Mechanical characterization of arterial tissue: Simultaneous confocal imaging and tensile testing

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***Abstract*—To avoid damage as a consequence of a surgical procedure, it is important to define loading thresholds that can safely be applied to soft tissues. The goal of this research is to determine the mechanical properties of cardiovascular tissue, more specifically the sensitivity of the tissue to damage. To be able to quantify this sensitivity to damage, an experimental setup was designed. This setup can stretch a piece of cardiovascular tissue stepwise. After each elongation of the tissue a microscopic image can be taken with a confocal microscope. These images can then be analyzed in order to determine the strain of the tissue, the orientation of the fibers and the damage to the tissue. To study the elasticity of the tissue, the stress-strain curve was calculated for one of the tissue samples. The orientation of the fibers was compared at different depths in the sample. To determine the damage of the tissue, the number of microruptures in the tissue were analyzed. These first results are promising and serve as a proof of concept of the experimental setup.**

I. Introduction

Accurate mechanical characterization of cardiovascular tissue at micro-level is necessary for numerous applications. One of these applications is robotic surgery, where there is currently still a lack of haptic feedback, with tissue overload as a consequent risk. By means of an online finite element simulation with a realistic material model, extra information about damage to the tissue can be handed to the surgeon, based on forces measured during the surgical procedure. Another application for this model is the prediction of the behavior of aneurysms, to avoid rupture [1].

To obtain realistic results from this model, it is important that the material parameters of the model are known. Several testing devices exist to acquire the material parameters for the different constituents of the aorta.

Commonly material parameters are obtained by performing mechanical experiments using a uniaxial or biaxial test bench or a pressure-diameter test setup. However, this method provides only information about the passive tissue response. The active behavior of the smooth muscle cells is examined with a myograph. The histology of the tissue can be examined with a microscope [1]. To determine the parameters that take the damage of the tissue into account however, not many methods are available.

To obtain more information concerning these damage parameters and to examine the stress-strain relationship of the aortic tissue, an experimental setup was designed. With this setup it is possible to look at cardiovascular tissue on a microscopic level. These microscopic images can be analyzed to determine some of the damage parameters.

II. Materials and methods

A. Tissue preparation

Rat aortic tissue is used since its dimensions are similar to those of human arteries often encountered during surgery. The rats used in the experiments were all male and weighed 500 g. After excising the aortic tissue, a segment of the aorta was cut open. The surface of each sample is approximately 5 mm by 5 mm. All samples are stained with propidium iodide (excitation 536 nm; emission 617 nm) and Hoechst33342 (excitation 346 nm; emission 460 nm) [3]. Propidium iodide (PI) will color only the dead cells, the Hoechst staining the living cells. The staining protocol starts with rinsing the sample twice in fresh phosphate buffered saline (PBS). Afterwards, the tissue is placed in a PI solution for 30 minutes. The PI is diluted with PBS (1/100). The sample is then rinsed again in PBS, before it is placed in a Hoechst solution for one minute. The Hoechst is diluted with PBS (1/500). Finally the tissue is rinsed once more with PBS. The samples are preserved in PBS at a temperature of 4°C.

Prior to staining the samples were punctured with a needle to provide some reference points. To determine the optimal diameter of this needle, 3 needles with different diameters (1.1 mm, 0.45 mm and 0.15 mm) were used to puncture three different tissue samples. The needle with diameter 0.45 mm gave the best result: clearly defined regions of dead cells that are easily recognized. These reference points can be used to calculate the strain of the tissue, when strain mapping based on image correlation fails. They can also be used to keep track of the orientation of the tissue, e.g. by placing two points along the axial axis.

B. Testing device

A testing device, consisting of a uniaxial tensile test bench and a microscope, was built. The goal of this device is to enable stretching of the tissue in small steps, while simulateneously allowing a confocal microscope to take an image after each elongation step. During the experiments, a load cell continuously records the forces on the tissue. This setup can be seen in figure 1.

