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Review

# Oxygen Transport during Ex Situ Machine Perfusion of Donor Livers Using Red Blood Cells or Artificial Oxygen Carriers

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**Abstract:** Oxygenated ex situ machine perfusion of donor livers is an alternative for static cold preservation that can be performed at temperatures from 0 °C to 37 °C. Organ metabolism depends on oxygen to produce adenosine triphosphate and temperatures below 37 °C reduce the metabolic rate and oxygen requirements. The transport and delivery of oxygen in machine perfusion are key determinants in preserving organ viability and cellular function. Oxygen delivery is more challenging than carbon dioxide removal, and oxygenation of the perfusion fluid is temperature dependent. The maximal oxygen content of water-based solutions is inversely related to the temperature, while cellular oxygen demand correlates positively with temperature. Machine perfusion above 20 °C will therefore require an oxygen carrier to enable sufficient oxygen delivery to the liver. Human red blood cells are the most physiological oxygen carriers. Alternative artificial oxygen transporters are hemoglobin-based oxygen carriers, perfluorocarbons, and an extracellular oxygen carrier derived from a marine invertebrate. We describe the principles of oxygen transport, delivery, and consumption in machine perfusion for donor livers using different oxygen carrier-based perfusion solutions and we discuss the properties, advantages, and disadvantages of these carriers and their use.

**Keywords:** machine perfusion; liver; gas transport; artificial oxygen carriers; oxygen; carbon dioxide; temperature

## 1. Introduction

Novel approaches are being explored to increase the number and quality of donor organs. These methods are aimed to decrease the donor organ shortages in relation to the number of candidate recipients on the waiting list [1]. One such approach is dynamic or machine preservation of donor organs. Machine perfusion (MP) is a procedure whereby organs are perfused ex situ after procurement. The aims of MP are multiple and include mitigation of ischemia-reperfusion injury (IRI), ability to prolong preservation time ex situ, improvement of organ condition and function, and assessment of organ viability prior to transplantation [2–4]. Currently, MP is widely studied and perfusion systems have been developed for various organs, including liver, heart, kidneys, and lungs [5].

MP of donor livers can be performed at different temperatures depending on the goal of the perfusion. For clinical use, temperature ranges are divided into three categories [6]. Hypothermic machine perfusion (HMP) ranges from 0 °C to 12 °C, at which the metabolic rate and energy expenditure of the organ is low. Oxygenated HMP mitigates IRI due to the resuscitation of mitochondria prior to warm reperfusion in the recipient. HMP can restore levels of intercellular adenosine triphosphate (ATP), reduce the production of reactive oxygen species (ROS), and confer protection to the bile ducts [7–9]. During HMP, organ viability and function cannot be assessed, because the liver is in a low metabolic state, but in addition it may prolong preservation time [10,11]. Subnormothermic machine perfusion (SNMP) ranges from 24 °C to 34 °C. During SNMP, at temperatures between 30 °C and 33 °C, the rate of metabolism increases to nearly 70% of the normal rate at body temperature. SNMP has the ability to increase ATP-levels and decrease mitochondrial injury and possibly assess viability [12,13]. In normothermic machine perfusion (NMP) organs are perfused at a temperature between 35 °C and 38 °C. During NMP, the metabolic rate is at normal physiological levels and the viability of the hepatocytes and cholangiocytes can be assessed [14–16]. A potential benefit of NMP is the use for therapeutic interventions [17]. NMP is technically more challenging, because the fully functioning liver has higher oxygen (O<sub>2</sub>) demands than during HMP. MP methods which are in experimental or preclinical evaluation such as subzero and hyperthermia conditions are beyond the scope of this review and are discussed elsewhere [18,19].

MP procedures are performed with different perfusion solutions depending on the temperature range. In HMP, an elementary perfusion solution contains the following ingredients: gluconate, phosphate, glutathione, allopurinol, and hydroxyethyl starch. Perfusion with this solution serves to provide O<sub>2</sub> and remove waste products continuously produced by the liver [20]. In NMP, the perfusion solution should contain at least an oxygen carrier (OC), nutrients to maintain metabolism, anticoagulation, and broad-spectrum antibiotics [21]. To obtain the preferred osmolality and colloid osmotic pressure, water, saline, and human albumin or another type of colloid are usually added [21,22].

In this descriptive review we discuss the principles of gas transport during ex situ MP. Our focus is on the impact of temperature on gas transport and the various natural and artificial oxygen carriers (AOC) that can be added to the perfusion solution during MP from hypothermia to normothermia. We reviewed the published literature regarding MP of human livers and principles of gas transport. Experimental and clinical studies are discussed.

