Oxygen diffusion and consumption of aortic valve cusps

K. L. WEIND,1,2 D. R. BOUGHNER,1,2,3 L. RIGUTTO,2 AND C. G. ELLIS2
1Heart Valve Laboratory, John P. Robarts Research Institute, London N6A 5K8; 2Department of Medical Biophysics, University of Western Ontario, London N6A 5C1; and 3Division of Cardiology, London Health Sciences Centre, University Campus, London, Ontario N6A 5A5, Canada

Received 27 March 2001; accepted in final form 9 August 2001

Weind, K. L., D. R. Boughner, L. Rigutto, and C. G. Ellis. Oxygen diffusion and consumption of aortic valve cusps. Am J Physiol Heart Circ Physiol 281: H2604–H2611, 2001.—To maintain tissue oxygenation, normal aortic valves contain a vascular bed where tissue thickness is greatest. Avascular “living” tissue-engineered heart valves have been proposed, yet little information exists regarding the magnitude of valve tissue metabolic activity or oxygen requirements. We therefore set out to measure the oxygen diffusivity (D_O2) and oxygen consumption (V_O2) of seven porcine aortic valve cusps in vitro at 37°C using a chamber with a Clark oxygen sensor. Mean D_O2 and V_O2 were 1.06 × 10^-5 cm^2/s and 3.05 × 10^-5 ml O2·ml tissue^-1·s^-1, respectively. When modeled as a three-layered structure by using these values and a boundary condition of 100 mm Hg at both surfaces, the average aortic cusp predicted a central mean Po2 of 27 mm Hg (range of 0–50 mm Hg). The D_O2 value obtained was similar to that found for other vascular structures, but because our studies were carried out in vitro, the V_O2 measurements may be lower than that required by the functioning valves. These values provide an initial understanding of the oxygen supply possible from the cusp surfaces and the oxygen needs of the tissue.

tissue engineering; metabolic activity

IT HAS BEEN SHOWN THAT HEART VALVES ARE LIVING TISSUE CONTAINING SEVERAL VARIETIES OF ACTIVELY METABOLIZING CELLS (16, 17), YET LITTLE INFORMATION EXISTS REGARDING THE MAGNITUDE OF THEIR METABOLIC ACTIVITY OR FACTORS THAT AFFECT IT. IN ADDITION, THE AMOUNT OF OXYGEN NEEDED BY THE TISSUE TO SUSTAIN ITS FUNCTION IS UNKNOWN AND LITTLE IS UNDERSTOOD REGARDING THE OXYGEN Supply ROUTES OF THIS TISSUE. WITH THE INCREASING INTEREST IN THE CREATION OF TISSUE-ENGINEERED BIOPROSTHETIC HEART VALVES (23, 24), SUCH DATA IS IMPORTANT IN THE DESIGN OF A SUCCESSFUL VALVE SUBSTITUTE.

Our previous work (30) has suggested that oxygen is supplied to aortic valve cusp tissue via two complementary pathways, in a manner similar to that seen in large arteries and veins. In those vessels, oxygen diffuses into the tissue from both the vessel lumen and also through a circulatory system within the adventitia. In contrast, for aortic valve tissue, oxygen can diffuse through both the fibrosal and ventriculissurfaces because both surfaces are exposed to arterial blood. However, when cusp thickness limits oxygen delivery to the cells by simple diffusion, our results imply that an intrinsic circulation is necessary within the central layer (the spongiosa) to compensate for unmet oxygen demands. That circulation may be the equivalent of the vasa vasorum of the aortic root.

To better appreciate the roles of these oxygenation pathways, we must first gain an understanding of the metabolic demands of the tissue. A good approximation of the overall metabolic rate and oxygen demands of a tissue can be provided by the measurement of its rate of oxygen consumption (V_O2) (25). The other major factor that influences oxygen delivery is the permeability of the tissue to oxygen. The amount of oxygen that can be transported through a tissue is the product of diffusivity (D_O2) and solubility (a) divided by the thickness of the tissue (L), i.e., D_O2·a/L. Therefore, by determining how thick the cusp is, by how readily oxygen can diffuse in from the surfaces of the cusp, and by having some knowledge of tissue oxygen solubility, we can quantify the ability of an oxygenation route to sustain the metabolic needs of this tissue. In addition, if both V_O2 and D_O2 of the tissue are known, we can also indirectly assess the role played by the intrinsic circulation of the cusps.