The two linear actuators ensure that the tissue can be stretched symmetrically up to 200% for a tissue sample of size 5 mm by 5 mm. The chosen actuators are the microslide series from Newmark Systems Inc, with steppermotor. Only one load cell is necessary due to the symmetric application

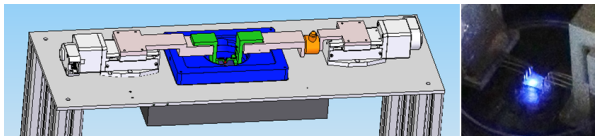

Fig. 1. (Left) Uniaxial tensile device: table with adjustable height (gray), stage of microscope (black), actuators (white), load cell (orange), clamps (green) and perfusion chamber (blue). The total length of the tensile test bench is 215mm.; (right) Snapshot of a mounted sample.

of force. The 'Model 31 mid-range precision load cell' from Honeywell is used. This load cell can measure forces up to 23 N, using four strain gauges in a full Wheatstone bridge. The tissue can be fixed to the tensile test bench with the aid of two clamps, see figure reffig:testsetup, which in turn are connected to the device through magnets. The clamps have several hooks (diameter: 0.9 mm) attached to them, used to mount the sample. The aortic tissue can be placed inside a heated perfusion chamber (the 'Heating Insert M' from Pecon) to keep it viable. The system is placed under an Olympus Fluoview1200 IX81- ZDC confocal microscope.

C. Imaging protocol

The wavelengths of the lasers of the microscope used during the experiments, are 405 nm and 552 nm. The beamsplitter splits the beam of light into two beams, one with frequencies above 490 nm, one with frequencies below 490 nm. Since the experiments are dynamic, the pinhole is completely opened. The image is magnified 10 times.

D. Data analysis

Three analyses are executed on the microscopic images. The stress-strain relationship is examined, as well as the orientation of the fibers and the damage to the tissue.

Stress-strain analysis: The one-dimensional second Piola-Kirchoff stress S_{11} and the lagragrian strain E_{lag} are calculated.

$$S_{11} = \frac{FL_0}{A_0 L} \tag{1}$$

$$\mathbf{E}_{lag} = \frac{1}{2}(\mathbf{U}^2 - \mathbf{I}) \tag{2}$$

with F being the force, L_0 the initial length of the tissue, L the current length of the tissue, A_0 the initial surface size of the tissue, $\mathbf{I}$ the identity tensor and U the right Green-Lagrange strain tensor. More information about these tensors can be found in [1].

The lagrangian strain is calculated based on digital image correlation with Vic-2D, a software from Correlated Solutions. The program tries to find a certain gray value pattern in consecutive images. Based on the correlation between two images, the software calculates the displacement vector for all points and consequently the strain in the images [4].

Orientation of the fibers: In the images, elastin and collagen fibers can be recognized. Since both contribute to the mechanical properties of the tissue, it is important to study them. The orientation of the fibers is studied with the program ImageJ, more specifically with OrientationJ. The hessian matrix is calculated for each point in the image. The