## 2. Overview of Gas Transport in Machine Perfusion

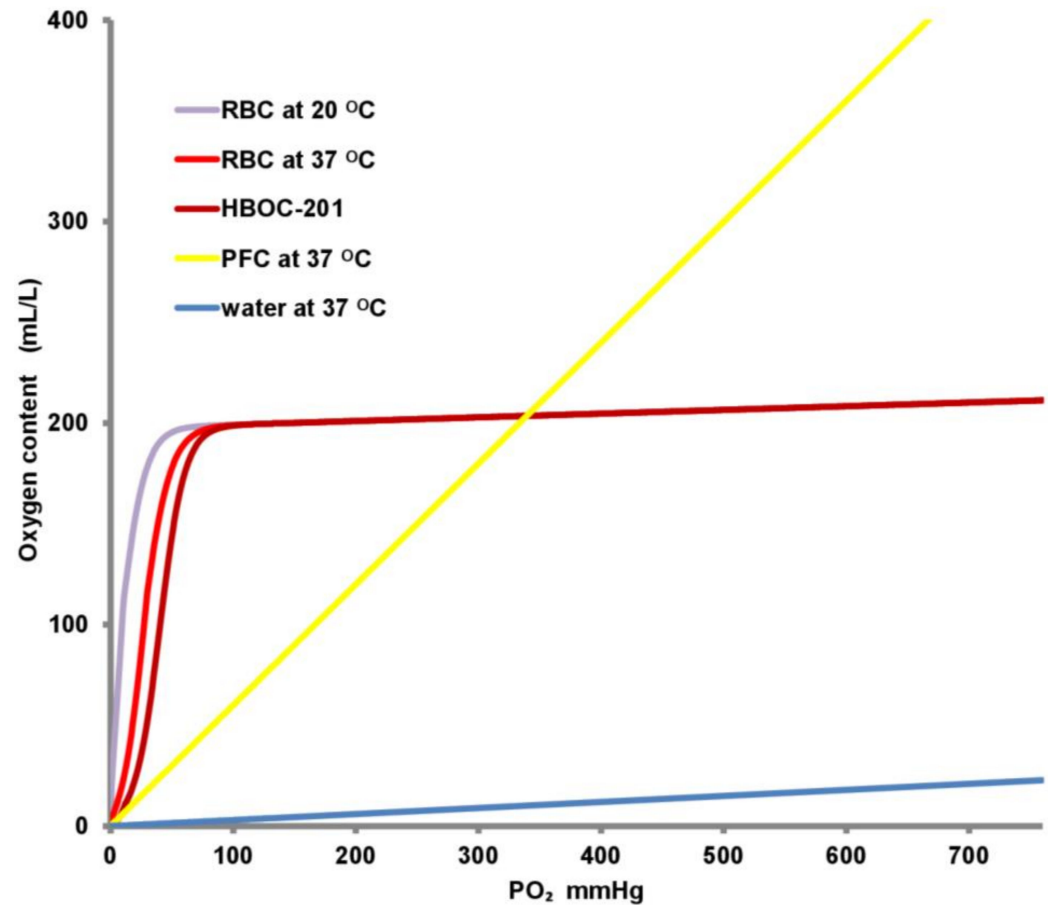
### 2.1. Physiological Gas Transport

In humans, gas exchange takes place by diffusion in the alveoli. The diffusing capacity of carbon dioxide (CO<sub>2</sub>) is 20 times higher than that of O<sub>2</sub> [23]. After diffusion, 97% of O<sub>2</sub> is absorbed by the red blood cells (RBC) where each hemoglobin (Hb) molecule binds four O<sub>2</sub> molecules to iron ions (Fe<sup>++</sup>) to form oxyhemoglobin. The remaining O<sub>2</sub> dissolves into the plasma. In the cell, O<sub>2</sub> is consumed by the mitochondria in aerobic respiration to produce ATP [24]. In the citric acid cycle, the acetyl group of acetyl-coA is oxidized and produces CO<sub>2</sub>, which is transported back to the alveoli in a dissolved state (approximately 7%), in the form of bicarbonate (70%), or bound to Hb to form carbaminohemoglobin (20%) [25,26]. Thus, the transport of CO<sub>2</sub> depends less on a carrier system than O<sub>2</sub>. Because CO<sub>2</sub> also has a better diffusing capacity across membranes than O<sub>2</sub>, transport of CO<sub>2</sub> is rarely a rate-limiting factor in MP.