D_O2 and V_O2 can be measured experimentally using an oxygen sensor. To measure diffusivity, the tissue is exposed to a step change in oxygen level at one surface and the transient change in oxygen at the other closed surface is measured with the oxygen sensor. D_O2 is proportional to the time constant of this oxygen transient. To measure V_O2, the exposed surface of the tissue is covered with an oxygen-impermeable barrier (e.g., glass coverslip) and the fall of the oxygen level with time is measured with the sensor. Consumption is proportional to the rate of oxygen decrease. It is important to remember that such measurements of D_O2 and V_O2 are temperature sensitive. Because “correction factors” for temperature can lead to poor representative values (2), such experiments should be carried out at a physiological temperature if they are to reflect the in vivo situation.

On the basis of the work by Ellsworth and Pittman (10), we designed a chamber to carry out oxygen mea-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
measurements of both aortic valve cusp DO$_2$ and VO$_2$ parameters at the physiological temperature of 37°C with the use of a Clark polarographic oxygen sensor. The current output of a Clark sensor is proportional to PO$_2$ in the medium at the surface of the sensor. We performed our experiments using porcine aortic valve cusps because these valves are similar to human valves (19) and are used as bioprosthetic replacement devices (9).

**MATERIALS AND METHODS**

**Cusp preparation.** Seven porcine hearts were freshly obtained from a local abattoir and returned (on ice slush) to the laboratory. One cusp from each aortic valve was randomly dissected and rewarmed up to 37°C in a physiological balanced salt solution immediately before being mounted in the sample chamber for DO$_2$ and VO$_2$ measurements.

The sample chamber. A sample oxygen chamber (Fig. 1) was designed for DO$_2$ and VO$_2$ measurements based on the design of Ellsworth and Pittman (10). The design consisted of an acrylic chamber to house the cusp with a gas inlet and outlet. A Clark PO$_2$ sensor (model 733, Diamond General Development; Ann Arbor, MI) entered through a tight-fitting O-ring at the base of the chamber. The surface of the Clark sensor was positioned to be flush with the bottom surface of the chamber so that the tissue could be placed directly on top of the sensor. The Clark sensor recorded changes in tissue PO$_2$ throughout the experiment. The chamber was built with a tightly fitting lid so that the chamber could be easily sealed. The temperature of the chamber was measured using a thermocouple. The thermocouple controlled the gas inlet heater, which maintained the chamber and the tissue inside it at 37 ± 0.2°C. All gases were humidified before entering the chamber by being bubbled through distilled water and heated to 33°C using a water bath. This ensured that the tissue moisture level remained constant throughout the experiments and the increase in gas temperature from 33 to 37°C prevented the buildup of excess condensation within the experimental setup.

A picoammeter (model 487, Keithley; Cleveland, OH) provided the polarizing voltage of −0.8 V to the polarographic sensor and measured the current output. The output from the picoammeter was digitized by an analog-to-digital interface board (model AT-MIO-16E-10, National Instruments; Austin, TX) and passed to a virtual strip-chart recorder designed and run on a personal computer using commercially available software (LabView, National Instruments). This procedure allowed raw data to be moved directly into Excel software (Microsoft; Redmond, WA) for analysis.

Before each experiment, the Clark sensor was calibrated using humidified 100% N$_2$, 21% O$_2$, and 30% O$_2$ heated to 37°C. These measurements were also used to create a calibration curve to change current into PO$_2$ for the VO$_2$ rate analysis. After this experiment, the cusps were laid flat over the sensor and the chamber was sealed for DO$_2$ and VO$_2$ measurements. All measurements were carried out at 37°C and performed in one location per cusp.

**DO$_2$ measurement.** The DO$_2$ measurement was determined using the method described by Mahler (14). The PO$_2$ on the lower surface of the cusp (ventricularis side) was continuously recorded while the cusp tissue was allowed to equilibrate with a heated humidified gas containing 30% oxygen. Once a constant current reading was recorded by the sensor, the PO$_2$ in the chamber (upper surface of the cusp) was changed in a stepwise fashion to 21% oxygen (Fig. 2). Recording of the polarographic sensor current continued until a new steady state was reached. The PO$_2$ measured by the sensor at the bottom surface of the chamber remained >6 mmHg, i.e., above the critical PO$_2$ necessary to maintain uniform O$_2$ consumption (5).