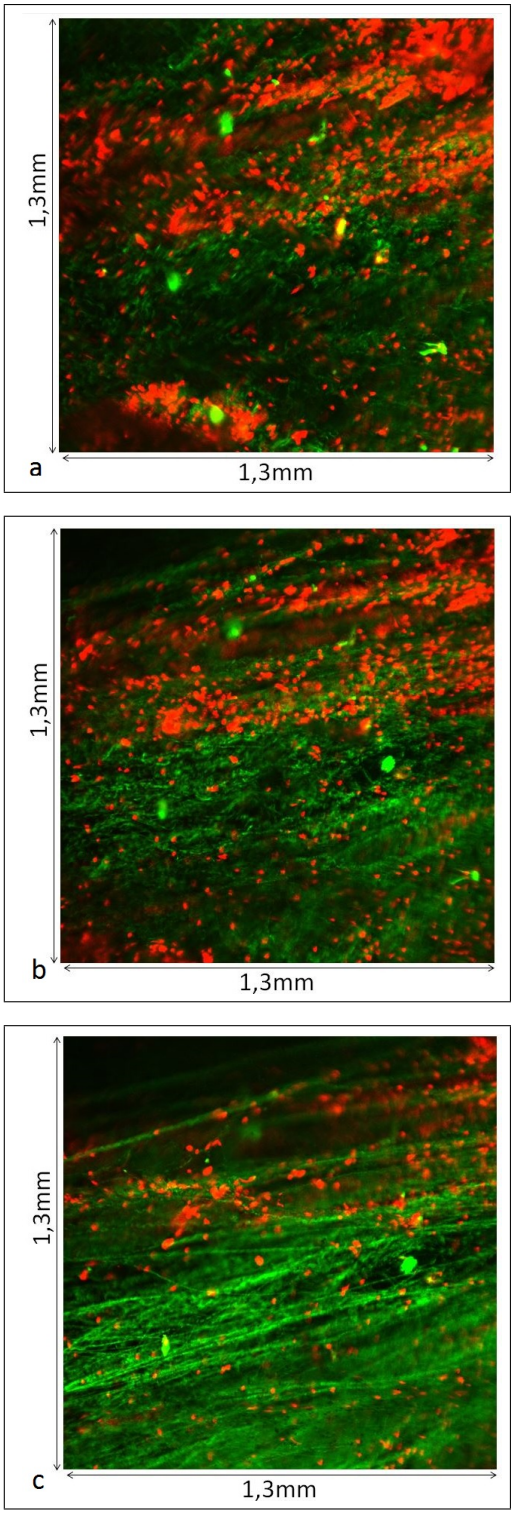

Fig. 2. Segment (1.3 mm by 1.3 mm) of a specimen (5 mm by 5 mm) stained with PI and Hoechst. The depth of the image is 125-150 micron. The tissue is stretched under the confocal microscope: a) unstretched, b) 1.3 mm stretched and c) 2.6 mm stretched.

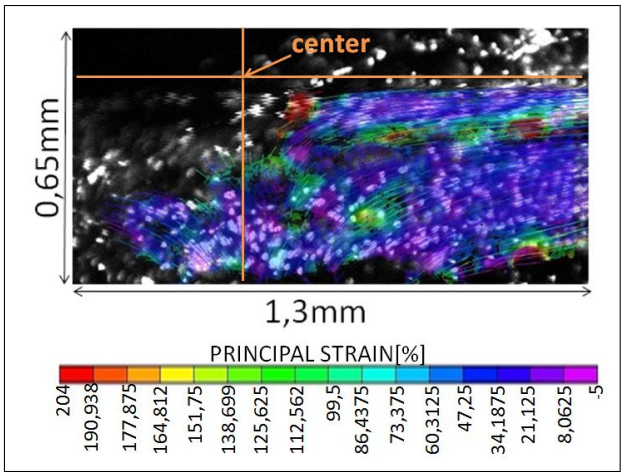

Fig. 3. Strain and displacement, calculated with Vic-2D for the image taken at a motor displacement of 1.3mm. The depth of the image is 125-150 micron.

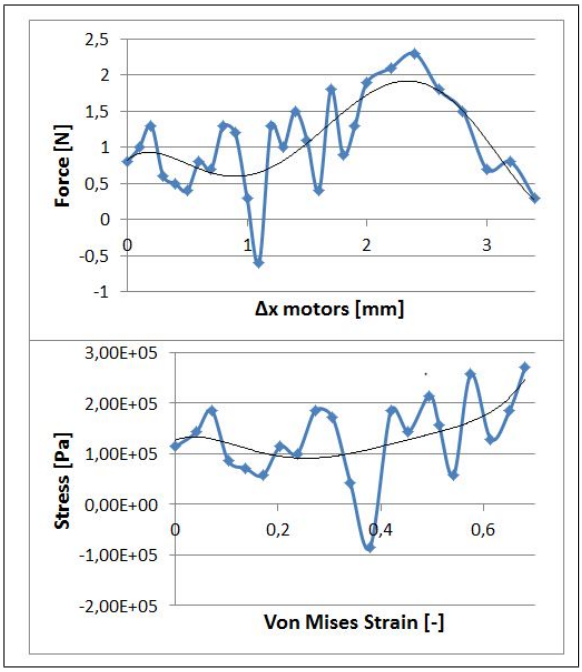

Fig. 4. (Top) Force as a function of motor displacement (Bottom)Stress-strain curve for a depth of 125-150 micron.

eigenvalues of this matrix correspond to the curvature values, the eigenvectors to the principal directions [5].

