On the role of the pericardium in the Physioheart experimental setup

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1 Introduction

This report is about my internship at HemoLab BV. HemoLab BV is a small company situated at the Biomedical Engineering Department of the Eindhoven University of Technology. It is specialized in research in the field of cardiovascular biomechanics, analysis of hemo-physics in and around cardiac devices and pathophysiological processes. One of their tools is Physioheart; an isolated heart experiment, performed in Langendorff perfusion or in a different working heart mode. A recent development is that the Langendorff experiment can be done with an intact pericardium. The aim of my study is to get more insight in the influence of the pericardium in the Physioheart setup.

1.1 Langendorff perfusion

The Langendorff perfusion is one of the oldest, but a still often used method in isolated beating heart experiments. It was first performed by Oscar Langendorff in 1895 [13]. As shown in Figure 1, it involves the cannulation of the aorta which is then attached to a reservoir containing oxygenated perfusion fluid. This fluid is then delivered in a retrograde direction down the aorta either at a constant flow rate (delivered by an infusion or roller pump) or a constant hydrostatic pressure (usually in the range of 60-100mmHg). In both instances, the aortic valve is forced to shut and the perfusion fluid is directed into the coronary ostia. Thereby it perfuses the entire ventricular mass of the heart, draining into the right atrium via the coronary sinus [11]. In Langendorff setup the heart can be kept alive for several hours.

HemoLab does constant flow Langendorff perfusion in porcine hearts. Directly after bleeding a stunned pig to death, the thoracic content is excised. Then the pericardium is removed and the aorta and pulmonary artery are excised. The aorta is cannulated to administer cold cardioplegia, to stop the heart from beating and thereby preventing tissue damage of the heart muscle by oxygen defect. Figure 1 shows a porcine heart without pericardium in a HemoLab perfusion experiment, supported by a plateau.

The removal of the pericardium may have an influence on the filling dynamics of and the general conditions (e.g. moistness) of the heart (see section 1.2). Furthermore, supporting the heart by a plateau might cause changes in the beating of the heart. Therefore it was attempted to create a more physiological setup by leaving the pericardium intact during the Langendorff perfusion experiment. The aim of my project was to investigate the role of the pericardium in these kind of experiments.

To do the Langendorff experiment within the pericardium two changes had to be made. Firstly, the cardioplegia had to be given to the heart within the pericardium. This caused difficulty since the first branches of the aorta are inside the pericardium. By cross clamping the aorta downstream the first bifurcation, the cold cardioplegia could be administered retrogradely through the first bifurcation. The second alteration was that the heart should be suspended in the pericardium without being supported by a plateau. Therefore the pericardium was attached to the experimental setup by bulldog clamps, as can be seen in Figure 2.
Figure 1: Schematical drawing of the Langendorff perfusion experimental setup, and a picture of a Physioheart in Langendorff mode at Hemolab.

Figure 2: Picture of the Physioheart with intact pericardium in Langendorff mode.
1.2 The pericardium

The pericardium is a membrane around the heart. It consists of a serous part and a fibrous part. The fibrous part is a strong, loosely fitting closed sac around the heart, consisting of elastin fibers and collagen fibers. The collagen fibers make the fibrous layer a strong tissue. On the inside of the fibrous layer a thin smooth layer is found. This layer is called the parietal layer. Together with the epicardium and pericardial fluid the parietal layer forms the serous part of the pericardium. The serous part provides smooth movement of the pericardial sac over the epicardium [7]. From now on with the word pericardium the loosely fitting sac is meant, consisting of the fibrous and parietal layer. Figure 3 shows a schematic drawing of the human heart in its pericardium and a picture of the porcine heart suspending on the remains of its pericardium. It can be seen here that the pericardium is attached to the upper part of the heart, and isolates the heart from its surroundings. Only the arteries and great veins run through the pericardium into the heart. Other membranes merge from the pericardium, and run to other organs. The pressure in pericardial space is normally -5 to -6 mmHg [4].

The most obvious functions of the pericardium are to provide smooth movement of the heart in its surroundings, to keep the heart in place and to protect it from infections in nearby organs. Beside these functions, the pericardium is also known to have influence on the filling dynamics of the heart. In this study we focussed on the latter function.