**Calculation of DO$_2$.** As described by Mahler (14), the time course for the new steady state after a step change in PO$_2$ is predominantly monoexponential in nature and the rate constant (k) of this function is related to the diffusivity of the tissue through the equation $k = \frac{\pi^2}{4L^2}D$, where $k$ is the rate constant (in s$^{-1}$), DO$_2$ is the diffusion coefficient (in cm$^2$/s), and $L$ is the thickness of the tissue (in cm). Therefore, a semilogarithmic plot was generated for each run by plotting the log of the PO$_2$ change versus time and $k$ was determined as the slope of the line of best fit through the data points (Fig. 1).
Calculation of \( V_{\text{O}_2} \). In a closed system (i.e., no external source of oxygen) \( V_{\text{O}_2} \) can be calculated by using the formula

\[
V_{\text{O}_2} = \alpha \Delta P/\Delta t,
\]

where \( V_{\text{O}_2} \) is the consumption rate of the tissue (in \( \text{ml O}_2/\text{ml tissue} \cdot \text{s}^{-1} \)), \( \alpha \) is the oxygen solubility (in \( \text{ml O}_2/\text{ml tissue} \cdot \text{mmHg}^{-1} \)), and \( \Delta P/\Delta t \) is the change in \( \text{PO}_2 \) over time (in mmHg/s). This method is based on the one used for measuring \( V_{\text{O}_2} \) in cell suspensions (13). Oxygen solubility of the aortic valve tissue was estimated based on the assumption that solubility of a tissue is equivalent to the solubility of the water fraction of a tissue. To get a closer approximation, the solubility of oxygen in plasma was used. This solution, which contains proteins, has a solubility of

\[
2.816 \times 10^{-5} \text{ ml O}_2/\text{ml tissue} \cdot \text{mmHg}^{-1}
\]

at 37°C, which is ~90% the value of water at 37°C (7), and is much more similar to tissue than water. Previous work in our laboratory (22) has shown that porcine aortic valve tissue is 90% water. Therefore, the oxygen solubility value used for the determination of the \( V_{\text{O}_2} \) within porcine aortic valve tissue was 90% that of oxygen solubility in plasma at 37°C (2.534 \times 10^{-5} \text{ ml O}_2/\text{ml tissue} \cdot \text{mmHg}^{-1})

To determine the rate of change in \( \text{PO}_2 \) over time, the recorded current was changed to \( \text{PO}_2 \) using the calibration curves generated at the beginning of the experiments. By using the rate of fall in \( \text{PO}_2 \) versus time and the solubility of the cuspal tissue, \( V_{\text{O}_2} \) was calculated.

**Finite difference modeling of tissue \( \text{PO}_2 \) profiles.** To gain an understanding of the oxygen supply within the native aortic valve cusps, a finite difference mathematical model was developed based on Fick’s Law for diffusion. This would allow for in vivo tissue oxygen profiles to be created, based on our experimental in vitro \( \text{DO}_2, V_{\text{O}_2} \), and thickness data. MATLAB, a commercially available numeric computation and visualization software package (The MathWorks; Natick, MA), was used to produce and run the simulations. The parameters of the model included a time step of 0.025 s with a node spacing of 2 \times 10^{-2} \text{ cm}. To evaluate the sensitivity of the approach, a model was designed to mimic the in vitro experiment. Results within 2% of the experimental values for \( \text{DO}_2 \) and \( V_{\text{O}_2} \) were obtained, giving validity to our modeling.
The assumption was made that $P_{O_2}$ in the surrounding environment of the cusp would be 100 mmHg in vivo. The first model was created assuming that the cusp was a homogenous one-layered structure. Matched experimental values for $D_O_2$, $V_{O_2}$, and tissue thickness for each sample were used and a solubility equal to 90% that of plasma at 37°C were incorporated to generate tissue $P_{O_2}$ profiles as shown in Fig. 4A.