#### 2.1.1. The Impact of Temperature on Gas Transport and Oxygen Requirements

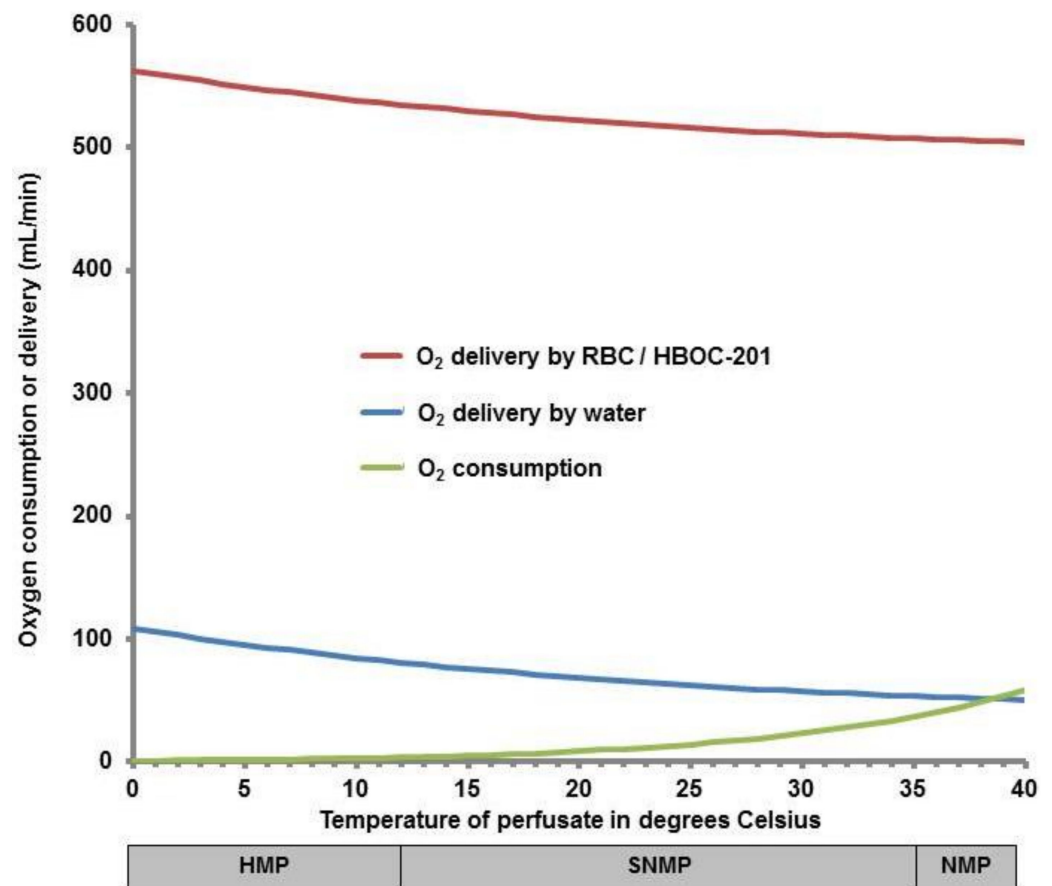
Increasing blood temperature causes a right shift in the O<sub>2</sub>-Hb dissociation curve (Figure 1), supporting O<sub>2</sub> release to the tissue. Increases in body temperature also lead to an increased metabolic rate, with higher O<sub>2</sub> requirements. As is shown by the Van 't Hoff equation and the Arrhenius relation derived from it [27], the relation between metabolic

rate and temperature is remarkably similar in various animal species, with an approximate 10% or 1.1-fold increase in metabolic rate per 1 °C (Figure 2) [28]. Likewise, increasing amounts of CO<sub>2</sub> will be produced at higher temperatures [29].



**Figure 1.** Graphical presentation of the relation between partial oxygen (PO<sub>2</sub>) pressure and the O<sub>2</sub> content of different solutions. Note how poorly O<sub>2</sub> dissolves in water (blue curve). To achieve a useful O<sub>2</sub> content without an oxygen carrier, a saturation of 100% O<sub>2</sub> is required, i.e., a partial pressure of 760 mm Hg. Perfluorocarbons (PFC; yellow curve) can dissolve 20 times more O<sub>2</sub>. The red blood cell (RBC) and hemoglobin-based oxygen carrier-201 (HBOC-201) curves assume a concentration equivalent with 7.76 mmol/L of O<sub>2</sub> binding places [30]. Additionally, note that the only small apparent shifts in the dissociation curves result from the supraphysiological PO<sub>2</sub> of 760 mmHg.

When glucose is fully oxidized under steady-state conditions, the production of CO<sub>2</sub> ( $\dot{V}CO_2$ ) will be equal to the consumption of oxygen ( $\dot{V}O_2$ ). The ratio between  $\dot{V}CO_2$  and  $\dot{V}O_2$  is called the respiratory quotient (RQ) and can be monitored *in vivo* or during *ex situ* MP. It is an important indicator of how ATP is generated. Glucose oxidation has an RQ of 1, whereas lipid oxidation has an RQ of <0.8 [23].



**Figure 2.** Graphical presentation of the relation between perfusion temperature and oxygen ( $O_2$ ) consumption (green curve) and  $O_2$  delivery. Metabolic rate and  $O_2$  consumption rise with approximately 10% for each increase in temperature measured in degrees Celsius. Note that the amount of  $O_2$  that is dissolved in water (blue curve) decreases at higher temperatures. At body temperature,  $O_2$  consumption/requirement will be larger than the amount that can be delivered by dissolved  $O_2$  alone, as indicated by the crossing green and blue curves. The addition of an oxygen carrier such as red blood cells (RBC) or hemoglobin-based oxygen carrier 201 (HBOC-201; red curve) can dramatically increase  $O_2$  content and delivery. Note that, as in whole body physiology, during organ perfusion oxygen delivery must be considerably higher than oxygen consumption, because oxygen consuming tissues such as the liver [31] can only extract a fraction of the delivered oxygen. The numbers displayed here assume oxygenation with 100% oxygen, a hemoglobin or HBOC-201 concentration equivalent to 7.76 mmol/L of  $O_2$ -binding sites and a perfusion flow of 2300 mL/min.

