BE 172 Spring 2018

Week V: Cardiac function: Experimental measures and computational modeling

Objective

The objective of this lab is to obtain experimental data for cardiac mechanics and function. Following the experimental procedures, measurements from the frog heart are used in a computational model of the heart to predict stresses and material properties of the myocardium.

Background

The pumping function of the heart depends on a complex interaction of many factors including passive and active material properties, electrical activation, geometry, loading conditions, and neural and hormonal stimulation. The ultimate goal of a biomechanical model would be to incorporate all of these features in order to study the normal and diseased heart. To simplify the analysis of cardiac function, we will look at a few specific aspects of regional mechanics, specifically passive or diastolic function, and use a very simplified computational model of the heart to examine mechanical function.

The cardiac cycle consists of systole (contraction) and diastole (relaxation). Both of the portions of the cycle are important from a mechanical standpoint: systolic function directly determines the ability of the heart to eject blood, but diastole does this indirectly through the Frank-Starling mechanism. Also, many cardiac diseases such as heart failure are thought to involve diastolic dysfunction, for example the inability of the heart to return normally to the passive state after each contraction. Thus passive or diastolic function has been the focus of much research in the area of cardiac dysfunction. One method of studying passive mechanics in an experimental preparation is the isolated, arrested heart (5, 6). The contraction of the heart can be stopped with an agent such as potassium, but the heart remains "viable" and the passive properties can be studied in tissue samples.

One simple measure of passive function of the heart is the pressure-volume relationship (3). This curve can give much insight into changes in passive material properties which can occur, for example after myocardial infarction, as well as geometrical changes seen in certain cardiac diseases such as hypertrophy and dilated cardiomyopathy. The pressure-volume curve can also be used as the basis for simplified mathematical models of the heart which can elucidate properties such as wall stress (which cannot be measured directly). Wall stress is very important for cardiac function since it is known to correlate with tissue oxygen consumption, which is one of the most important regulators of myocyte function. Other aspects of passive mechanics such as preconditioning (2), strain softening (1) and residual stress (4) will also be examined in this week's experiment. All of these factors are important in determination of passive wall stress in the heart, which can only be found with mathematical models.

Equipment

- Oscilloscope
- Pressure gauge, validyne signal conditioner
- Stopcocks, tubing, syringes, razor blades
- Aortic cannula for retrograde infusion
- Mitral cannula with ventricular balloon, tubing, syringe for inflation and pressure measurement
- Video acquisition (camera and software) for imaging heart during and after inflation
- Continuity Finite Element modeling software

Tissue

- Arrested heart of the bullfrog

Prelab Questions

• What factors affect the shape and magnitude of the passive pressure volume curve of the heart?

• Myocardium can go into “contracture” when coronary blood flow is interrupted, this is similar to rigor mortis in dead skeletal muscle. Explain the cellular mechanism of contracture. How do you expect a passive PV curve to change when contracture sets in?

• Why is in important to have a ring of tissue “floating” in fluid when attempting to experimentally measure residual stress? Why do we make a radial cut in an intact short-axis ring of tissue to relieve residual stresses?

• In a computer model simulating inflation of the heart, we define the initial geometry of the heart (from experimental measures), and assume a value for the stiffness of the material. What are the boundary conditions that will be applied to this model in order to simulate passive inflation? How do we get those values needed for the analysis described here?

The isolated, arrested frog heart

As with other biological specimens, it is important to keep the heart moist and not induce overt external loads (i.e. don't drop it on the floor and step on it!) Once the heart is isolated and arrested, it will remain viable for at least an hour. A drawing of the heart from both ventral and dorsal views is shown in Figure 1. Note the large atria, single ventricle and bifurcation of the aorta. Venous systemic blood enters the right atrium from the sinus venosis, and oxygenated blood from the lungs enters the left atrium from the pulmonary veins. Blood from both atria then enter the ventricle, although does not mix thoroughly as the ventricle contracts. The spiral valve tends to direct oxygenated blood to the carotid and systemic branches of the truncus arteriosis, while unoxygenated blood mostly goes to the pulmonary arteries and then to the lungs.

The heart is located in the thoracic cavity and is found via a midsternal thoracotomy. Obtain a pithed frog, and with the frog on its back, make an incision through the skin and underlying muscle layer (starting about 1/2" below the rib cage) along the midline of the ribs. Use the large scissors to cut the ribs along their midline, and the heart should be visible and may be still beating. You should remove the pericardium to see the heart directly.