In reality, the cusp is actually a three-layered structure (3, 11). The two outer layers contain substantially more collagen and elastin than the middle layer, which is composed predominantly of glycosaminoglycans (22). Because tissue composition determines tissue permeability, it is likely that the two outer layers of the cusp will have lower diffusivity and solubility values for oxygen, than those determined experimentally for the cusp tissue cross section, whereas the spongiosa values will be higher. If the oxygen supply route were in fact solely from the surfaces of the cusp, this would create a situation where the effective $D_O_2$ to the center of the tissue is actually lower than the value we have obtained. Therefore, to provide a better representation of the oxygen profile in the in vivo situation, a second model was developed taking into account the three-layered structure of the aortic valve cusp. To run this model and determine tissue $P_{O_2}$ profiles, several assumptions were made. First, it was assumed that the ventricularis and fibrosa would have thicknesses equal to 0.25 of total measured cusp thickness, whereas the spongiosa layer would account for the remaining 0.5. On the basis of examination of histological aortic valve cusp cross sections, this seemed reasonable for the area of the cusp where our experimental measurements had been performed. Second, it was assumed that the central spongiosa layer, based on its composition, would have $D_O_2$ and solubility values equal to those values of plasma at 37°C which are reported to be $2.0 \times 10^{-5}$ cm$^2$/s (21) and $2.816 \times 10^{-6}$ ml O$_2$/ml tissue$^{-1}$·mmHg$^{-1}$ (7), respectively. The $D_O_2$ value of plasma is approximately two-thirds of the $D_O_2$ value of water at 37°C (27). The two outer layers, the fibrosa and ventricularis, were modeled as having identical composition and therefore identical $D_O_2$ and $\alpha$-values. To determine these values, plasma values representing the spongiosa, were subtracted from our experimentally derived values in the proper thickness ratios (see APPENDIX). Finally, $V_{O_2}$ was assumed to be uniform throughout the tissue based on the report (18) that cellular density is even throughout the three cuspal layers.

**RESULTS**

Rate constants of the diffusivity measurements were in the range of $4.2-9.6 \times 10^{-8}$ s$^{-1}$ for the seven samples. Mean thickness of the tissue over the oxygen sensor, where the measurements were made, ranged from 4.55 to 8.63 $\times 10^{-2}$ cm (Table 1). With the use of matching rate constants and mean thickness values for each sample, the mean $D_O_2$ for porcine aortic valve tissue was found to be $1.06 \times 10^{-5} \pm 1.535 \times 10^{-6}$ cm$^2$/s. A sample recording of the change in current with time reflecting the step change in oxygen used to determine $D_O_2$ measurements is shown in Fig. 3. The graph inlay shows the semilogarithmic plot of this data from which $k$ can be acquired.

After a coverslip was applied to the exposed surface of the cusp, oxygen is consumed by the tissue until a level of 0 $P_{O_2}$ is reached throughout the tissue. This rate of fall in oxygen was recorded by the Clark oxygen sensor in contact with the lower surface of the tissue and was determined to be within a range of 0.68–2.13 mmHg/s. By using a solubility value of $2.534 \times 10^{-5}$ ml O$_2$/ml tissue$^{-1}$·mmHg$^{-1}$, the mean $V_{O_2}$ for the tissue was found to be $3.05 \times 10^{-5} \pm 1.37 \times 10^{-5}$ ml O$_2$/ml tissue$^{-1}$·s$^{-1}$.

The oxygen profiles for the one-layer model were determined for each sample using their individual experimental values for thickness, $D_O_2$, and $V_{O_2}$. The predicted minimum $P_{O_2}$ values at the center of the spongiosa in vivo for a surface $P_{O_2}$ of 100 mmHg for each cusp can be found in Table 2. The mean minimum $P_{O_2}$ at the cusp center for the one-layer model under these conditions was 42 mmHg. In contrast, when the cusps were each modeled as a three-layered structure, more closely representing the in vivo situation, and solubility and diffusivity values reflected tissue layer composition, their individual oxygen profiles returned lower minimum $P_{O_2}$ values at the maximum distance from the cusp surfaces. These values are also reported in Table 2. The mean minimum $P_{O_2}$ value of 27 mmHg predicted using the three-layered model created a 26% larger $P_{O_2}$ gradient than that determined using the one-layer model and a paired Student’s $t$-test found this difference to be statistically significant ($P \leq 0.001$). Examples of the mean oxygen profiles for both the homogenous and heterogeneous tissue models can be seen in Fig. 4.
DISCUSSION