### 2.1.2. The Impact of Temperature on Gas Solubility and Pressure in Fluids

Blood consists for approximately 92% of water and  $O_2$  dissolves poorly in water [32]. The solubility of a gas into a fluid is determined by Henry's law: "At a constant temperature, the amount of a given gas dissolved in a given type of liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid." This implies that a rise in the partial pressure of  $O_2$  ( $PO_2$ ) leads to proportionally more dissolved  $O_2$  in the fluid if the temperature is constant [33]. Delete blank row under the Equation (use backspace)

$$\text{Henry's law : } c = k \cdot P \quad (1)$$

$c$  = concentration dissolved gas,  $k$  = Henry's law constant,  $P$  = partial pressure (in mm Hg or kPa).

However, when the temperature increases,  $O_2$  solubility decreases. Thus, at low temperatures, more  $O_2$  can be dissolved in the water compartment of a solution [32]. To

maintain the same O<sub>2</sub> concentration in a high-temperature solution without an OC in comparison to a low-temperature solution, the PO<sub>2</sub> should be increased (see Figures 1 and 2).

### 2.1.3. Gas Transport in Machine Perfusion

During MP, O<sub>2</sub> diffuses into the perfusate in the oxygenator, with a controlled gas flow and a set O<sub>2</sub> fraction. The oxygenated perfusion solution enters the liver through the cannulated portal vein and, when dual perfusion is applied, also via the hepatic artery. The CO<sub>2</sub> produced diffuses into the perfusate and circulates back to the oxygenator(s). The O<sub>2</sub> content of the perfusate depends on the perfusate temperature, the administered O<sub>2</sub> flow, and the concentration and use of either a cell-free solution or an OC-based solution.

Oxygenated HMP reduces IRI in contrast to non-oxygenated HMP or static cold storage (SCS) [7,16]. In hypothermic conditions the liver still maintains a low metabolic rate, therefore mitochondrial oxidative phosphorylation generates ATP when O<sub>2</sub> is supplied. During oxygenated HMP the mitochondrial respiration decreases and ceases altogether after approximately 90 min [34]. At this point, the mitochondria have switched from a high-flux to a low-flux electron transfer stage. When O<sub>2</sub> is not supplied to the solution, the mitochondria remain in the high-flux state electron transfer stage which causes the release of ROS during reperfusion [34–36].

The O<sub>2</sub> requirements in HMP and SNMP can be met by adding dissolved O<sub>2</sub> in the perfusion fluid [13,34]. In contrast, at 37 °C, the freely dissolved O<sub>2</sub> concentration in blood is only 3%. As indicated in Figure 2, at higher temperatures, the O<sub>2</sub> requirements exceed the maximal O<sub>2</sub> delivery by dissolved O<sub>2</sub> alone. It should also be noted, that the total delivery of O<sub>2</sub> to any organ should be considerably larger than its consumption because of heterogeneity in O<sub>2</sub> demand and metabolism. Consequently, a perfusion solution without an OC cannot supply sufficient O<sub>2</sub> to the liver to maintain aerobic metabolism at normothermia [37,38].

In MP, oxygenation and CO<sub>2</sub>-removal are adjustable by controlling the gas flow and its O<sub>2</sub> concentration (FiO<sub>2</sub>). The O<sub>2</sub> and CO<sub>2</sub> levels can be measured via arterial and venous blood gas analysis. In the arterial blood, the partial pressure of oxygen (aPO<sub>2</sub>), partial pressure of CO<sub>2</sub> (aPCO<sub>2</sub>) and the percentage of Hb saturated with oxygen (asO<sub>2</sub>) can be measured. Likewise, the venous blood gas parameters vPO<sub>2</sub>, vPCO<sub>2</sub> and vsO<sub>2</sub> can be measured. Oxygen consumption can be reasonably accurately derived by subtracting the venous O<sub>2</sub> content from the arterial O<sub>2</sub> content.

$$\text{Oxygen consumption} = \text{VO}_2 \text{ (mL/min)} = \text{blood flow} \cdot (1.36 \cdot \text{Hb} \cdot (\text{asO}_2 - \text{vsO}_2) + 0.0031 \cdot (\text{aPO}_2 - \text{vPO}_2)) \quad (2)$$

VO<sub>2</sub> = oxygen consumption, bloodflow (L/min), Hb = hemoglobin (g/dL), sO<sub>2</sub> = saturated oxygen (%), PO<sub>2</sub> = perfusate partial pressure (mm Hg).

Similarly determining CO<sub>2</sub> consumption is more challenging, as CO<sub>2</sub> content is more difficult to accurately estimate in the arterial and venous samples.