Figure 4 illustrates that the pericardium encloses both ventricles and both atria. In resting volume, the pericardium is loosely fitting. However, when the heart fills it grows in volume and the pericardium becomes tight. Since the collagen fibers give the pericardium strength the pericardium gets a constraining effect on the heart. Thereby the hearts influences the left-right interaction of the heart. This function has been investigated and demonstrated in several articles (see subsection 1.3).
1.3 Earlier research on the pericardium

Several researches have been done on the pericardium, which can be divided in two main categories; research on its mechanical properties and on its influence on cardiac function and filling. In research on the influence of the pericardium on cardiac function, Bergelund proved that the pericardium has an effect on diastolic filling dynamics during overfilled state of the left ventricle caused by aortic resistance. The right ventricular filling and work are hindered when the left ventricle is dilated, and atroventricular regurgitation is prevented [2]. Gibbons showed that the pericardium modulates left and right ventricular stroke volumes to compensate for sudden changes in atrial volume [1]. In that way it keeps left and right ventricle output equal, protecting the body from disturbed blood distribution. After removal of the pericardium, little or no direct ventricular interaction and limited ability to compensate rapidly for sudden changes were shown.

As for material properties, it was shown that the collagen bundles in the pericardium are distributed over two main directions, at an angle of 80 degrees from each other. Therefore stress strain measurements are always done multi axially. Freeman and Little were the first to get the pressure and volume data of an intact pericardium. They made clear that the pressure volume behavior of the pericardium depends on more than its mechanical properties. Boundary conditions such as natural attachments also affect the pericardium’s influence [6]. Lee and Boughner found an almost bilinear stress-strain curve of canine pericardium [5]. They examined the effect of rapidly and slowly applied loads on the pericardium for a better understanding of the adaptation of the pericardium to slow accumulation of fluid in the intrapericardial space. They concluded that in response to chronic increased volume, the pericardium stretched predominantly in circumferential di-
rection. Also, in their experiments they found "strain-rate insensitivity for small strains, little hysteresis, substantial stress relaxation, little creep and anisotropy". The anisotropy was significantly larger in the overloaded pericardium. The influence on filling dynamics caused by the pericardium were also ascribed to the composition of the pericardium, i.e. elastic fibers and collagen fibers by Nelemans [8].
2 Materials and Methods

A good way to measure the influence of the pericardium on the heart, would be to compare the pressure-volume loop of the heart with and without pericardium to determine at what pressures the pericardium affects the pressure-volume relation of the ventricles, and how much. These experiments would be done best in a living heart, but since the in vitro working heart is still a big challenge, only dead hearts were used in this project. The pressure-volume loop of a dead heart is not very representative for the living heart, since rigor mortis will stiffen the heart. Strain softening could appear, but representative outcome is not guaranteed. Therefore the pressure-volume loop of the pericardium itself was determined. After some problems were encountered (see section ‘Failed Methods’) it was decided to measure on samples of the pericardium only.

2.1 Data acquisition

Hearts were removed from pigs (female, ±160 days old, ±100 kg) within twenty minutes after death, trying to keep the pericardium rather intact. The heart was transported to the lab in a cooling box, and arrived within half an hour. The pericardium was removed from the heart and preserved in physiological salt solution. Then a part of the pericardium was stretched over a rigid tube (outer diameter = 2.9 cm) and bonded with a string. The tube was filled with physiological salt solution and connected to a syringe and a pressure sensor. The system was free of air. A schematical drawing and a picture of the experimental setup can be seen in figure 5.

Then 1.0 ml of physiological salt solution was added with steps of 0.1 ml at a time, with a following pause of half a second. In the same way the volume was withdrawn again. The pressure was measured constantly and all data were saved. The data were corrected for the initial pressure, caused by the difference in height of the pericardium sample and the pressure sensor. Then, the pressure was converted into Pascal (conversion factor: 100 mm Hg = 13.3 kPa).

Then the pressure was plotted against time. For each volume the corresponding pressure was read out, immediately after the pressure peak caused by the change of the volume. The pressures plotted against the corresponding volumes presented the pressure-volume relation. As expressing the behavior of a biological material in material properties is more convenient, the pressure volume relations were now used to calculate the elastic moduli of the samples (see section 2.2).

2.2 Pressure-Volume data to E-modulus

To convert the pressure-volume data into material properties, an equation of Fichter was used. He made an equation for calculating the deflection \( w \) of circular isotropic inflated membrane \[3\]:

\[ \text{Conversion factor: } 100 \text{ mm Hg} = 13.3 \text{ kPa}. \]
Figure 5: Schematic representation of the experimental setup seen from side, and a picture of the setup seen from top

\[ w(\rho) = a\left(\frac{pa}{Eh}\right)^{\frac{1}{2}} \sum_{n=0}^{\infty} a_{2n}(1 - \rho^{2n+1}) \]  
(1)

in which \( \rho \) is the dimensionless coordinate \( \frac{r}{a} \), with \( a \) is the radius and \( r \) the radial positions. The equation for \( n=1 \) is:

\[ w(\rho) = a\left(\frac{pa}{Eh}\right)^{\frac{1}{2}} \cdot [a_{0}(1 - \rho^{2}) + a_{2}(1 - \rho^{4})] \]  
(2)

