Characterizing Tissue Growth through Residual Strain

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Introduction

Tissue engineering is an important growing field in medicine, biology, and engineering. Tissue engineering is used for the purpose of restoring and maintaining tissue and/or organ function[1]. Currently, tissue engineering faces obstacles from the body rejecting the engineered tissue, mass transport limitations, and growth limitations. In order to succeed in restoring tissue function, engineered tissue should mimic native tissue as best as possible. To achieve this, the native behavior of the tissue must be thoroughly studied.

One key aspect of tissue engineering that is being studied is how native tissue grows. An important application of this is for pediatric right ventricular outflow tract (RVOT) replacements. Currently, children who receive RVOT replacements will need multiple replacements as they grow because the engineered tissue being used is a non-living, foreign material with limited long-term function. What is desired is to be able to use an engineered tissue that can grow as the patient grows, thus eliminating the need for subsequent replacements as the patient outgrows the engineered tissue.

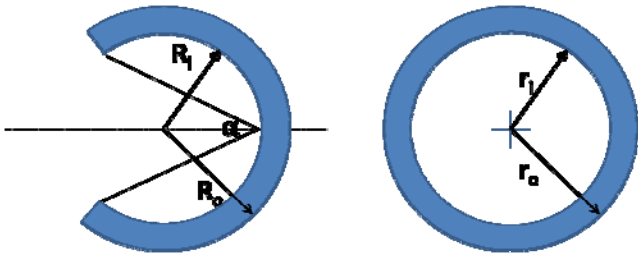
There are several ways to characterize tissue growth. Some properties that may help give an understanding of tissue growth are residual strain, tissue geometry, structure, and mechanics. My research will focus on the study of residual strain, specifically in the ovine pulmonary artery.

Residual strain is the strain present in a solid when all external loads have been removed[2]. Residual strain is an important property that has many effects. It homogenizes the stress distribution (circumferential, longitudinal, and radial stresses), which provides a uniform local mechanical

environment for the vascular smooth muscle cells (VCSM) throughout the arterial wall. This uniform environment is desirable in the tissue since smooth muscle cells respond to stress and non-uniformity would cause changes in their activity. Residual strain also serves to decrease wall shear and increase compliance, which implies that residual strains make elastic arteries more compliant, making them improved elastic reservoirs. It has been shown that residual strain changes with growth and remodeling by various experiments. For example, a study done on rats has indicated that the opening angle (see methods section), which is used to measure residual strain, increases from puberty to some finite age[2]. It has also been observed that induced hypertension results in increased residual strain. These studies show that residual strain is important to the function and performance of VSCM. We hope to gain a better understanding of its importance and role in growth through our study.

Methods

Residual strain can be measured by measuring the opening angle that is formed after a single radial cut is made in a ring of arterial tissue. The opening angle (OA) is the angle that is formed by the intersection of lines from the ends of the open inner arc at the midpoint of the inner arc (see figure 1).



a b

Figure 1: (a) shows the zero stress state in which a radial cut has been made. Opening angle (OA) is measured by the angle alpha (α). (b) shows the arterial ring in the no load state

To measure the OA, a camera is used to capture an image of the arterial ring in the no load state (closed ring) and also in the zero stress state after a radial cut is made in the closed ring. The experimental setup can be viewed in figure 2. The tissue ring in the no load state is placed in a neutrally buoyant medium and an image is obtained. The ring is then radially cut once, attached to a tube for stabilization, replaced in the medium, and another image is obtained of the tissue in this zero-stress state. When the

ring is initially cut, there is an initial rapid opening of the ring followed by a more gradual opening. This gradual opening takes about 20-30 minutes before the tissue has reached the equilibrium state and no longer opens. It is at this time that an image can be obtained and the tissue is considered to have fully reached the zero-stress state.