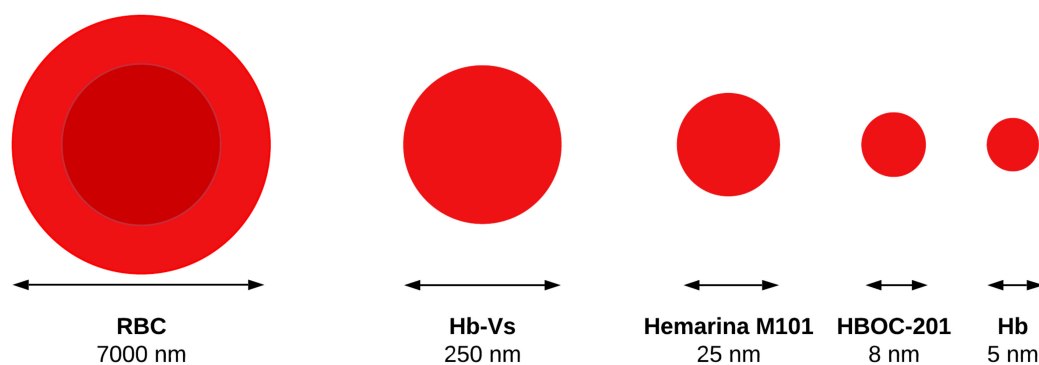
### 3. Overview of Oxygen Carriers

As described above, ex situ MP of an isolated organ at normothermia requires the addition of an OC to the perfusion fluid to enable sufficient O<sub>2</sub> delivery. Various OCs are currently used during machine preservation of donor organs. OCs have various characteristics resulting in different properties per carrier. In most studies on liver NMP, investigators have used RBCs as OC. Artificial alternatives are hemoglobin-based oxygen carriers (HBOCs) and natural extracellular OCs (Table 1, Figure 3).

**Table 1.** Overview of the advantages and disadvantages of different oxygen carriers.

| Oxygen Carrier                           | Advantages  | Disadvantages   | Pharmacokinetics  | Possible Toxicity in Humans                 |
|--|---|---|---|---|
| Hemoglobin in red blood cells (RBC)      | Within its physiological microenvironment<br>Human blood product<br>Low methemoglobin production<br>Dynamic shift of O <sub>2</sub> -hemoglobin dissociation curve                                    | Immune-mediated phenomena<br>Blood-borne infection transmission<br>RBC hemolysis during hypothermic machine perfusion (HMP)<br>Cross-matching difficulties<br>Precious resource | T <sub>1/2</sub> = 115 days<br>MW = 64 kDa<br>[Hb] = 12–15 g/dL<br>P <sub>50</sub> = 27 mm Hg | ABO incompatibility                         |
| Hemoglobin-based oxygen carrier-201 [39] | Easy O <sub>2</sub> release to tissue<br>Sterile and pyrogen-free<br>Large temperature range (4 °C–37 °C)<br>Less viscous than RBC<br>Long shelf life: three years<br>Compatible with all blood types | Formation of methemoglobin<br>Short half-life<br>Systemic vasoconstriction<br>Lower O <sub>2</sub> affinity than RBC  | T <sub>1/2</sub> = 20 h<br>MW = ~250 kDa<br>[Hb] = 13 g/dL<br>P <sub>50</sub> = 38–40 mm Hg   | Systemic vasoconstriction                   |
| Hemoglobin vesicles [40,41]              | Absence of RBC antigens<br>Smaller than RBC<br>sDo not generate colloid osmotic pressure<br>Do not rupture<br>Long shelf life: two years  | Only used in animal models  | T <sub>1/2</sub> = 2–3 days<br>[Hb] 10 g/dL<br>P <sub>50</sub> = 9 mm Hg                      | Release of free Hb can cause renal toxicity |
| Hemarina M101 [42,43]                    | Preliminary evidence in static cold storage<br>Large temperature range (4 °C–37 °C)<br>Simple gradient release O <sub>2</sub><br>High O <sub>2</sub> affinity<br>Non-immunogenic                      | Only used in preclinical HMP and clinically in static cold storage  | MW = 3600 kDa<br>P <sub>50</sub> = 7 mm Hg  | None reported                               |
| Perfluorocarbons [44,45]                 | High O <sub>2</sub> solubility<br>Inexpensive<br>Obey Henry's law<br>O <sub>2</sub> uptake and release insensitive to environment   | Formulated as emulsion, which reduces O <sub>2</sub> content<br>Needs high PO <sub>2</sub> to maximize O <sub>2</sub> content   | T <sub>1/2</sub> = 8–24 h<br>Emulsion dependent   | Visual loss                                 |
| Water                                    | Delivers sufficient O <sub>2</sub> below 20 °C<br>Inexpensive   | O <sub>2</sub> content decreases with higher temperature leading to a mismatch above 20 °C  | T <sub>1/2</sub> = ∞<br>MW = 18 Da  | None  |

MW = molecular weight, T<sub>1/2</sub> = half-life, [Hb] = concentration of Hb or its equivalent for other carriers, P<sub>50</sub> = O<sub>2</sub> tension where 50% of Hb is saturated with O<sub>2</sub>.



**Figure 3.** Visualizing the large differences in size between various oxygen carriers: red blood cells (RBC), hemoglobin vesicles (Hb-Vs), hemoglobin-based oxygen carrier 201 (HBOC-201), human hemoglobin (Hb).

### 3.1. Hemoglobin in Red Blood Cells

The human OC, Hb is transported by RBCs. Hb has a high affinity for O<sub>2</sub> and a low affinity for CO<sub>2</sub>. One molecule of Hb can adsorb four molecules of O<sub>2</sub>. A higher concentration of Hb in the blood results in a higher capacity to transport O<sub>2</sub>. When an O<sub>2</sub> molecule binds to Hb, the binding of additional O<sub>2</sub> molecules is facilitated, which results in the S-shape of the O<sub>2</sub>-Hb dissociation curve (Figure 1). Human Hb further depends on 2,3-biphosphoglycerate, pH and temperature for its affinity for O<sub>2</sub> [46].

