The Groningen hypothermic liver perfusion system for improved preservation in organ transplantation
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Chapter 7

Hypothermic Machine Preservation of the Liver: A Comparison of Miniature Oxygenators using Belzer’s UW solution


7.1 Introduction

Despite improvements and better quality of organ preservation due to the University of Wisconsin (UW) solution and similar cold storage (CS) solutions, which were subsequently developed following the UW concept, organ preservation has remained an Achilles heel of transplantation. Due to the persistent shortage of donor organs nowadays older and more marginal donor livers are accepted for transplantation by most centres. This results in less organ viability and a higher chance of early dysfunction with lower graft survival after transplantation. Thus, to maintain or even improve organ viability during preservation and reduce the effect of ischemia/reperfusion injury another mode of preservation has become mandatory. Hypothermic machine preservation (HMP) is a method of organ preservation in
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which, in contrast to the CS technique, the donor organ is continuously perfused with the preservation solution under hypothermic conditions, allowing continuous supply of nutrients and removal of waste products. In the past HMP has been successfully applied in kidney transplantation with improved clinical results in comparison to CS\textsuperscript{7,8,9}. Thus, we assumed that HMP would be beneficial for the liver as well. This assumption was supported by experimental work in the eighties by Belzer and Southard’s group\textsuperscript{10,11}.

A controversial point in HMP in general, and for the liver in particular, is the question whether adding oxygen to the Belzer University of Wisconsin machine preservation solution (UW-MP) is necessary or not (Table 7.1). During his experiments, Pienaar et al\textsuperscript{10} did not use any additional oxygen, and achieved a 90% short term survival after 72 hrs HMP of dog livers. In his experiments the follow-up, however, was only 14 days and no long-term results could be obtained due to the design of the study, including thus the possibility of ischemia type biliary lesions or other late graft dysfunction due to lack of oxygen which would require retransplantation in clinics. On the other hand, Dutkowski et al\textsuperscript{12,13} argued, that it is beneficial to actively include oxygen during preservation. Fujita et al\textsuperscript{14,15} came to a similar conclusion, when they determined the oxygen consumption ($VO_2$) during continuous perfusion of rat livers as a function of temperature ($T$) [°C]: $VO_2 = 0.21 \cdot 10^{0.029 \cdot T}$ [mmol/min/gr liver]. Oxygen consumption at 0°C is 0.21 mmol/min/g liver, which is, compared to oxygen consumption of the liver at 37°C, a twelve-fold decrease.

A rat liver slice study, performed recently by our group, reconfirmed that oxygen

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration [mM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>5</td>
</tr>
<tr>
<td>Adenosine</td>
<td>5</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>1</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>0.5</td>
</tr>
<tr>
<td>Gluconate-K</td>
<td>10</td>
</tr>
<tr>
<td>Gluconate-Mg</td>
<td>5</td>
</tr>
<tr>
<td>Gluconate-Na</td>
<td>80</td>
</tr>
<tr>
<td>Glucose</td>
<td>10</td>
</tr>
<tr>
<td>Glutathione</td>
<td>3</td>
</tr>
<tr>
<td>HEPES</td>
<td>10</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>15</td>
</tr>
<tr>
<td>Raffinose</td>
<td>30</td>
</tr>
<tr>
<td>Ribose</td>
<td>5</td>
</tr>
<tr>
<td>HydroxyEthyl Starch</td>
<td>50 [g/l]</td>
</tr>
</tbody>
</table>

Table 7.1: Composition of the Belzer University of Wisconsin machine preservation solution (UW-MP).
supply during preservation is indeed important and should be included during HMP with UW-MP solution\textsuperscript{16}.

Thus, in our attempt to develop a portable HMP system in which donor livers are stored and can be easily transported, we decided that it was necessary to include an oxygenation facility to fulfil the need of oxygen during HMP. Data of oxygenator performance under hypothermic conditions (0–4°C) or when an a-cellular preservation solution such as UW-MP (Table 7.1) is used is lacking in the literature. The aim of this study was therefore to compare commercially available miniature oxygenators for their ability to sufficiently oxygenate the UW-MP solution. Additional criteria were a low pressure drop and small dimension to be able to fit the oxygenator in the portable HMP system.