Present recommendations for the creation of a tissue engineered bioprosthesis valve involve a biodegradable scaffold seeded with the cells of the recipients (31). The long-term goal is to create a self-sustaining valve implant containing living cells enabling ongoing tissue repair without immune rejection. To achieve this goal, several issues are being investigated, including the most suitable harvest source for these cells, the cell density necessary to produce a stable structure, and the appropriate scaffolding material. The final device must embody the flexibility and mechanical strength needed to withstand the substantial physical forces applied during the cardiac cycle (24). Because the ultimate goal is also to create a durable implant that will survive the lifetime of the patient, careful consideration must be given to all parameters of native aortic valve function. For example, not only is it important to seed the implant with the appropriate cells and density, it is also crucial that an adequate oxygen supply be available to these cells to allow for optimal function.

To date, the three-layered structure of aortic valve cusps is well defined, as are the physical properties and orientation of its major structural components, collagen and elastin (3, 6, 22). The cellular content of the cusps is less well understood, but several recent studies (16–18) have provided clarification. For these interstitial cells, an oxygen delivery system is certainly necessary, and aortic valve metabolic requirements have not yet been quantified. Blood supply within the valve has been identified previously and discussed intermittently over the past several decades. Its distribution and relation to tissue requirements has been unclear, but we (30) have recently shown that the aortic valve capillary network is contained within the thickest regions of the cusps, where oxygen diffusion from the cusp surfaces alone, is unlikely to provide an adequate environment to sustain cellular activity. The addition of such a vascular bed to a tissue-engineered valve would substantially increase the level of complexity and it would therefore be desirable to avoid that requirement if possible. A full understanding of the metabolic activity of valve cusps is therefore necessary to address this issue.

Using the Clark oxygen sensor technique, we have been able to determine both oxygen diffusion and consumption values of porcine aortic valve tissue. The mean value of \(1.06 \times 10^{-5} \text{ cm}^2/\text{s}\) we found for \(D_O2\) at 37°C was well within the range of in vivo values reported in the literature for other vascular structures such as the dog femoral artery (8) and the aorta of various species (4). Our reported \(V_O2\) values ranged from \(1.73–5.39 \times 10^{-5} \text{ ml O}_2/\text{ml tissue} \cdot \text{s}^{-1}\) and were anywhere from 59% to 87% lower than the mean in vivo value reported for the dog femoral artery (8). Because of the similar nature of these vascular structures (18) and the reported rates of turnover within the valves (20, 26), it is possible that our in vitro measurement has provided an underestimation of aortic valve cusp \(V_O2\) values.

To better understand the implications of our experimental results, we developed the finite difference

Table 1. Experimental results for porcine aortic valve cusp thickness, diffusion, and consumption values

<table>
<thead>
<tr>
<th>Sample</th>
<th>(k, 10^{-3}/\text{s})</th>
<th>(L, 10^{-2} \text{ cm})</th>
<th>(D_O2), 10^{-5} cm^2/s</th>
<th>(\Delta P/\Delta t), change in PO2 over time; V_O2, oxygen consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.2</td>
<td>8.63 ± 0.782</td>
<td>1.270</td>
<td>1.14</td>
</tr>
<tr>
<td>2</td>
<td>5.8</td>
<td>6.74 ± 0.639</td>
<td>1.090</td>
<td>0.76</td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
<td>7.28 ± 0.876</td>
<td>1.070</td>
<td>0.76</td>
</tr>
<tr>
<td>4</td>
<td>5.1</td>
<td>7.08 ± 0.378</td>
<td>1.030</td>
<td>0.68</td>
</tr>
<tr>
<td>5</td>
<td>9.2</td>
<td>4.55 ± 0.822</td>
<td>0.770</td>
<td>1.67</td>
</tr>
<tr>
<td>6</td>
<td>9.6</td>
<td>5.49 ± 0.823</td>
<td>1.170</td>
<td>2.13</td>
</tr>
<tr>
<td>7</td>
<td>5.6</td>
<td>6.78 ± 0.510</td>
<td>1.040</td>
<td>1.29</td>
</tr>
<tr>
<td>Mean values</td>
<td>6.6646 ± 1.31</td>
<td>1.063 ± 0.154</td>
<td>3.054 ± 1.37</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD for \(k, \text{ rate constant; } L, \text{ tissue thickness; } D_O2, \text{ oxygen diffusivity; } \Delta P/\Delta t, \text{ change in PO2 over time; } V_O2, \text{ oxygen consumption.}