It should be noted that when clinical laboratories report Hb concentrations in mmol/L, this concentration refers to the number of O<sub>2</sub>-binding sites and not to the actual number of Hb molecules. One molecule of Hb can bind to four O<sub>2</sub> molecules and thus, in reality, a reported Hb of 10 mmol/L is only 2.5 mmol/L of Hb molecules. Accordingly, when Hb is expressed in mmol/L units, in order to calculate the total O<sub>2</sub> content in a solution containing Hb, the amount of Hb should *not* be multiplied by 4 to arrive at the amount of O<sub>2</sub> bound to Hb. This is, however, a common mistake in formulas that are used to calculate O<sub>2</sub> consumption and it leads to an overestimation of O<sub>2</sub> consumption by a factor 4. So, there is at least one advantage to the older formula that was based on the conventional unit g/dL: max O<sub>2</sub> bound to Hb (mL/dL) = 1.36 Hb [47]. RBC-based perfusion solutions are only used in SNMP and NMP, due to hemolysis of the RBCs in a hypothermic environment. RBC are the most widely used OC in NMP [15,48–50]. Apart from Hb, RBCs also contain enzyme systems relevant for Hb protection and enhanced CO<sub>2</sub> transport, such as methemoglobin-reductase and carbonic anhydrase. Some disadvantages of the use of RBCs are the relative scarcity of precious resources, immune-mediated phenomena, blood-borne infection transmission, and logistical difficulties with cross-matching. Considering that RBCs are a limited resource, alternative OCs have been examined for use in MP (Table 1) [51].

### 3.2. Hemoglobin-Based Oxygen Carrier

For decades, the development of AOCs has been a topic of extensive research. The first objective of AOCs was to substitute RBCs in blood transfusions to eliminate RBC-related side effects and to decrease the use of a scarce resource. Although the theoretical rationale for AOCs still exists, on account of the relatively short half-life and potential side effects when administered intravenously, the *in vivo* use of AOCs was never really successful. MP serves as a new niche for the application of AOCs because important systemic side effects are not relevant in MP. Clinical trials are currently using AOCs as possible substitutes for RBCs in the MP of single organs (Table 2) [52].

**Table 2.** Overview of *ex situ* (sub)normothermic machine perfusion using artificial oxygen carriers.

| Author and Year                              | Oxygen Carrier | Temperature Range | Machine      | Sample Size n | Transplanted n | Species and Organ |
|--|----------------|-------------------|--------------|---------------|----------------|-------------------|
| Fontes et al. [53]<br>AJT 2015               | HBOC-201       | SNMP              | Liver Assist | 6             | 6              | Porcine livers    |
| Sadowsky et al. [54]<br>Front Pharmacol 2016 | HBOC-201       | SNMP              | Liver Assist | 6             | 6              | Porcine livers    |
| Matton et al. [55]<br>Liver Transpl 2018     | HBOC-201       | NMP               | Liver Assist | 24            | -              | Human livers      |
| Laing et al. [51]<br>Transplantation 2017    | HBOC-201       | NMP               | Liver Assist | 5             | -              | Human livers      |
| Boteon et al. [56]<br>AJT 2018               | HBOC-201       | NMP               | Liver Assist | 10            | -              | Human livers      |



Table 2. Cont.

| Author and Year                                   | Oxygen Carrier | Temperature Range | Machine       | Sample Size n | Transplanted n | Species and Organ |
|---|----------------|-------------------|---------------|---------------|----------------|-------------------|
| De Vries et al. [57]<br>AJT 2019                  | HBOC-201       | HMP to NMP        | Liver Assist  | 7             | 5              | Human livers      |
| Aburawi et al. [58]<br>AJT 2019                   | HBOC-201       | NMP               | Kidney Assist | 7             | -              | Human kidneys     |
| Vrselja et al. [59]<br>Nature 2019                | HBOC-201       | NMP               | -             | 32            | -              | Porcine brains    |
| Van Leeuwen et al. [60]<br>Ann Surg 2019          | HBOC-201       | HMP to NMP        | Liver Assist  | 16            | 11             | Human livers      |
| Bhattacharjee et al. [61]<br>Transplantation 2020 | HBOC-201       | SNMP              | -             | 5             | -              | Porcine kidneys   |
| Shonaka et al. [62]<br>Transplantation 2018       | Hb-Vs          | SNMP              | -             | 3             | -              | Porcine livers    |
| Shonaka et al. [63]<br>Plos one 2019              | Hb-Vs          | SNMP              | -             | 5             | -              | Porcine livers    |
| Brasile et al. [64]<br>Biotechnol. 1994           | PFC            | SNMP              | -             | 4             | -              | Canine kidney     |
| Inci et al. [65]<br>Cells 2020                    | PFC            | SNMP              | -             | 11            | 4              | Porcine lungs     |

### 3.2.1. Hemoglobin-Vesicles

Hemoglobin-vesicles (Hb-Vs) are phospholipid vesicles containing human-derived Hb. The diameter of the vesicles is 250–280 nm, which is smaller than that of RBCs. Hb-Vs are saturated for 50% at an O<sub>2</sub> pressure between 9 mm Hg and 30 mm Hg. They do not contain clinically relevant RBC antigens and have a longer shelf life than RBCs. Although Hb-Vs are small in comparison to RBCs, they are large compared to macromolecules (Figure 3) and accordingly do not generate a colloid osmotic pressure. The half-life of Hb-Vs is two to three days, which limits their use [66,67]. A few studies have reported on the use of Hb-Vs in porcine and rat MP models for livers and limbs [63,68,69]. The porcine models showed increased O<sub>2</sub> consumption during SNMP and decreased alanine aminotransferase and lactate dehydrogenase levels after reperfusion compared to HMP and SNMP, without an additional OC [62,63].