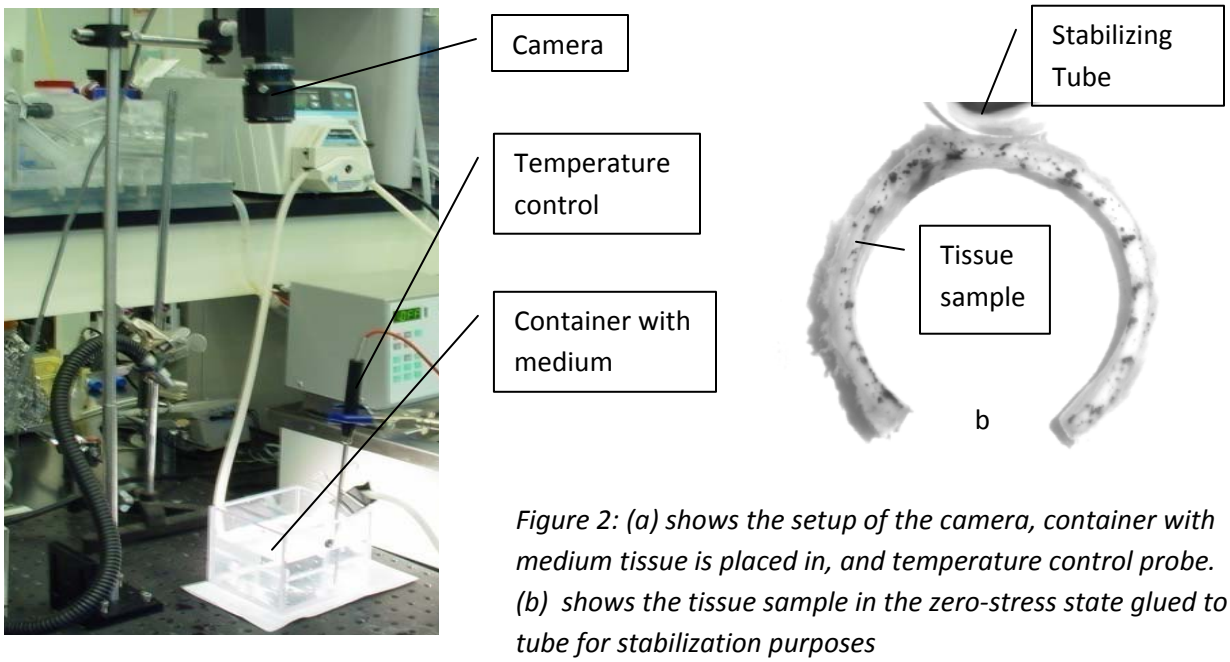


Figure 2: (a) shows the setup of the camera, container with medium tissue is placed in, and temperature control probe. (b) shows the tissue sample in the zero-stress state glued to a tube for stabilization purposes

a

For our experiment, we will measure the opening angle of two different sections of the RVOT, one near the sinus and one just before the bifurcation. Before being placed in the neutral medium, which is kept at 37°C, the tissue sample will be coated with many black water-insoluble ink dots. We will track the change in movement of these ink dots, which will be used to measure the local circumferential residual strain. We will then follow the methods outlined above to obtain images of the tissue sample.

From the dimensions obtained (see figure 1), the residual circumferential stretch ratio (λ_θ) can be calculated using the equation[2]:

$$\lambda_\theta = \frac{\pi \cdot r}{(\pi - \alpha)R}, \qquad \text{where} \quad r = \sqrt{r_i^{\,2} + \frac{(\pi - \alpha)}{\pi} \cdot \left[R^2 - R_i^{\,2}\right]} \qquad R_i \le R \le R_o$$

From the residual stretch, residual circumferential strain can be calculated by:

$$e_\theta = \frac{1}{2} \cdot \left({\lambda_\theta}^2 - 1 \right)$$

Another factor that is important to know is residual stress. Residual stress cannot be measured and must be calculated. To calculate residual stress, a 3-point bending test (see figure 3) is performed on the tissue sample.

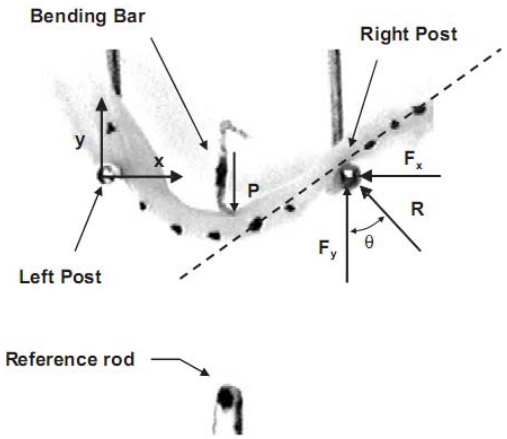


Figure 3: This shows an example of a 3-point bending test[3]. The two endpoints are secured on the left and right posts and a force is exerted in the middle at point P.

The bending test is used to characterize tissue properties in the low strain range. In this low strain range, the tissue exhibits a linear behavior. From the bending test, curvature deflection can be measured and the strain distribution can be analyzed by treating the tissue as a beam and applying beam theory[3].

A 3-point bending test is where the two endpoints of a beam-like structure of a tissue sample are secured and a force is applied to the center of the "beam". Using a reference bar of known curvature and deflection amounts per unit of force, the change in curvature of the tissue sample can be measured. Then, using equation $M = \Delta KEI$ (where M = known moment, E = Young's Modulus, I = geometric

constant, and ΔK = change in curvature), Young's modulus (E) can be calculated and thus, residual stress can be calculated using E and the previously measured strain.

Expected Results

From previous studies, it has been shown that longitudinal and radial residual strains are relatively negligible[2]. Because of this, we will be focusing on measuring circumferential residual strain. Y.C. Fung has shown that circumferential residual strain differs along the length of an aorta[4], decreasing as one moves further from the heart. Since the aorta has non-uniform dimensions (i.e. varying thickness) this indicates that tissue makeup affects residual strain. As tissue ages, there is an increasing quantity of collagen and elastin fibers that form within the tissue. Thus, we expect to see increasing residual strain with increasing tissue age. We also expect to see differences in residual strain measurements along the pulmonary trunk, which will be observed by measuring the residual strain at the end-sinus as well as just before the bifurcation, as Fung observed decreasing residual strain down the length of the aorta.

References

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