Damage to the tissue: The damage can be determined in two ways. A first possibility is to look at the evolution of the number of dead cells. A second possibility is to look at the evolution of the number of micro-ruptures in the tissue.

III. RESULTS

The analyses described above have been performed on data from one tissue sample. The results in this section are therefore *not* statistically relevant, but serve as a proof of concept. Microscopic images of this sample in the unstretched condition and in two stretched conditions can be seen in figure 2.

A. Stress-Strain

Lagrangian strain From the microscopic images the strain can be calculated with Vic-2D. The strain in the principal direction is depicted in figure 3 with a colormap. Only the top half of the microscopic image is depicted, since only at the top half of the image enough dead cells were visible to calculate the correlation between consecutive images. The small vestors in this figure depict the displacement compared to the unstretched condition. The strain calculated by Vic-2D is on average 0.129 mm smaller than the displacement of the motors. This is due to the fact that the sample ruptures around the hooks of the clamps. To determine where the center of the tissue is in the image, one can look at the orientation of the displacement vectors on figure 3. The y-axis (the axial axis) can be found in the region where the displacement vectors move apart. One part of the displacement vectors moves towards the left side, the other part towards the right side. The displacement vectors don't move horizontally, but a slightly upwards. This is due to the poisson effect. The x-axis (the transversal axis) lies at the top of the image where the the displacement vectors run horizontally.

Second Piola-Kirchoff stress Using the force data recorded during the experiments, the second Piola-Kirchoff stress can be calculated. The measured force has a large variability (see top figure 4), this can be explained by the fact that the experiments were performed pseudo-statically. It took about 60 seconds to take an image after displacement of the motors. During this time the tissue had time to relax.

Stress-strain curve The stress-strain curve can be seen in figure 4.

B. Fiber orientation

In the microscopic images (figure 2), it can clearly be seen that the fibers start to align during stretching. The fibers visible on the images are elastin and collagen fibers. They are autofluorescent and their emission spectra are in the same region as that of the Hoechst staining (elastin: 405 nm; collagen: 390 nm) [6]. Therefore they are visible in the green spectrum.

The orientation can be calculated with OrientationJ. Since it is interesting to compare the orientation of the fibers at different depths, this analysis was executed for three slices, 25 micron between each slice (figure 5).

The measured fiber angles of these slices are similar, therefore it is probable that the fibers from the three different depths belong to the same fiber family. In literature, the angles of the fibers with reference to the axial axis are located between $-5°$ and $-5°$ [8]. As can be seen on the figure, the values obtained with OrientationJ are in line with these results.

C. Damage to the tissue

The damage to the tissue can be quantified by looking at the evolution of the number of dead cells and the number of

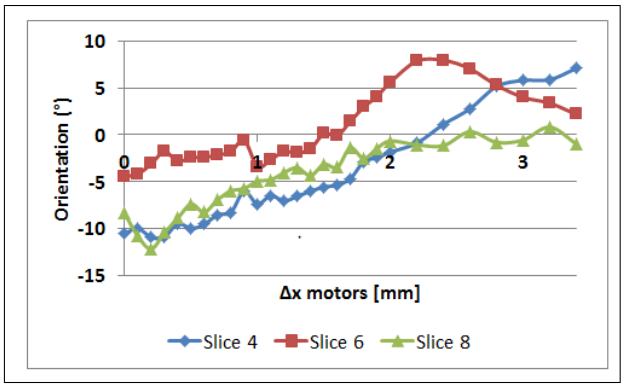

Fig. 5. Comparison of the orientation of the fibers: slice 4 (depth: 75-100 micron), slice 6 (depth: 125-150 micron) and slice 8 (depth: 175-200 micron).