Notice the bifurcation of the truncus arteriosis (aorta). Since only one branch is needed, tie off one side of the truncus arteriosis with suture before the heart is removed, and cut the truncus arteriosis distal to the tie. Excise the heart, making sure not to cut the ventricle or atria, and leaving about 1 centimeter of the truncus arteriosis intact. Place the heart in cooled, hyperkalemic solution to initiate arrest.

Since frog hearts do not have a coronary circulation, the muscle is perfused via the blood in the chambers and outside the heart. Thus to fully arrest the heart, the truncus arteriosis is cannulated and tied with suture, then perfused with arresting solution. Slowly perfuse the arresting solution into the heart, 2-3 ml total, to stop any cardiac contractions. This procedure will essentially produce a "diastolic" heart, but a few contractions may persist and will not alter the results substantially. Keep the heart in cooled arrest solution while you are not using it, and periodically spray or drip perfusate on the outside of the heart once it is in place in the measuring apparatus.

Passive mechanics: Experimental Data

The goal of this lab is to experimentally measure the passive mechanics of the heart. Some of the data will be used the computational analysis to estimate mechanics of the diastolic heart. There are many ways to characterize the passive mechanics of the heart, in this lab we will examine 4 aspects of passive mechanics: preconditioning, the pressure-volume relationship, strain softening and residual stress. All 4 of these results should be included in your write-up with data and interpretive text.