### 3.2.2. Hemoglobin-Based Oxygen Carrier-201

The first-generation HBOCs prepared from modified tetramer Hb molecules, has been associated with vasoconstriction and renal dysfunction when administered intravenously. Clinical trials were suspended because of increased mortality, myocardial infarction, and stroke [70,71]. Hemoglobin-based oxygen carrier-201 (HBOC-201) can cause vasopressor effects due to a tetramer Hb, possibly through the binding of nitric oxide (NO) in the interstitial space, which leads to vasoconstriction and platelet aggregation. Additionally, HBOC-201 reduce the NO concentration due to its scavenging effects, which may explain the increased risk of myocardial infarction when administered intravenously [72,73]. However, many studies reevaluated the use of HBOC-201 and reported no evidence of NO-related toxic effects applicable to all HBOCs [74–76]. Later, clinical trials were performed using HBOC-201 in patients with severe anemia who could not receive whole blood products. Results indicated that patients with acute bleeding and hemolysis survived relatively longer than patients who did not receive HBOC-201 [77]. Currently, HBOC-201 is also used in US and European programs as a blood substitute for patients who do not accept transfusion with RBCs on account of their religious background [78]. In these compassionate

use programs numerous patients have repeatedly received administration of HBOC-201 without clinical side effects (Z. Zafirelis, personal communication).

HBOC-201 or Hemopure<sup>®</sup> (HbO<sub>2</sub> Therapeutics LLC, Cambridge, MA, USA) is the most frequently used HBOC in MP. HBOC-201 is a polymerized Hb synthesized from bovine Hb. The Hb is extracted from bovine RBCs, purified, and cross-linked with glutaraldehyde to increase the stability and the molecular size. A purification process excludes possible harmful blood borne transmissions so that the end-product is a sterile pyrogen-free solution. These OCs are smaller than RBCs, resulting in a less viscous fluid. The affinity for O<sub>2</sub> in HBOC-201 depends on the chloride ion concentration. It releases O<sub>2</sub> easier than human Hb, because of a right shift in the Hb-dissociation curve (Figure 1). The in vivo half-life of HBOC-201 is approximately 20 h, which is much shorter than the half-life of RBCs, but sufficient for most cases of ex situ organ MP. The O<sub>2</sub> pressure required for 50% saturation of the O<sub>2</sub>-binding sites in HBOC-201 is 38–40 mm Hg, which is higher than for human Hb in RBCs. When completely saturated, HBOC-201 can bind 1.36 mL O<sub>2</sub> per gram of Hb. It has a molecular weight of 250 kDa [51,55,79]. A disadvantage of HBOC-201 is the formation of methemoglobin (metHb), which is formed when Fe<sup>++</sup> is oxidized to Fe<sup>+++</sup>. Because the natural metHb-reductase in the RBCs that reduces Fe<sup>+++</sup> back to Fe<sup>++</sup> is absent, this leads to a gradual increase in the metHBOC-201 concentration in the perfusion fluid. MetHBOC-201 is longer available for O<sub>2</sub> transport, leading to a lower saturation in prolonged machine preservation [80]. MetHBOC-201 can be converted back to normal, functional HBOC-201 by (repeated) addition of glutathione or ascorbic acid [79].

In several clinical studies HBOC-201 served as the OC in the MP of donor organs (Table 2). Laing et al. concluded that HBOC-201 could be used as an alternative for RBCs in NMP [51]. Van Leeuwen et al. used HBOC-201 in the DHOPE-COR-NMP trial, rewarming discarded human donor livers from hypothermic to normothermic conditions. In this trial suboptimal livers were continuously perfused and oxygenated, which increased the utilization of initially discarded donor organs. An advantage of HBOC-201 is that it can be used during the HMP phase which avoids the need to change the perfusion fluid when one switches from hypothermia to normothermia [55,57,60]. After MP, the perfused organ is flushed, leaving only minimal amounts of HBOC-201 to reach the recipient. Apart from visceral organ MP, Vrselja et al. used HBOC-201 to perfuse porcine brains to study if brain circulation and cellular functions could be restored (Table 2) [59].

### 3.3. Natural Extracellular Oxygen Carrier Hemarina M101

The natural extracellular Hb equivalent Hemarina M101 (HEMO2life<sup>®</sup>, France) is obtained from a marine invertebrate: *Arenicola marina*, a lugworm. The molecule is quite large with a molecular weight of 3600 kDa. It is composed of 156 globins and 44 non-globin linker chains that can carry up to 156 O<sub>2</sub> molecules when saturated, which results in a high O<sub>2</sub>-binding capacity. Hemarina M101 is active over a large range of temperatures (4 °C to 37 °C) and releases O<sub>2</sub> according to a simple gradient that does not require any allosteric effector. The molecule possesses intrinsic Cu/Zn-superoxide dismutase antioxidant activity that, to a certain extent, protects tissue from superoxide radicals [81].

