The Effect of Temperature and Glutaraldehyde Fixation on the Mechanical Properties of Bovine Pericardial Tissues

Adja RADIEMAN and Koon Ong LIM

School of Physics, Universiti Sains Malaysia, Penang, Malaysia

Abstract A comparison is made of the elastic response of fresh pericardial tissues at 25, 37, and 42°C, and also of fresh and glutaraldehyde-fixed tissues. Strips of bovine pericardial tissues cut perpendicular to the base-apex axis of the heart were used. An Instron machine was used for uniaxial tensile tests, and the strain-rate used was 666.7%·min⁻¹. No significant differences in tissue mechanical properties were observed for temperature values of 25, 37, and 42°C. However tissues fixed in a glutaraldehyde solution were more extensible than fresh tissues. The elastic responses of tissues preserved for 1 day in glutaraldehyde are not very different from those preserved for up to 10 days.

Key words: mechanical properties, bovine pericardium.

The mechanical properties of tissues have been cited as a possible factor in determining the success or failure of biological materials used in human heart valve substitutes. The mechanical properties of fresh bovine pericardial tissues have been reported by RADIEMAN et al. (1985). WRIGHT et al. (1977) and WALLACE (1975), indicated that glutaraldehyde preservation of heterograft valves increased their durability as heart valve substitutes; IONESCU et al. (1974) reported and suggested that preserved bovine pericardial tissues have the potential qualities needed for use in heart valve substitutes. In this paper we report the effect of temperature and glutaraldehyde fixation on the mechanical properties of bovine pericardial tissues. Stress-strain responses were investigated at 25, 37, and 42°C, the latter being taken as the highest temperature for cases of high fever. Uniaxial tensile tests were also performed at room temperature on tissues fixed in glutaraldehyde for 1 day and for up to 10 days.

MATERIALS AND METHODS

The preparations of the materials for this study were similar to that done for...
the stress-strain response study of fresh bovine pericardial tissues as reported by 
RADJEMAN et al. (1985). Six bovine pericardial sacs were procured from the local 
abattoir and were removed within 2 h after the animals were slaughtered. Sacs from 
male animals (ages 4–5 years, weight 150–220 kg) were used, as only male animals 
are slaughtered in the local abattoir. The 6 pericardial sacs were divided into 2 
groups of 3 each. From each sac, 12 rectangular strips, 6 from the anterior portion 
and 6 from the posterior portion of the heart were cut perpendicular to the base-
apex axis of the heart as shown in Fig. 1 (H₁ to H₁₂). The width of each strip was 
0.5 cm and the length was 5 cm. Strips from the first group of sacs were used for 
temperature effect studies and were kept in a physiological solution containing 
0.01% Merthiolate at a temperature of 5°C for at most 2 days until studies could be 
made. This antibacterial solution was found to preserve the mechanical properties 
of elastin and collagen (ROACH and BURTON, 1957).

Strips from the second group of sacs were used for glutaraldehyde fixation 
studies. Pericardial strips from this group were divided into 3 subgroups of 12 each. 
The first subgroup of strips were kept in a physiological solution containing 0.01% 
Merthiolate at a temperature 5°C and were tested within 5 h after removal from the 
animal. Each strip from the second and the third subgroups was placed flat on a 
piece of rigid styrofoam and clamped at both ends at its natural length (see Fig. 2)
before it was fixed in 0.625% glutaraldehyde in 0.15 phosphate buffer at a pH 7.2 (BROOM, 1978), and a temperature of 5°C. One subgroup of strips was stored in the glutaraldehyde solution for 1 day and the other subgroup for 10 days.

The stress-strain response of all the prepared pericardial strips, were examined by subjecting each unpreconditioned strip to a uniaxial tensile stress at a strain rate of 666.7%·min⁻¹ until rupture occurred. A floor model of the Instron machine (model 1114) was used and for each specimen strip, only one test was performed. Due to technical limitations, experiments at higher strain rates could not be performed. This strain rate was chosen as LIM and BOUGHNER (1975) estimated that the average maximum rate in which chordae tendineae are strained in vivo is about 2000%·s⁻¹, though lower rates are possible.