7.2 Materials and Methods

Four types of commercially available miniature hollow fibre gas exchangers were selected based on size and cost and were evaluated for the use in a hypothermic liver machine perfusion system. In addition to the specifications listed in Table 7.2, the gas exchangers and a number of specific features are discussed below.

<table>
<thead>
<tr>
<th></th>
<th>HILITE</th>
<th>Baby-RX</th>
<th>MiniModule</th>
<th>FiberFlo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter</td>
<td>55 mm</td>
<td>87 mm</td>
<td>27 mm</td>
<td>25.5 mm</td>
</tr>
<tr>
<td>Length</td>
<td>150 mm</td>
<td>130 mm</td>
<td>195 mm</td>
<td>175 mm</td>
</tr>
<tr>
<td>Build-in vol.</td>
<td>3495 mm$^3$</td>
<td>3809 mm$^3$</td>
<td>3183 mm$^3$</td>
<td>2776 mm$^3$</td>
</tr>
<tr>
<td>Area</td>
<td>3200 cm$^2$</td>
<td>5000 cm$^2$</td>
<td>1200 cm$^2$</td>
<td>2300 cm$^2$</td>
</tr>
<tr>
<td>Priming vol.</td>
<td>50 ml</td>
<td>43 ml</td>
<td>10 ml</td>
<td>15 ml</td>
</tr>
<tr>
<td>Price</td>
<td>€600</td>
<td>€550</td>
<td>€140</td>
<td>€100</td>
</tr>
<tr>
<td>Flow range</td>
<td>0-1 l/min</td>
<td>0-2.5 l/min</td>
<td>0-1 l/min</td>
<td>0-3 l/min</td>
</tr>
<tr>
<td>$\Delta P$</td>
<td>0-70 mmHg</td>
<td>0-190 mmHg</td>
<td>0-233 mmHg</td>
<td>0-520 mmHg</td>
</tr>
<tr>
<td>Type</td>
<td>800 LT</td>
<td>Capiox RX05</td>
<td>0.75 x 5</td>
<td>MV-C-030-L</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>Medizintechnik, Tokyo, Jpn</td>
<td>Terumo, Membrana</td>
<td>Celgard, Fibercor</td>
<td>Stolberg, D, Charlotte, USA</td>
</tr>
</tbody>
</table>

Table 7.2: The tested oxygenators with their geometric and physical properties, price and type and manufacturer details.
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The following gas exchangers were evaluated:

**HILITE** The HILITE infant hollow fibre oxygenator (HILITE 800LT, MEDOS Medizintechnik AG, Stolberg, Germany) is designed for extracorporeal blood-gas exchange for infants. Special features of the 800LT version are the integrated heat exchanger and the plasma-tight hollow fibre membranes (poly-methylpentene) that make the oxygenator especially useful for long term applications.

**Baby-RX** The Baby-RX (CAPIOX RX05, Terumo, Tokyo, Japan) oxygenator is developed for blood oxygenation of neonates and infants during extracorporeal circulation. The oxygenator has a low priming volume yet a large flow range. The design combines an integrated heat exchanger with compact outer dimensions.

**MiniModule** The MiniModule (Liqui-Cel MiniModule 0.75x5, Membrana, Charlotte, USA) is a membrane gas exchanger of polypropylene fibres. It is developed for degassing aqueous fluids by a vacuum or strip gas stream around the fibres. There is no FDA approval for contact with blood. However, since liver HMP uses UW-solution, gas exchange with this type of exchanger could be applicable.

**FiberFlo** The FiberFlo gas exchanger (MV-C-030-L, Minntech Fibercor, Minneapolis, USA) includes a hydrophobic polypropylene hollow fibre. It is originally developed for gas removal from fluids, but filtration and oxygenation are possible applications as well.