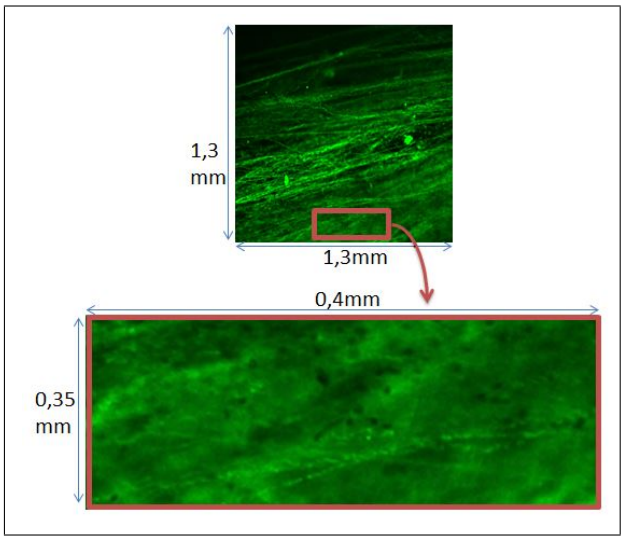

Fig. 6. Black dots appearing in images: possible micro-ruptures.

micro-ruptures.

Evolution of micro-ruptures It is possible to see more and more black dots appearing in the images, as the tissue gets stretched further, see figure 6. It is hypothesized that these dots are micro-ruptures. The decrease in the force around the time these dots appear, seems to support this theory. Further testing is necessary to confirm this.

Assuming that these black dots are indeed micro-ruptures, the evolution in the number of micro-ruptures can be seen in figure 7. The drop in the curve can be explained by several smaller micro-ruptures combining into some larger micro-ruptures.

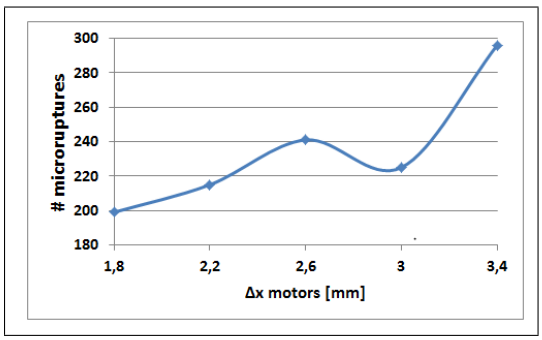

Fig. 7. Damage to the tissue: evolution in the number of micro-ruptures

Evolution of dead cells To be able to see more dead cells appearing as the tissue gets stretched and thus damaged more, the staining protocol needs to be adapted as described in [2]. The PI is than diluted in Hank's Balanced Salt Solution (HBSS) with a concentration of 1.5 M. The staining happens inside the perfusion chamber and is added 30 minutes prior to the start of the experiments. The tissue is not rinsed after applying the staining.

This protocol was not yet applied in the executed experiments.

D. Determination of damage-parameters

With the information from the images and the recorded forces, several parameters of the material model can be derived. The material model requires for example information about the angle of the collagen fibers. The results from the analysis in OrientationJ can directly be implemented here. Information about the damage parameters can also be accessed with the testing device. For example, one of the parameters that needs to be defined, is a dimensionless variable that brings the damage to the fibers into account. This parameter can be determined by looking at the occurrence of micro-ruptures. Another damage parameter is a dimensionless variable that brings the damage to the extracellular matrix into account. To fill this parameter in, more tests need to be performed with PI staining, continuously surrounding the tissue, as described above. That way the ratio of the number of dead cells and the total number of cells can be used to derive this dimensionless parameter.

IV. CONCLUSION

The designed setup was able to capture the deformation in the aortic tissue on a micro-level during stretching. This setup is of great assistance in determining realistic material parameters for cardiovascular tissue.

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