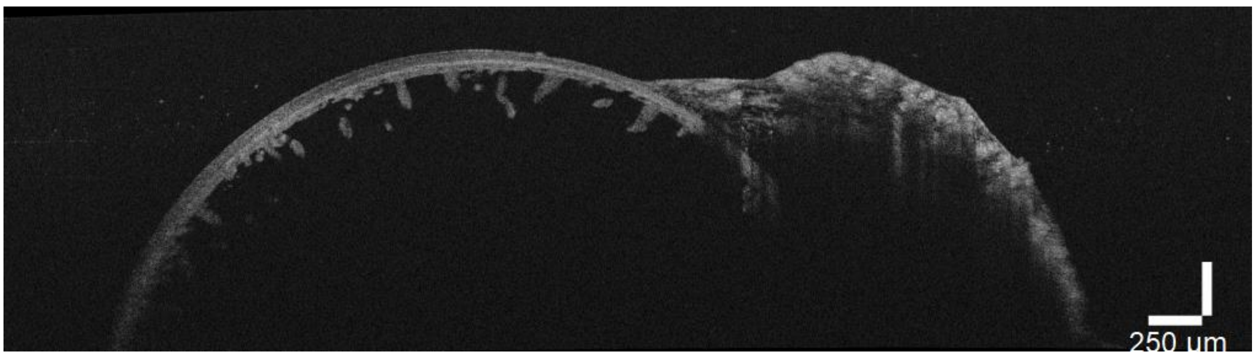

Figure 13: Diseased tissue at 0.1 PSI

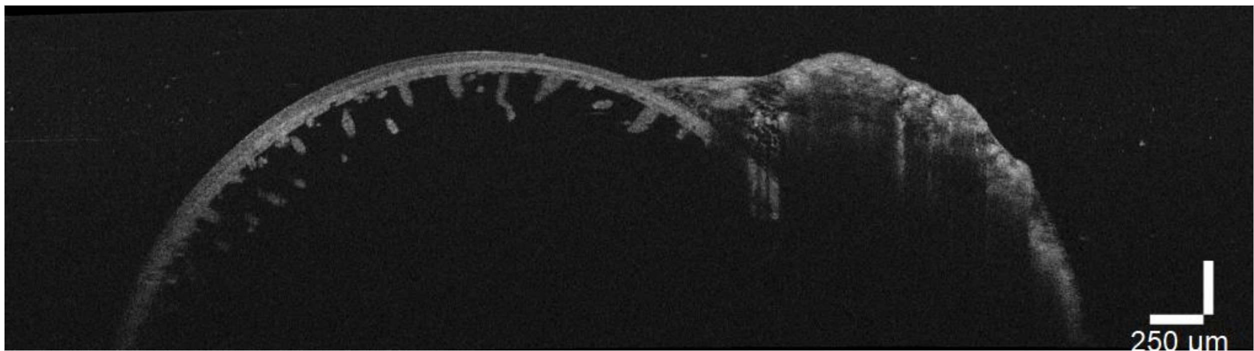

Figure 14: Diseased tissue post 0.1 PSI at pressure of 0 PSI

As shown in figure 14, post pressurized images were taken to serve as a comparison to the original diameter of the intestine at 0 PSI. Little difference was observed between pre- and post-pressurizated images, suggesting that 0.1 PSI is not sufficient to induce tissue damage. Future

studies will examine the loading conditions at which the tissue goes from being elastic to inelastic using a similar approach of OCT image collection at controlled pressure values.

Conclusion

Three primary experimental methods were utilized to geometrically characterize mouse small intestinal tissue in health (control) and disease (AngII). OCT imaging revealed the difference in intestinal wall thickness and villi structure between the diseased and healthy tissue. Histological analysis demonstrated that AngII treatment results in a lack of undulated villi projections and the pressure cannulation indicated a first step towards characterizing the mechanical properties of the small intestine. Each of the different techniques revealed more information about structure and function of intestinal tissue. From this work, further studies can be performed to better understand and characterize the mechanical properties of these tissues and begin to explain how they relate to chronic diseases such as colon cancer and hypertension.

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