Basically the Instron consists of a fixed crosshead and a movable crosshead with jaws facing each other. Since the specimen could not be mounted directly onto the jaws of the Instron, it was instead held vertical by 2 parallel plate clamps that were attached to the jaws of the Instron. These clamps were found to held the pericardial tissue firmly without damaging it. The amount of stretch and the force applied were measured simultaneously and continuously on a chart-recorder. The details of this experimental procedure have been reported previously (LIM and BOUGHNER, 1975; LIM, 1980).

Prior to testing, the mean width and thickness of the pericardial strips were measured using a travelling microscope. Their cross sectional areas were determined by assuming that the strips have rectangular cross sections. The cross sectional areas of the specimens tested ranged from 0.025 to 0.055 cm². All specimens were mounted with an initial length of about 3 cm. The tension-time traces were recorded continuously by a chart-recorder with speeds that provided a suitable resolution of

Fig. 3. Arrangement used for the study of effect of temperature on the mechanical properties of bovine pericardial tissues.
the different straining conditions. From a knowledge of the initial cross sectional areas and the strain rate, stress-strain curves could be obtained from these continuous tension-time records. Here stress is defined as force per unit original area of cross section, and strain is the change in length over the original length, the length between the clamps at zero stress. Throughout the duration of the test the specimens were kept moist by immersing them in a 0.01% Merthiolate solution held in a specially designed chamber (see Fig. 3).

For temperature effect studies, a helical copper tube was also immersed in the chamber holding the solution of 0.01% Merthiolate as well as the specimen under study. This is also shown in Fig. 3. The ends of the helical copper tube were connected to a temperature controller by 2 rubber tubings as shown in the figure. With the temperature controller on, warm water circulated through the helical copper tube so that specimens could be tested at controlled temperatures.

RESULTS

Figure 4 shows the average stress-strain response of bovine pericardial tissues at 25, 37, and 42°C. Each curve represents the average of 6 specimens. The figure shows that the 3 curves nearly superimposed upon one another. The secant and final moduli as well as other parameters obtainable from these curves are shown in Table 1. Final modulus is defined as the gradient of the final linear portion of the stress-

![Stress-strain curve](image)

**Fig. 4.** Stress-strain response of bovine pericardial tissues tested at 3 different temperatures. Strain rate: 666.7%·min⁻¹. Tissues were strained perpendicular to the base-apex axis of the heart.
TEMPERATURE AND FIXATION EFFECTS

Table 1. Average mechanical response of bovine pericardial tissues strained perpendicular to the base-apex axis of the heart at a strain rate of 666.7% min⁻¹ for temperature values of 25, 37, and 42°C.