The constants \( a_{0} \) and \( a_{2} \) can be calculated by using the equilibrium equation, the Poisson ratio and the boundary condition \( w(\rho = 1) = 0 \) [9]. For the case of \( n = 1 \) and Poisson ratio \( \nu = \frac{1}{2} \), the following applies: \( a_{0} = \frac{1}{b_{0}^{2}} \), and \( a_{2} = \frac{1}{2b_{0}^{4}} \) with \( b_{0} = \sqrt[3]{5} \).

With use of the formula for the volume within the sample

\[ V = \int_{0}^{r} 2\pi rw(r)dr \]  
(3)

the following formula is formed for volume:

\[ V = \pi a^{3}\left(\frac{ap}{Eh}\right)^{\frac{1}{2}}(a_{0} + a_{2} - \frac{1}{6}) \]  
(4)

which, with inclusion of the constants \( a_{0} \) and \( a_{2} \), leads to

\[ Eh = k^{3}\left(\frac{p}{V^{3}}\right) \]  
(5)
with

\[ k = \frac{28}{30\sqrt{5}} \pi a^{\frac{10}{3}} \]  

(6)

For each volume \( V \) added to the experimental setup, the matching pressure \( p \) is known from the measurement data. By dividing the pressures by the third power of the matching volume \( V^3 \), \( Eh \) can be calculated. Thickness \( h \) of the pericardium was not measured in this experiment, so an estimation had to be made in order to calculate the elastic modulus \( E \) for each sample.
3 Results

The results of the measurements on the three samples are shown in Figure 6, 7 and 8. The upper figures show the pressure [kPa] against time [ms] for the stepwise addition of fluid to the system. The pressure shows a decay after each addition of fluid. The lower figures show loops of the pressures $p$ plotted to the third power of the matching volumes $V^3$. According to equation 5, the material property $Eh$ was calculated from linear fits in these loops. These fits are shown in red. The slopes of the fits per sample, in [kPa/ml$^3$], are: 13.67 for sample 1, 6.889 for sample 2 and 6.019 for sample 3. Table 1 shows the final results of the samples for $Eh$, and $E$ for an estimation of $h = 0.5mm$ and $h = 0.3mm$.

Table 1: Results for $Eh$ and $E$ on basis of estimation of $h$, for all three samples

<table>
<thead>
<tr>
<th></th>
<th>$Eh[kPa \cdot m]$</th>
<th>$E_{h=0.5mm}[MPa]$</th>
<th>$E_{h=0.3mm}[MPa]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>12.67</td>
<td>25.33</td>
<td>42.22</td>
</tr>
<tr>
<td>Sample 2</td>
<td>5.92</td>
<td>11.84</td>
<td>19.73</td>
</tr>
<tr>
<td>Sample 3</td>
<td>5.58</td>
<td>11.15</td>
<td>18.59</td>
</tr>
</tbody>
</table>
Figure 6: Results for Sample 1. Blue line show interpolated pressure to time (upper figure) and pressure to volume$^3$ loop (lower figure) data, red line is linear fit
Figure 7: Results for Sample 2. Blue line show interpolated pressure to time (upper figure) and pressure to volume$^3$ loop (lower figure) data, red line is linear fit.
Figure 8: Results for Sample 3. Blue line show interpolated pressure to time (upper figure) and pressure to volume$^3$ loop (lower figure) data, red line is linear fit
4 Discussion

As for the method used, this method was decided upon after several attempts to measure intrapericardial pressure in the intact pericardium (see Appendix). Instead of doing a tensile test on pericardial samples, it was chosen to stretch the pericardium over a rigid tube. A disadvantage of this method is that it is hard to be sure the string and sample are fully fixed, and do not shift slightly. Also it was hard to determine the tension free state of the tissue. Some measurement failed because the sample slipped, or air was detected in the setup.

The pericardium is anisotropic and has different strengths on different locations and in different directions. The latter is averaged by the experimental setup. Since the samples were rather small, they only give insight to very local material properties. To have a better idea about the pressure volume relation of the pericardium, several samples should be taken from the same pericardium, with registration of the original location and orientation of the samples. Furthermore, subsequent measurements on the same sample and registration of the end volume would give an idea about relaxation and plastic deformation of the tissue. Also, measuring the thicknesses of the samples would have been better than an estimation on basis of literature.