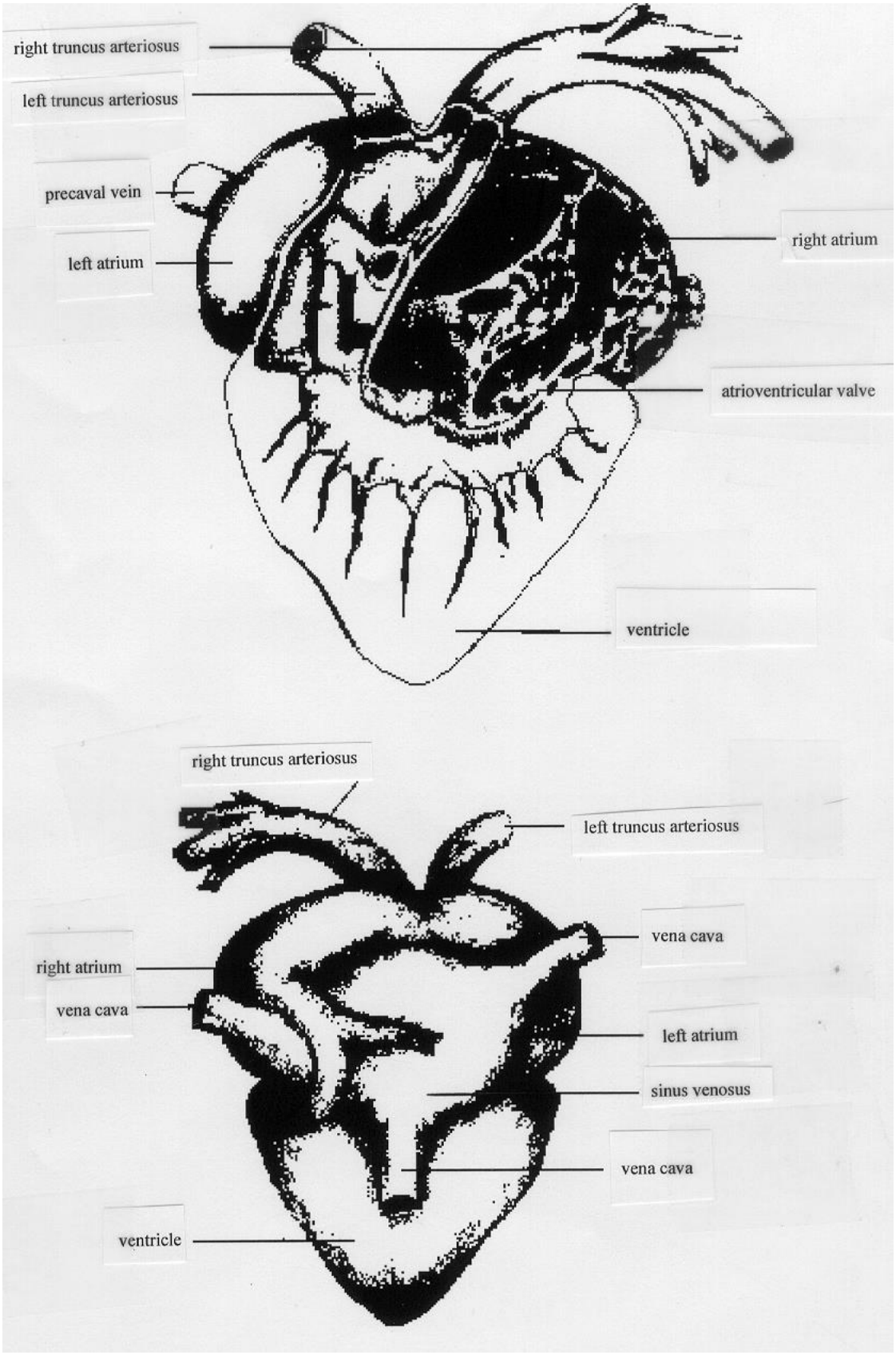

Figure 1: Ventral (top) and dorsal views of the frog heart

Pressure-volume curve for the arrested ventricle. The volume inside a frog ventricle is very small (measured in fractions of a milliliter), thus it is important to keep track of every drop of fluid. We will measure the pressure in the heart by inserting a balloon into the ventricular chamber, and inflating the balloon, which will stretch the passive myocardium around the balloon. The pressure is found from the pressure gauge connected to the closed system, and the volume in the ventricular chamber will be estimated in 2 ways: from video images of the heart combined with a simple geometric model, and also directly from the infused volume of a syringe. To find the starting (zero-pressure) volume of the ventricle, you must measure the inner and outer radii after you have completed the experimental inflation tests.

Preconditioning and strain softening. These 2 phenomena are examined from the pressure-volume data which have already been recorded. Make sure to plan your pressure volume experiments properly so you can quantify these properties after you are all finished with the data acquisition.

Residual stress. We will not be finding the residual stress directly, but rather quantify the stress-free state of the ventricle. This is done after the PV curves are finished, by cutting the ventricle such that is essentially stress-free, and measuring the deformations that occur.

Experimental Methods

The bulk of the experimental equipment is used for the pressure-volume experiments outlined below.

(1) Calibrate the pressure transducer. This calibration is done with a static pressure head just like in Lab I. First balance the gauge by adjusting the balance potentiometer on the strain gauge amplifier to read zero volts with zero pressure. You may assume a linear calibration, thus obtain 1 point for the calibration at approximately 50 mmHg, and compute the calibration factor assuming a linear system. You should obtain a calibration factor which will convert voltage seen on the oscilloscope to pressure, and write that factor on the white-board in the lab. In your write-up use mmHg vs. ml for the plot of pressure-volume, although the engineering FEM may need other units for pressure. After the calibration, remove the static pressure head.

(2) Set up the system for the PV experiment. Set up the experimental apparatus as shown in Figure 2 (without the heart). The calibrated pressure gauge and volume infusion devices are connected with a "T" connector tube which will go into the heart. The balloon will be on the orange tubing adaptor and inside the heart. Try to remove the large air bubbles from the system. Small bubbles will not affect the measurements taken here (Why not?). Remember to "zero" the system by opening a stopcock to the atmosphere (without letting air in!) and adjust the pressure offset to be zero at that pressure level. Set up the video acquisition system to run the video cameras and capture images to files.

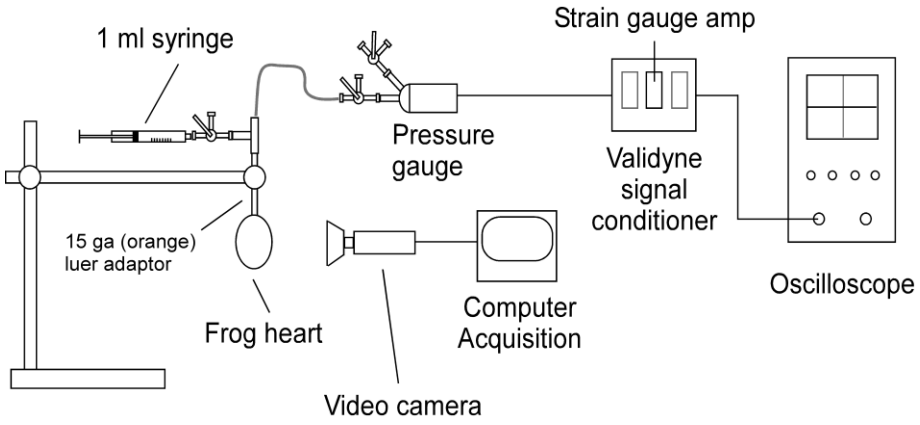

Figure 2: Experimental setup for the isolated frog heart

(3) Secure heart and obtain pressure/volume/diameter data. The entire balloon inflation system should be setup and tested, with the balloon secured and in place at your station with a clamp. Cut a large hole in the top of the atrium of the heart, and then pull the heart up over the balloon. Loosely tie a suture around the atrio-ventricular groove which will help keep the balloon in place. With the heart in place, the aortic cannula is not needed and should be cut off. Pressure-volume data will be found with the following protocol:

Inflate the ventricle with 6-8 pressure load increments, up to a maximum pressure of 15-20 mmHg. Repeat this curve 3 times, recording the PV data for each. You only need to record the PV data for loading, not unloading. You should read the volume infused directly from the syringe markings. After each loading/unloading cycle, open the tubing system to ambient air pressure to relieve any residual negative pressure. You will examine the preconditioning effects by looking at the changes in these 3 PV loading curves.

For a final, preconditioned curve to a maximum of 15-20 mmHg, record a video image of the heart at each static pressure from which the volume will also be estimated. Record a calibration ruler in one of your video images. Again, only do this for the loading part of the cycle.

To examine strain-softening, repeat the loading curve, but this time inflate to a higher pressure on the order of 30-50 mmHg maximum. Repeat this curve twice. You do not need to record the video for this curve.

(4) Weigh the ventricle. Remove all cannulae, balloon, and excess fluid from the heart. Use a single-edge razor blade to cut off the atria, and weigh just the ventricle. Assume a density for muscle to find the volume of the ventricular tissue.

(5) Measure the unloaded geometry of the heart. Clean up the frog and all the PV equipment if you have not done so. Use the video acquisition system to take pictures of the tissue, and a video analysis program to measure the lengths of interest. First record a picture of the entire heart to measure external diameter and apex-base length. Next, use the razor blade to cut a “ring” from the ventricle (about 2 mm thick). The cut will be perpendicular to the long axis of the heart, a short-axis slice of the ventricle. Measure the inner and outer radii of the ring. Your picture will look better with the ring submerged in fluid and with proper lighting. You can adjust the aperture opening on the camera lens to adjust for lighting. Remember to include a calibration image (a ruler) to get calibrated distances. Sometime the inner diameter is difficult to see, look for a flattened "crack" and use its length for the inner diameter. These values will be used to find the residual, unloaded volume of the heart, and used in the computational model of the heart. If we assume the heart is a sphere and that the material in incompressible, then the volume inside the unloaded heart can be determined from the radius information. You can also find the volume (and inner radius) of the heart tissue from the weight above, compare these 2 values and comment on the limitations of each.

(6) Measure the “opening angle” of a stress-free ring. Cut the ring across the radius and record the opening angle immediately with video. To see the effects of residual stress, you must have the ring submerged in fluid, so the frictional effects are minimized. The opening angle is an indication of the residual stress present in the intact ring.

Computational modeling

Computational modeling of cardiac function takes on many forms, but a powerful computer method known as finite elements (7) has been used to look at the mechanics of ventricular deformation and pressure generation. The passive inflation of the heart can be modeled as inflation of a thick-walled pressure vessel (sphere, cylinder or ellipsoid) by simple analytic models of an elastic material, for example as shown by Timoshenko (8). Even the simplest analytic model may be difficult to implement for quick results. Thus the finite element technique offers an alternative to such analytic models which is readily available and easy to run on most computers. We will employ a simple finite element scheme to model the inflation of the passive heart. In such a model, the quantities necessary to run the model are the initial geometry, the boundary conditions, and the material properties. By comparing the results of the model (for example the ventricular volume as a function of load) with the experimental findings, one can alter the material properties such that the model more closely approximates the inflation of the actual heart. Thus the material properties are "optimized" (9) and the resulting model can then be used to predict mechanical features of the heart, for example, the stresses in the wall during inflation (10).

Use Continuity to model inflation of the heart. Continuity is an academic use only, public domain finite element modeling tool developed by UCSD researchers for biological modeling. This finite element program can be used to model inflation of the heart as a thick-walled ellipsoid, replicating the experimental result and providing additional information about the mechanics problem. The program is installed on the computers in PFBH 161, or you can register and install it on your own computer (for academic use only). The Continuity Web Site is located at http://www.continuity.ucsd.edu/Continuity and the analysis for the heart lab in BE172 is located under “Documentation:Tutorials:Biomechanics Module: Frog Ventricular Pressure-Volume Model”. All of the instructions for setting up the finite element model and completing the analysis are in this documentation.