<table>
<thead>
<tr>
<th>Mechanical response</th>
<th>25°C</th>
<th>37°C</th>
<th>42°C</th>
<th>25°C</th>
<th>25°C</th>
<th>37°C</th>
<th>37°C</th>
<th>42°C</th>
<th>42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress at 5% strain</td>
<td>3.61 ± 0.28</td>
<td>3.45 ± 0.51</td>
<td>3.23 ± 0.58</td>
<td>&gt;0.70</td>
<td>&gt;0.50</td>
<td>&gt;0.70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(× 10⁶ N·m⁻²)</td>
<td>(× 10⁶ N·m⁻²)</td>
<td>(× 10⁶ N·m⁻²)</td>
<td>(× 10⁶ N·m⁻²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress at 10% strain</td>
<td>9.65 ± 0.47</td>
<td>9.05 ± 0.66</td>
<td>8.80 ± 0.78</td>
<td>&gt;0.40</td>
<td>&gt;0.30</td>
<td>&gt;0.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(× 10⁶ N·m⁻²)</td>
<td>(× 10⁶ N·m⁻²)</td>
<td>(× 10⁶ N·m⁻²)</td>
<td>(× 10⁶ N·m⁻²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage strain at transition</td>
<td>5.45 ± 0.26</td>
<td>5.70 ± 0.75</td>
<td>5.80 ± 0.74</td>
<td>&gt;0.70</td>
<td>&gt;0.60</td>
<td>&gt;0.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress at transition</td>
<td>4.25 ± 0.31</td>
<td>4.20 ± 0.19</td>
<td>4.19 ± 0.59</td>
<td>&gt;0.80</td>
<td>&gt;0.80</td>
<td>&gt;0.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(× 10⁶ N·m⁻²)</td>
<td>(× 10⁶ N·m⁻²)</td>
<td>(× 10⁶ N·m⁻²)</td>
<td>(× 10⁶ N·m⁻²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secant modulus</td>
<td>7.79 ± 0.30</td>
<td>7.37 ± 0.50</td>
<td>7.20 ± 0.70</td>
<td>&gt;0.40</td>
<td>&gt;0.40</td>
<td>&gt;0.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(× 10⁷ N·m⁻²)</td>
<td>(× 10⁷ N·m⁻²)</td>
<td>(× 10⁷ N·m⁻²)</td>
<td>(× 10⁷ N·m⁻²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final modulus</td>
<td>12.40 ± 0.76</td>
<td>12.14 ± 0.71</td>
<td>11.81 ± 0.42</td>
<td>&gt;0.80</td>
<td>&gt;0.40</td>
<td>&gt;0.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(× 10⁷ N·m⁻²)</td>
<td>(× 10⁷ N·m⁻²)</td>
<td>(× 10⁷ N·m⁻²)</td>
<td>(× 10⁷ N·m⁻²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage strain at rupture*</td>
<td>16.25 ± 0.21</td>
<td>16.16 ± 1.29</td>
<td>17.20 ± 0.82</td>
<td>&gt;0.90</td>
<td>&gt;0.40</td>
<td>&gt;0.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breaking stress**</td>
<td>13.54 ± 0.87</td>
<td>12.97 ± 1.05</td>
<td>12.49 ± 0.87</td>
<td>&gt;0.60</td>
<td>&gt;0.40</td>
<td>&gt;0.70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(× 10⁶ N·m⁻²)</td>
<td>(× 10⁶ N·m⁻²)</td>
<td>(× 10⁶ N·m⁻²)</td>
<td>(× 10⁶ N·m⁻²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* A point on the stress-strain curves at which the axially loaded specimen ruptures under a load. ** The stress corresponding to the rupture point.

strain curve, while secant modulus is defined as the gradient of the straight line joining the transition point of the stress-strain curve to the origin. The transition joint corresponds to the point on the curve where linearity begins. By using a simple Student's t-test (CAMPBELL, 1967), it was found that there is no significant difference, at the 5% level, in the mechanical response of bovine pericardial tissues at 25, 37, and 42°C. The response was also independent of the location on the pericardial sac.

The response to mechanical stress of fresh as well as glutaraldehyde-fixed bovine pericardial tissues was also found to be independent of the location on the bovine pericardial sac and the average response curves are as shown in Fig. 5 while the various parameters of this response are tabulated in Table 2. As expected, the stress-strain curves of these tissues are non-linear. The curves in Fig. 5 indicate that fixed tissues are more extensible than fresh ones. While the data of Table 2 show that the strain at rupture and strain at transition of fixed tissues are significantly higher than those for fresh tissues, the final moduli and the breaking stress of all tissues, whether fresh or fixed, on the other hand, are not significantly different. It
Fig. 5. Stress-strain response of fresh and glutaraldehyde-fixed bovine pericardial tissues. (a) Fresh, (b) fixed for 1 day, (c) fixed for 10 days. The broken lines denote the standard error for (a) and (c), and the dotted lines for (b). Strain rate: 666.7% min⁻¹ and temperature: 25°C. Tissues were strained perpendicular to the base-apex axis of the heart.