In the time-pressure plots, a decay of pressure at constant volume can be seen in time. This is the effect of two phenomena; relaxation and permeability. As the pericardium is a biological material, consisting of a matrix with elastin and collagen fibers, relaxation is expected. Permeability, however, was not expected but seemed to occur if pressure was applied by physiological salt solution. These effects are undistinguishable and may have distorted the results for elastic moduli of the pericardium samples. Figure 9 illustrates the leaking of physiological solution through the pericardium during the experiment. Permeability of the pericardium was not expected because of the pressure that can be build up in the pericardium at a cardiac tamponade. There are several possible explanations for the occurrence of this permeability. For example, blood is more viscous than physiological solution and contains cells that may block pores. Also, cells may normally cause impermeability but may have died during the measurements. The results cannot be assumed to apply for the human pericardium since the measurements were done on that of a pig. The porcine heart is said to be comparable to the human heart in several experiments [10], but Naimark et al [12] showed that there are differences in pericardia of species. He showed porcine and canine pericardium to be thinner (200 to 400 µm) than human pericardium (± 550 µm). He also stated that thinner pericardium is stronger, probably due to greater cross-link densities in the type III collagen. The comparison of human and canine pericardium is a good example for this; 20.4 MPa and 198 MPa respectively [6]. No such data is known for porcine pericardia, but the range of the results (11 MPa - 43 MPa) is in the same magnitude. It should also be regarded that the samples used in this experiment were room temperature in stead of body temperature. This lower temperature will lower the rate of relaxation in the tissue. [5]
Figure 9: Permeability of pericardium for physiological solution: under pressure, physiological salt solution leaks through the membrane and forms drops on the surface.
5 Conclusion

The method of measuring the pressure to volume loop and therefrom calculating the elastic modulus using the method of Fichter yields results in a range expected for a biological material (11 MPa - 43 MPa). In literature large difference in moduli is given for human (20.4 MPa) and canine (198 MPa) pericardia. The results for our three samples of porcine pericardium are in the same magnitude. Since in this study only three samples were tested, it is impossible to say more about these results.

In all measurements, the permeability of the pericardium for physiological salt solution under pressure caused a problem. The pericardium is probably not permeable to blood (think of a tamponade), possibly because of the viscosity of blood or obstruction by the blood cells. Exploration of this as well as measurements with blood in stead of physiological salt solution would be a possibility.
References


6 Appendix: Failed Measurements

The described experiment was the third in a row. The previous two attempts were done on hearts with an intact pericardium. The first try to measure the pressure in the intrapericardial volume while adding volume was by adding physiological salt solution through the right heart cavity. The right side of the heart was closed with stitches except for the vena cava inferior, which was cannulated. A small hole was made through the vena cava superior making a connection between the inner right heart and the volume between the epicardium and the pericardium. The left heart was filled with physiological salt solution and completely closed with stitches, thus maintaining a constant volume. Then the heart was hung in a physiological salt solution, keeping the pericardium free from stretching. Through the cannula 50 ml of physiological salt solution was added at a time. After each injection the pressure showed a rise which decayed quickly. The presence of leaks in the pericardium was excluded. It was concluded that due to the higher pressure in the right atrium, the solution flew through the coronaries into the aorta. Thereby it changed the volume in the left heart cavity and also stretched the aorta, causing a pressure decay and at the same time making the intrapericardial volume unknown.

The second try was to fill the right and left cavities with 1.5 percent agarose gel. Then a needle was put through the pericardium and bonded tightly. A pressure wire was guided through the needle. The physiological salt solution was injected into the intrapericardial space through the needle with 50 ml at a time and pressure was measured. After the measurements the heart was weighed before and after emptying the pericardium, to determine the original amount of solution inside the pericardium. Then the heart and agarose were cut open over the attachment line of the pericardium to the epicardium to determine the volume of the heart muscle inside the pericardium (Vrest). Next, the gel was be taken out and the heart was weighed again to determine the muscle volume of the heart (Vmuscle). (including possible blood clots). The volume within the pericardium (V) is then defined as the total amount of solution added (Vadded) plus the rest volume of the heart (Vrest): Vtotal=Vadded+Vrest. The total volume within the pericardium was normalized to the volume of the heart muscle: Vindex=Vtotal/Vmuscle

Both methods showed a decrease in pressure. At first it was assumed that this was due to loss of volume, through holes in the pericardium itself, through the holes creates by the stitches or indirectly by the compliance of the aorta. When small tests were done, the volume loss seemed to be caused by by the permeability of the pericardium. Another cause of the decaying of pressure could be visco-elastic behavior. Another big problem in these two methods was that it was very hard te keep the pericardium intact when removing it from the pig.