Hemarina M101 was first described as a new and potentially promising blood substitute in 1997. The initial transplantation-related research was performed by Thuillier et al. They demonstrated that Hemarina M101 has a beneficial effect during SCS before kidney transplantation by decreasing chronic fibrosis and organ dysfunction [82]. Alix et al. compared SCS solution with Hemarina M101 versus SCS solution alone during porcine liver graft preservation, showing higher ATP levels in the SCS with Hemarina M101 group after 80 min [42,83]. M101 was also used in preclinical trials as an additive to the perfusion solution during HMP in a marginal kidney porcine model. A reduction of short-term function loss and no loss of function or tissue integrity were observed (Table 1) [43,84].

### 3.4. Perfluorocarbons

Perfluorocarbons (PFCs) are hydrocarbons in which practically all hydrogen atoms are replaced by fluoride. They have a high capacity for dissolving respiratory gasses and have an O<sub>2</sub> solubility that is 20 times higher than that of water. As in the case of water, the amount of O<sub>2</sub> that can be dissolved in PFCs is determined by Henry's law. A high partial O<sub>2</sub> pressure is necessary to maximize O<sub>2</sub> content of the PFCs (Figure 1). The O<sub>2</sub> dissociation curve of PFCs is a straight line in contrast to the sigmoid curve of Hb in RBCs. For the intravascular use of PFCs, the lipophilic molecule should be formulated as an emulsion, which inevitably will reduce its overall O<sub>2</sub> content. PFCs have been used in static cold preservation with active and non-active oxygenation [85,86]. In 1994 PFCs were already used in a pulsatile subnormothermic setting for the preservation canine kidneys [64]. Recently, PFCs were used during porcine ex vivo lung perfusion, which showed better preservation of mitochondrial function, glucose consumption and neutrophil infiltration [65].

### 4. Future Perspectives

The majority of OCs used in clinical MP of donor organs are RBCs. HBOC-201 may be a promising alternative to RBCs in MP. The ideal perfusion solution should contain a cell-free OC that can be used for MP at all temperatures, and it should have a long shelf life. Future research should aim to elucidate the relation between metabolic rate and required O<sub>2</sub> delivery in donor organs.

### 5. Summary

MP is a dynamic organ preservation platform technology used to increase the number and quality of donor organs. It can be performed at different temperatures of the perfusate solution—varying from hypothermic to normothermic perfusion. Low temperatures lead to a decreased metabolic rate, while at 37 °C the organ is metabolically active at a physiological level.

At normothermia, oxidative phosphorylation is required to generate sufficient ATP. In MP, O<sub>2</sub> is added to the perfusion solution through the oxygenators, while CO<sub>2</sub> diffuses out passively through the oxygenators. The O<sub>2</sub> requirements in HMP and SNMP can be met with dissolved O<sub>2</sub> in the perfusion fluid. Because O<sub>2</sub> solubility decreases with increasing temperatures, in NMP a perfusion solution containing an OC is required.

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### Abbreviations

|                   |   |
|-------------------|---|
| AOC               | artificial oxygen carrier                       |
| aPCO <sub>2</sub> | arterial partial pressure of carbon dioxide     |
| aPO <sub>2</sub>  | arterial partial pressure of oxygen             |
| ATP               | adenosine triphosphate                          |
| asO <sub>2</sub>  | arterial percentage of Hb saturated with oxygen |
| CO <sub>2</sub>   | carbon dioxide                                  |

|                   |   |
|-------------------|---|
| Fe <sup>++</sup>  | iron ion                                      |
| FiO <sub>2</sub>  | fraction of inspired oxygen                   |
| Hb                | hemoglobin                                    |
| HBOC              | hemoglobin-based oxygen carrier               |
| HBOC-201          | hemoglobin-based oxygen carrier-201           |
| Hb-Vs             | hemoglobin-vesicles                           |
| HMP               | hypothermic machine perfusion                 |
| IRI               | Ischemia reperfusion injury                   |
| metHb             | methemoglobin                                 |
| MP                | machine perfusion                             |
| NMP               | normothermic machine perfusion                |
| NO                | nitric oxide                                  |
| O <sub>2</sub>    | oxygen  |
| OC                | oxygen carrier                                |
| PCO <sub>2</sub>  | partial pressure of carbon dioxide            |
| PFCs              | perfluorocarbons                              |
| PO <sub>2</sub>   | partial pressure of oxygen                    |
| RBC               | red blood cell                                |
| ROS               | reactive oxygen species                       |
| RQ                | respiratory quotient                          |
| SCS               | Static cold storage                           |
| SNMP              | subnormothermic machine perfusion             |
| SO <sub>2</sub>   | oxygen saturation                             |
| VCO <sub>2</sub>  | production of CO <sub>2</sub>                 |
| VO <sub>2</sub>   | consumption of O <sub>2</sub>                 |
| vPCO <sub>2</sub> | venous partial pressure of carbon dioxide     |
| vPO <sub>2</sub>  | venous partial pressure of oxygen             |
| vsO <sub>2</sub>  | venous percentage of Hb saturated with oxygen |

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