Table 2. Calculated diffusivity values for aortic valve collagenous layers and summary of minimum PO2 values at the center of the tissue as predicted by the one- and three-layer models

<table>
<thead>
<tr>
<th>Sample</th>
<th>Collagenous Layers (D_O2) (10^{-5} cm^2/s)</th>
<th>Minimum PO2 Values at the Center of the Tissue Results, mmHg</th>
<th>Difference in Relative PO2 Drop</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>One layer  Three layer</td>
<td>(100 − three-layer drop)/(100 − one-layer drop), mmHg</td>
</tr>
<tr>
<td>1</td>
<td>9.99E-06</td>
<td>17.1          0</td>
<td>1.217</td>
</tr>
<tr>
<td>2</td>
<td>8.08E-06</td>
<td>59.5          49.3</td>
<td>1.252</td>
</tr>
<tr>
<td>3</td>
<td>7.90E-06</td>
<td>53.8          41.9</td>
<td>1.256</td>
</tr>
<tr>
<td>4</td>
<td>7.58E-06</td>
<td>59.6          49.6</td>
<td>1.248</td>
</tr>
<tr>
<td>5</td>
<td>5.25E-06</td>
<td>42.7          22.3</td>
<td>1.356</td>
</tr>
<tr>
<td>6</td>
<td>8.96E-06</td>
<td>34.0          19.7</td>
<td>1.217</td>
</tr>
<tr>
<td>7</td>
<td>7.67E-06</td>
<td>28.5          9.8</td>
<td>1.261</td>
</tr>
<tr>
<td>Mean values</td>
<td>7.919E-06</td>
<td>42.16</td>
<td>27.38</td>
</tr>
</tbody>
</table>
model to examine oxygen profiles within the tissue. In our experiments, we treated the aortic valve cusps as homogeneous structures by determining an overall diffusion and consumption value. When these experimental results were incorporated into a one-layer cusp model and oxygen profiles within the cusp tissue were determined, it would seem that oxygen supplied by diffusion from the surfaces of the cusp was adequate to sustain the metabolic function of the tissue. However, examining the cusp as a homogeneous tissue is a gross oversimplification and much information exists with regard to the composition of the aortic valve as a three-layered structure (3, 11). Therefore, we developed a second model comparing the oxygen profile when the three layers of the cusp were taken into account. This latter model required that diffusivity and solubility values of the three layers be approximated based on layer composition. Because of a recent report by Rocca et al. (18) on uniform cellular distribution within the tissue, tissue \( V_{\text{O}2} \) was taken as uniform throughout the structure. The resulting oxygen profiles confirmed that there was a statistically significant difference in oxygen permeability if the valve were modeled as a three-layered structure. Furthermore, even with a conservative \( V_{\text{O}2} \) value, a \( P_{\text{O}2} \) of 0 was reached in the center of a cusp. These results predict that oxygen diffusion from the cusp surfaces alone may be inadequate to support cell viability and that the microcirculation previously identified within the spongiosa is necessary to act as an additional oxygen source. If myoglobin (or another \( O_2 \) carrier) were present within the tissue, the impact of facilitated diffusion would have to be added to the model. To date, we have found no reports in the literature identifying myoglobin within aortic valve cusp tissue. It is also possible that there is a nonlinear distribution of \( D_{\text{O}2} \), solubility, and \( V_{\text{O}2} \) across the layers of the cusp and that a three-layered model is still an oversimplification.

Because our studies were necessarily carried out in vitro rather than in vivo, our estimates of \( V_{\text{O}2} \) may be lower than that present in a functioning valve. In addition to the transport time of the whole heart on ice, the time delay between tissue excision to tissue assessment of \( \sim 90 \text{ min} \) may be responsible for stunning the interstitial cells, resulting in an overall decrease in \( V_{\text{O}2} \). Furthermore, there have been reports demonstrating the presence of contractile smooth muscle bundles (1) and innervation within the valve (15). This suggests an active component to valve motion in vivo that would not be present in the in vitro studies, which could also contribute to an underestimation of \( V_{\text{O}2} \).