7.2.1 Experimental set-up

The measuring set-up to test the different gas exchangers consists of a reservoir, a rotary pump (Deltastream, MEDOS Medizintechnik AG, Stolberg, Germany), a de-oxygenator (Lilliput, Sorin/Dideco, Mirandola, Italy) and the test oxygenator (Figure 7.1). The UW-MP preservation solution (Table 7.1) is cooled with melting ice to maintain a temperature of 0°C. A 100% nitrogen stream at maximum flow rate through the de-oxygenator was used to de-oxygenate the solution.

The capacity of the tested oxygenator is determined by measuring the pO$_2$ [kPa] difference between the inlet and outlet of the oxygenator. For this purpose, 0.5 ml samples of the perfusate are taken and analysed in a blood gas analyser (ABL 700, Radiometer A/S, Brønshøj, Denmark). To determine the pressure drop (Δ P) [mmHg] over the test oxygenator, the difference between inlet and outlet pressure
was measured with pressure transducers (Truwave, Edwards Lifesciences, Irvine, USA). Flow was measured after the pump using an ultrasonic clamp-on flow probe (H7C, Transonic Systems, Ithaca, USA).

![Test Circuit Diagram]

Figure 7.1: The test circuit with the de-oxygenator, test oxygenator, reservoir, pump and measuring points (P=pressure sensor, pO_2=sampling for partial oxygen pressure, Q=flow sensor).

The test oxygenators were subjected to an UW-MP flow of 250, 500 and 1000 ml/min, representing the range of expected flow rates in the liver HMP system. 100% oxygen was used. At each fluid flow rate, a ratio fluid flow:oxygen flow of 2:1, 1:1 and 1:2 was established. At each fluid flow rate and ratio, partial oxygen pressure (pO_2) [kPa] and perfusion pressure (P) [mmHg] were measured at the inlet and outlet of the test oxygenator. The measurements were performed according to AAMI/ISO standards\textsuperscript{17}, using 0°C UW-MP solution instead of 37°C bovine blood.

### 7.2.2 Error discussion

According to specifications, the inaccuracy of the pressure transducers was < 2 mmHg, of the pO_2 measurements with the blood gas analyser < 1 kPa and of the flow measurements < 7%. Values of measured pressure drop [mmHg] and oxygenation capacity [kPa] were assumed to be different for differences larger than the measured value ±2× inaccuracy.
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7.3 Results

The HILITE oxygenator showed an oxygenation capacity of 90 kPa at a fluid flow rate of 250 ml/min, regardless of the fluid/gas flow-ratio (Figure 7.2a). The capacity decreased with increasing fluid flow to 83 kPa at 500 ml/min and 68 kPa at 1000 ml/min. Again, the fluid/gas flow-ratio showed no influence at these higher flow rates.

The Baby-RX showed an oxygenation capacity of 72.7 kPa at 250 ml/min and a ratio of 2:1, while it was higher for the 1:1 and 1:2 ratios, 91.3 kPa and 88.6 kPa, respectively (Figure 7.2b). With increasing flow rates, oxygenation capacity decreased to 75 kPa. No clear capacity difference was found between the ratios at flow rates of 500 ml/min and 1000 ml/min.

![Figure 7.2: Oxygenation capacities of the test oxygenators (a=HILITE, b=Baby-RX, c=MiniModule, d=FiberFlo) at fluid flow rates of 250, 500 and 1000 ml/min and fluid flow/gas flow-ratios of 2:1, 1:1 and 1:2. (* = not measured).]

At a flow rate of 250 ml/min, the MiniModule showed an increased capacity for a fluid/gas-ratio of 1:1 and 1:2, 60.1 kPa and 62.2 kPa, respectively, compared to a ratio of 2:1, which is 56.1 kPa (Figure 7.2c). At 500 ml/min the capacity was lower for each fluid/gas flow-ratio, 43 kPa for 2:1, 39.5 kPa for 1:1 and 38.6 kPa.
for 1:2. Given the high pressure drop of the Minimodule, the rotary pump used was not able to produce a flow of 1000 ml/min through the oxygenator, so, no measurements of oxygenation capacity at this flow rate could be obtained.