Table 2. Effect of glutaraldehyde fixation on the mechanical properties of bovine pericardial tissues, strained perpendicular to the base-apex axis of the heart at a strain rate of 666.7% min⁻¹ and at a temperature of 25°C.

<table>
<thead>
<tr>
<th></th>
<th>Fresh</th>
<th>Fixed for 1 day</th>
<th>Fixed for 10 days</th>
<th>$p$ value by $t$-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage strain at transition</td>
<td>5.45 ± 0.26</td>
<td>15.26 ± 0.72</td>
<td>16.40 ± 1.65</td>
<td>&lt;0.001 &lt;0.001 &gt;0.1</td>
</tr>
<tr>
<td>Secant modulus ((×10^7 \text{ N} \cdot \text{m}^{-2}))</td>
<td>7.79 ± 0.30</td>
<td>3.11 ± 0.26</td>
<td>2.84 ± 0.39</td>
<td>&lt;0.002 &lt;0.001 &gt;0.2</td>
</tr>
<tr>
<td>Final modulus ((×10^7 \text{ N} \cdot \text{m}^{-2}))</td>
<td>12.40 ± 0.76</td>
<td>11.52 ± 0.21</td>
<td>11.38 ± 0.34</td>
<td>&gt;0.02 &gt;0.04 &gt;0.05</td>
</tr>
<tr>
<td>Percentage storage at rupture</td>
<td>16.25 ± 0.21</td>
<td>23.22 ± 1.17</td>
<td>26.86 ± 1.77</td>
<td>&lt;0.001 &lt;0.001 &gt;0.01</td>
</tr>
<tr>
<td>Breaking stress ((×10^8 \text{ N} \cdot \text{m}^{-2}))</td>
<td>13.54 ± 0.87</td>
<td>13.17 ± 0.60</td>
<td>12.49 ± 0.62</td>
<td>&gt;0.4 &gt;0.02 &gt;0.1</td>
</tr>
</tbody>
</table>
should also be noted that the secant modulus of fresh tissues is significantly higher than that of fixed tissues. The data in Table 2 also indicate that the mechanical response of tissues fixed for 1 day are not significantly different from those that have been fixed for up to 10 days.

**DISCUSSION**

The independence of temperature in relation to the mechanical properties (at least for the 25 to 42°C temperature range), implies that bovine pericardial tissues are suitable, from the mechanical properties point of view, for use in tissue valve substitutes. Thus situations of extreme fever or hypothermia would not present problems.

However the observation that unpreconditioned tissues fixed in glutaraldehyde are more extensible than unpreconditioned fresh tissues, when stretched along the line perpendicular to the base-apex axis direction, indicate that some changes to the constituent elastic fibers of the tissues may have taken place. Whether the change is a result of actual changes to the intrinsic properties of collagen fibers or just mere changes to the configuration of these fibers is still uncertain, though the latter is suspect. These changes may in fact be related to the increased durability of preserved heterograft valves as reported by Wright et al. (1977) and the glutaraldehyde-prepared heterografts reported by Wallace (1975). The increase in extensibility of glutaraldehyde-fixed tissues implies that at a similar strain value, the stress level experienced by fixed tissues will be lower than that experienced by fresh tissues. This decrease in the stress level could perhaps account for the increase in durability of fixed tissues. In addition, the increase in strain values at rupture of glutaraldehyde-fixed tissues also allows these tissues a greater degree of free play on valve closure. Since differences in mechanical properties exist between fresh and glutaraldehyde-fixed tissues, it is therefore imperative that these differences be taken into consideration when use is made of either fresh or fixed tissues as the material in tissue valve substitutes. For instance, would the increase in extensibility and the consequent decrease in secant modulus of fixed tissues give rise to regurgitation problems when these tissues are used? However, as found, storage time in glutaraldehyde of between 1 to 10 days need not be a point of contention.

The authors are grateful to the Deans of the School of Physics and the School of Engineering Sciences and Industrial Technology, University of Science of Malaysia, for the use of the laboratory facilities.

**REFERENCES**


Vol. 36, No. 6, 1986