---

Fig. 5. A thickness map of an aortic valve cusp (sample 6) using the radiographic technique discussed in MATERIALS AND METHODS. The thickness area where the polarographic oxygen sensor was approximately located is outlined in black. The mean thickness of this region was used for the calculation of tissue \( D_{\text{O}2} \).
these reasons, our results likely reflect the minimum oxygen requirements of the valve tissue and the oxygen needs in vivo may be significantly higher.

In addition, cusp thickness is extremely variable and tissue thickness has a significant impact on both the calculation of $\text{DO}_2$ and the tissue oxygen profiles. In our experiments, we only made thickness measurements of tissue lying directly over the oxygen sensor and thereby only utilized tissue thickness in a small area of the cusps. If the thickness of the entire cusp is examined, as shown for one sample in Fig. 5, it becomes apparent that there are much thicker regions than those we have analyzed. The impact of these larger diffusion distances would be lower $\text{PO}_2$ values within the center of the cusps.

If an optimal bioengineered replacement valve were to be developed with a potential durability matching patient lifespan, that tissue must have the ability to be developed with a potential durability matching native tissue. And, native tissue.

These values of oxygen diffusion and consumption in mammalian striated muscle, with lower diffusivity than oxygen, may be used to carry away potentially harmful metabolic products, from the deeper tissue. Of course, the microcirculation may also be used to carry away potentially harmful metabolic products, with lower diffusivity than oxygen, from the deeper tissue. These values of oxygen diffusion and consumption will also allow for comparison to be made with tissue-engineered prototypes before implantation to examine similarities between the designed implants and native tissue.

**APPENDIX**

**Determination of values for three-layer cusp finite difference model.** Oxygen transport through the three layers of the tissue acts in a similar manner to resistors in series

$$
\frac{L_{\text{total}}}{\text{DO}_2_{\text{total}} \cdot \alpha_{\text{total}}} = \frac{0.5 L_{\text{total}}}{\text{DO}_2_{\text{plasma}} \cdot \alpha_{\text{plasma}}} + \frac{0.25 L_{\text{total}}}{\text{DO}_2_{\text{spongiosa}} \cdot \alpha_{\text{spongiosa}}} + \frac{0.25 L_{\text{total}}}{\text{DO}_2_{\text{fibrosa}} \cdot \alpha_{\text{fibrosa}}}$$

Where

$$L_{\text{total}} = L_{\text{measured}}$$

$$\text{DO}_2_{\text{total}} = \text{DO}_2_{\text{measured}}$$

$$\alpha_{\text{total}} = 0.9 \alpha_{\text{plasma}}$$ (based on the water content of the cusp)

$$\text{DO}_2_{\text{spongiosa}} = \text{DO}_2_{\text{plasma}}$$

$$\alpha_{\text{spongiosa}} = \alpha_{\text{plasma}}$$

$$\text{DO}_2_{\text{fibrosa}} = \text{DO}_2_{\text{ventricularis}} = \text{DO}_2_{\text{collagen layer}}$$

$$\alpha_{\text{fibrosa}} = \alpha_{\text{ventricularis}} = \alpha_{\text{collagen layer}}$$

Therefore,

$$
\frac{L_{\text{measured}}}{\text{DO}_2_{\text{measured}} \cdot 0.9 \alpha_{\text{plasma}}} = \frac{0.5 L_{\text{total}}}{\text{DO}_2_{\text{plasma}} \cdot \alpha_{\text{plasma}}} + \frac{0.5 L_{\text{total}}}{\text{DO}_2_{\text{collagen layer}} \cdot \alpha_{\text{collagen layer}}}
$$

The authors thank Michael Thornton for assistance with the radiographic thickness measurements and Mount Bridges Abattoir for help with specimen retrieval.

This work was supported by Grants-In-Aid T-3573 from the Heart and Stroke Foundation of Ontario and the Medical Research Council of Canada. K. L. Weind was supported in part by the Ontario Graduate Student for Science and Technology Scholarship Programme.

**REFERENCES**


8. Crawford DW, Cole MA, and Back LH. Evidence for the blood oxygen boundary layer $\text{PO}_2$ gradient (change in $\text{PO}_2$) as a significant determinant of intimal ($P_i$) and lowest medial $\text{PO}_2$ ($P_m$) in the vivo dog femoral artery. Adv Exp Med Biol 358: 197–209, 1983.