The FiberFlo, finally, had a capacity of 64.8 kPa at 250 ml/min fluid flow and a ratio 2:1 (Figure 7.2d). Capacity increased to 75.7 kPa for 1:1, but decreased again for 1:2 to 69.6 kPa. At a flow rate of 500 ml/min the oxygenation capacity decreased for every ratio, 55.1 kPa, 48.7 kPa and 48.8 kPa for 2:1, 1:1 and 1:2, respectively.

The HILITE and Baby-RX showed the highest oxygenation capacity for a fluid/gas flow-ratio of 1:1 using flow rates of 250 ml/min, 500 ml/min and 1000 ml/min, respectively. The capacity of the FiberFlo was lower and the MiniModule showed the lowest (Figure 7.3).

Pressure drop was lowest in the two oxygenators (HILITE and Baby-RX) compared to the two gas exchangers (MiniModule and FiberFlo) over the total flow range (Figure 7.4). For the HILITE the pressure drop ranged from 12.8 mmHg at 250 ml/min to 69.3 mmHg at 1000 ml/min. Pressure drop in the Baby-RX ranged from 19.2 mmHg at 250 ml/min to 94.9 mmHg at 1000 ml/min. Pressure drop in the MiniModule could only be measured at a flow rate of 250 ml/min and was 176.1 mmHg. At higher flow rates the pressure exceeded the limits of the pressure transducer (>200 mmHg). This was also the case for the FiberFlo at flow rates

![Figure 7.3: Comparison of oxygenation capacity between the test oxygenators at flow rates of 250, 500 and 1000 ml/min at a fluid flow/gas flow-ratio of 1:1. (* = not measured).](image-url)
7. Comparison of Oxygenators

500 ml/min and 1000 ml/min, at 250 ml/min the pressure drop was 142.7 mmHg. The de-oxygenator established a mean partial oxygen pressure of 14.6 kPa at the efferent side of the test oxygenator.

Figure 7.4: Pressure drop over the test oxygenators at fluid flow rates of 250, 500 and 1000 ml/min. (* = too high to measure).

7.4 Discussion

It is our aim to develop a portable hypothermic liver perfusion system that allows better and longer preservation than with CS while maintaining liver viability. Such a device would allow the use of marginal, older and even non-heart-beating donor livers and could contribute to the expansion of the donor liver pool, reducing organ shortage. The necessity of oxygen supply in a HMP system has been indicated in several experiments\textsuperscript{12−16}. To fulfill the need for oxygen in a preserved liver at 0-4°C hollow fibre oxygenators may be suitable. As oxygenators are not specified for oxygenation of an a-cellular electrolyte solution such as the UW-MP preservation solution at 0-4°C, four commercially available hollow fibre gas exchangers were tested for use in hypothermic liver perfusion. The oxygenators were selected on the basis of their dimensions, cost and fluid flow rate of 0-1000 ml/min (Table 7.2). Our comparison of oxygenation capacity showed a better functioning of the HILITE and Baby-RX than the Minimodule and FiberFlo (Figure 7.2 and 7.3). The FiberFlo exceeded the Minimodule in capacity with 15 kPa at 250 ml/min and 9 kPa at 1000 ml/min. Whether the capacity of the tested oxygenators is sufficient for
additional oxygen support in HMP depends on the established flow rate during perfusion. The necessary partial oxygen pressure to fulfil the oxygen consumption of the liver, as determined by Fujita et al\textsuperscript{14}, can be expressed as a function of fluid flow rate:

\[
pO_2 = \frac{VO_2 \cdot H \cdot V_{\text{mol}}}{Q} \quad [Pa]
\]