Use the measured geometry of your group’s frog heart (radius, apex-base length and wall thickness, and make an initial guess for the scaling factor in the material law (stiffness of the tissue), and load the model heart with multiple load steps up to the maximum pressure obtained in the experiment. Make a plot of the pressure-volume curve for the model, and compare it to your experimental results. Since your guess for the material law was probably incorrect, modify the single scaling factor parameter in the model in order to change the stiffness, run it again, and plot the PV curve. Repeat this process until you have the best match between experiment and model (maximum of 5 iterations).

Once the model of the heart is complete (the ‘best’ material found), use the results to plot circumferential and radial stresses as a function of location from the inside to the outside of the wall. Plot these values at one loading condition. Comment on this distribution, and any physiologic implications you can think of for cardiac mechanics, including residual stress.

Write-up notes

Introduction:

Same as ususal: problem statement and objective of the lab. Background for passive mechanics of the heart, and mention each type of measurement that is taken here.

Materials and methods:

Again as usual: describe your system for the experiment and how you will use it to determine the desired quantities. Include at least a sentence for the different parts of the lab: the pressure-volume curve, the strain softening and the residual stress. Also briefly mention the finite element method and software.

Data/Analysis/Results/Discussion:

Volume estimation: Plot the preconditioned PV curves using both methods of volume estimation on the same plot with the same units for both. Include an estimated Vo (unloaded volume) in your plots. Comment on the differences in the ventricular volumes from the syringe readings and those calculated assuming a spherical geometry, incompressibility and a known starting volume. Comment on the limitations and sources of errors from each of the 2 methods. You may or may not use the ventricular weight in this calculation, but in any case compare the measured volume (weight) to the volume estimated from the measured geometry.

Indicate your "final" PV curve, i.e. which one represents the best data for the passive mechanics of the heart in your experiment. Comment on why this one is the best.

Preconditioning: Show changes in initial PV curves and how these changes become smaller with each repeated curve. Comment on deviations from the expected changes.

Strain softening: Look for changes in the ventricular compliance after inflation to a higher pressure. Comment on the possible mechanisms of this phenomenon. Remember compliance is non-linear and does not depend on the initial volume (at pressure = 0).

Residual stress: Give the magnitude of the opening angle, and compare to known values (in other species) and the possible implications of a non-zero opening angle for cardiac stresses.

Finite element modeling: Give 2 graphs in your writeup, (1) Plot of the pressure volumes curves, there should be up to 6 plots on this graph, 1 is the experimental data, and up to 5 from the model showing results with the different material properties. (2) Plot of circumferential and radial stresses as functions of radius at any one loading condition for the final model.

References

1. Emery, J. L., J. H. Omens, and A. D. McCulloch. Strain softening in rat left ventricular myocardium. *J Biomech Eng* 119: 6-12, 1997.

2. Fung, Y. C. *Biomechanics: Mechanical Properties of Living Tissues*. New York: Springer-Verlag Inc., 1981.

3. Gilbert, J. C., and S. A. Glantz. Determinants of left ventricular filling and of the diastolic pressure-volume relation. *Circ Res* 64: 827-852, 1989.

4. Omens, J. H., and Y. C. Fung. Residual strain in rat left ventricle. *Circ Res* 66: 37-45, 1990.

5. Omens, J. H., D. A. MacKenna, and A. D. McCulloch. Measurement of strain and analysis of stress in resting rat left ventricular myocardium. *J Biomech* 26: 665-676, 1993.

6. Omens, J. H., K. D. May, and A. D. McCulloch. Transmural distribution of three-dimensional strain in the isolated arrested canine left ventricle. *Am J Physiol* 261: H918-H928, 1991.

7. Becker, E. B., G. F. Carey, and J. T. Oden. *Finite Elements: An Introduction*. Englewood Cliffs, New Jersey: Prentice-Hall, 1981.

8. Timoshenko, S. P., and J. N. Goodier. *Theory of Elasticity*. Tokyo: McGraw-Hill Kogakusha Ltd, 1970

9. Emery, J. L., and J. H. Omens. Mechanical regulation of myocardial growth during volume-overload hypertrophy in the rat. *Am J Physiol* 273: H1198-H1204, 1998

10. Costa, K. D., P. J. Hunter, J. M. Rogers, J. M. Guccione, L. K. Waldman, and A. D. McCulloch. A three-dimensional finite element method for large elastic deformations of ventricular myocardium: I - cylindrical and spherical polar coordinates. *J Biomech Eng* 118: 452-463, 1996.