Biaxial Testing of Cadaveric and Decellularized Rat Heart Ventricles

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Introduction

Whole organ decellularization is a promising technique to create 3D scaffolds necessary to generate bioartificial organs. However, due to the vital role of tissue mechanics in the contraction of the heart [3], it is necessary to assess the changes to the myocardium caused by decellularization.

Previous studies have demonstrated that heart tissue is anisotropic [2]. Ventricle tissue fibers are aligned in the circumferential direction, and that orientation is preserved after decellularization [1]. The circumferential orientation of fibers is responsible for a higher stiffness in that direction than the longitudinal direction [1]. It has also been proved that decellularized tissues have a higher tangential modulus than cadaveric tissues [1].

Objective: Determine how the mechanical properties of heart ventricles compare in cadaveric and decellularized tissues, and left and right ventricles.

Hypothesis: We predict that decellularized tissue will be stiffer than the cadaveric tissue. It is also expected that the left ventricle will be stiffer than the right ventricle, because the left ventricle must attain a systolic pressure of almost 5 times as much as the right ventricle.

Methods

The experiments were performed on heart ventricles from young female SD rats with a mean mass of 254g. The samples that were decellularized were from the Taylor Lab in the Center for Cardiovascular Repair and decellularized at a perfusion pressure of 80%.

After dissecting the heart, the ventricles were cut into a cruciform shape using a template. A circular punch was used for the corners and a scalpel for all other edges. The thickness of the sample was then measured with a laser micrometer.

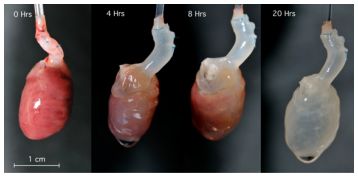
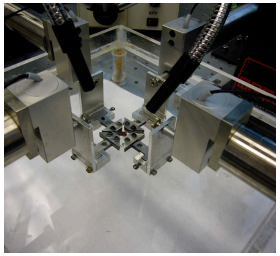


Figure 1 (Left): Pictures of the rat heart during the decellularization process.

Figure 2 (Below): Sample after being clamped into the biaxial machine.



Samples were tested on an Instron biaxial machine within 48 hours of dissection. The samples were placed in custom grips and speckled with Verhoeff stain for strain tracking. The stain was allowed to dry and the sample placed in the clamps attached to the load cells. The sample was immersed in 1% phosphate buffered saline at room temperature for the duration of testing.

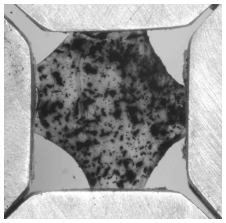
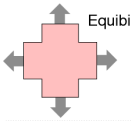


Figure 2 (above): Decellularized right ventricle, after being stained and clamped.

Figure 3 (below): Visual of the equibiaxial test used to calculate stress and strain.



The samples were tested using a protocol in which the displacement of the arms is set and the force measured on 5N load cells. The tissue was initially stretched to add a preload of approximately 0.01 N. The test starts with ten cycles of equibiaxial preconditioning. Ten cycles are used because the tissue is not perfectly elastic and therefore the last equibiaxial stretches exhibit lower forces than the ones occurring previously. By the tenth equibiaxial stretch, the force on each pull is no longer decreasing.

The test continues with single arm, adjacent arm, strip biaxial, and three arm stretches. One equibiaxial stretch concludes the test to ensure that no damage has been done to the sample in the multi arm stretches. Videos of each test were recorded.

To compare the tissues, the tenth and last cycle of equibiaxial preconditioning was the focus of our calculations. Longitudinal and circumferential stresses were calculated by dividing the total force on each axis by the total length of the axis multiplied by the thickness of the sample.

$$\text{First Piola-Kirchhoff Stress} = \frac{F_{Total}}{Length \cdot Thickness}$$

Stretch (λ) was then calculated using the following formula:

$$\lambda = \left(\frac{Q}{5}\right)t + 1$$

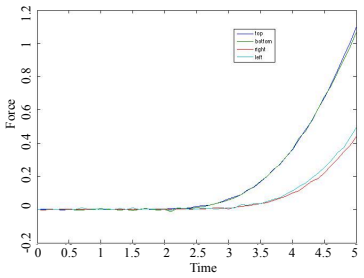
where Q is the grip strain that was input into the biaxial machine, is divided by 5 because the sample was pulled from zero to maximum strain in 5 seconds.

Green Strain was calculated using the following formula:

$$\varepsilon = 0.5\left(\lambda^2 - 1\right)$$

Results

Figure 4: Graph depicting the force on each arm of a decellularized left ventricular sample during equibiaxial extension. The top and bottom arms were the circumferential direction, and show higher forces than the longitudinal arms (left and right), but no two arms on the sample are identical.



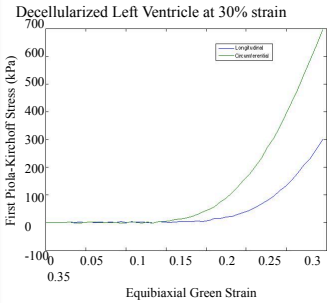
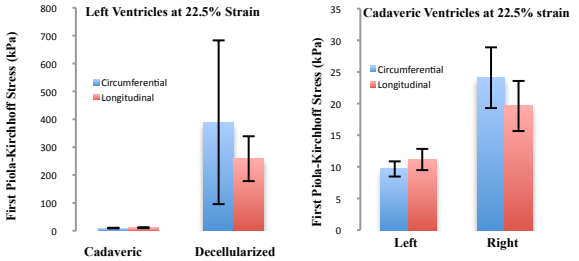


Figure 5 (left): Graph of First Piola-Kirchoff Stress v. Green Strain from the same test as Figure 4. Material behavior is nonlinear. The circumferential direction is stiffer than the longitudinal.

Figure 6 (below): A. Bar graph showing the average PK1 stress for cadaveric and decellularized tissue, with standard deviation. Decellularized tissue was found to be 20-40x stiffer than cadaveric. B. Bar graph showing the average PK1 stresses for left and right cadaveric ventricles with standard deviation. Right ventricles exhibited stress values about 2x higher than left ventricles.



Cadaveric and decellularized right ventricles behaved similarly to the left ventricles.

Discussion

Decellularized ventricles have been proven to be 20-40x stiffer than cadaveric ventricles. However, cadaveric samples are approximately 8x thicker than decellularized samples. Therefore, thickness does not account for the differences in stiffness. Decellularized tissues are stiffer than cadaveric, as predicted.

Right cadaveric ventricles are 2x stiffer than left, but left cadaveric ventricles are 2x thicker than right ventricles. In this case, the difference in thickness accounts for the difference in stiffness.

The experiment has also affirmed that the tissue of the heart is stiffer in the direction of fiber alignment, or the circumferential direction. The fiber orientation is preserved during the decellularization process.

However, there was much variety in the data, due to the the differences in the the original rat hearts, and the way the samples were cut and clamped.

Acknowledgements

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[3] Tendulkar, A.P. et al., 2006, Jour. Card. Surg., 21: 615-20