where \(Q\) is the normalised flow in ml/min/gr liver, \(H\) is Henry’s constant, denoting the solubility of oxygen in water and \(V_{\text{mol}}\) is the volume of 1 mol water (=18 ml)\textsuperscript{18}. This relation is visualised in Figure 7.5 for the range of human liver weight of 1400-1800 gr\textsuperscript{19,20,21} at 0\(^\circ\)C, Henry’s constant of 2.58 \times 10^9 Pa and an oxygen consumption of 0.21 mmol/min/gr liver. For each flow rate and fluid/gas flow-ratio, the oxygenation capacity of the test oxygenators is also shown in Figure 7.5. Figure 7.5 shows that at low fluid flow rates the MiniModule is not able to oxygenate the UW-MP sufficiently since the capacity lies beneath the pO\(_2\) demand range. The FiberFlo oxygenator lies just inside the pO\(_2\) demand range, the HILITE and Baby-RX have a clear over-capacity. For increasing flow rates all oxygenators are able to satisfy the required pO\(_2\), as measured by Fujita\textsuperscript{14}, although the large difference in efficacy between the HILITE and Baby-RX in comparison to the MiniModule and FiberFlo remains.

An estimation of the required flow rates in the liver HMP system was based on

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure7_5.png}
\caption{Comparison of oxygenation capacities of the test oxygenators at fluid flow rates of 250, 500 and 1000 ml/min and fluid flow/gas flow-ratios of 2:1 (1), 1:1 (2) and 1:2 (3). Required oxygen supply for a 1400-1800gr liver at 0\(^\circ\)C according to Fujita et al\textsuperscript{19} as a function of flow is shown as a grey area.}
\end{figure}
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physiologic pressure and flow relations in the orthotopic human liver. For the hepatic artery a mean pressure of 100 mmHg and a mean flow of 500 ml/min and for the portal vein a mean pressure of 12 mmHg and a mean flow of 1000 ml/min can be assumed\textsuperscript{\textregistered}22. As a consequence of the viscosity of UW-MP which is 3 times higher compared to blood\textsuperscript{\textregistered}23, perfusion with UW-MP preservation solution yields a 3 times lower flow (at the same pressure). To prevent flow-induced injury under hypothermic conditions a lowered perfusion pressure is used and flow rates of approximately 100 ml/min and 300 ml/min for the hepatic artery and portal vein, respectively, are expected. At these flow rates, both the HILITE and Baby-RX are able to fulfil the oxygen demand of the liver (Figure 7.5).

To enable the development of a portable perfusion system, it is of crucial importance to minimise the weight, and use only a limited number of batteries and lightweight pumps. This means that the pressure drop over the oxygenator has to be as low as possible, and result in the necessity of small and little energy consuming pumps. Our comparison of the four oxygenators showed that there was a large difference in pressure drop. The HILITE and Baby-RX showed a low and acceptable pressure drop (12.8-69.3 mmHg and 19.2-94.9 mmHg, respectively), while the MiniModule and FiberFlo showed a much larger pressure drop (176.1 mmHg and higher and 142.7 and higher, respectively). For increasing flow, the measured efferent pressures of the MiniModule and FiberFlo were higher than 200 mmHg, the maximal measuring pressure of the probe. As a consequence, the HILITE and Baby-RX are both suitable for the HMP system, with the HILITE having a slightly lower pressure drop than the Baby-RX. Although the MiniModule and FiberFlo are the lowest priced oxygenators with the smallest dimensions, the large pressure drop and insufficient oxygenation capacity rule out their use in the HMP system. Both the HILITE and Baby-RX are applicable in the HMP system. The Baby-RX has the disadvantage that at a flow rate of 250 ml/min the capacity at 2:1 is less than at 1:1 and 1:2. This means that at 250 ml/min this oxygenator works optimal at a 1:1 or 1:2 ratio. The HILITE does not show this effect, indicating that sufficient oxygenation is possible at an even higher fluid/gas-ratio.

In conclusion, this study shows that commercially available oxygenators, designed for oxygenating blood at 37°C, as well as gas exchangers designed for more industrial applications, are capable of oxygenating the a-cellular UW-MP preservation solution under hypothermic conditions (0-4°C). The limiting factor in applying an oxygenator in a liver HMP system is the pressure drop, which therefore should be as low as possible. Therefor, we have selected the HILITE oxygenator for application in our portable hypothermic liver perfusion system.
7.5 References


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22. Milnor WR. Hemodynamics, Williams & Wilkins 1989, Maryland USA.