Minireviews

Artificial Oxygen Carriers—Past, Present, and Future—a Review of the Most Innovative and Clinically Relevant Concepts

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ABSTRACT

Blood transfusions are a daily practice in hospitals. Since these products are limited in availability and have various, harmful side effects, researchers have pursued the goal to develop artificial blood components for about 40 years. Development of oxygen therapeutics and stem cells are more recent goals. Medline (https://www.ncbi.nlm.nih.gov/pubmed/?holding=iduedelib), ClinicalTrials.gov (https://clinicaltrials.gov), EU Clinical Trials Register (https://www.clinicaltrialsregister.eu), and Australian New Zealand Clinical Trials Registry (http://www.anzctr.org.au) were searched up to July 2018 using search terms related to artificial blood products in order to identify new and ongoing research over the last 5 years. However, for products that are already well known and important to or relevant in gaining a better understanding of this field of research, the reader is punctually referred to some important articles published over 5 years ago. This review includes not only clinically relevant substances such as heme-oxygenating carriers, perfluorocarbon-based oxygen carriers, stem cells, and organ conservation, but also includes interesting preclinically advanced compounds depicting the pipeline of potential new products. In-depth insights into specific benefits and limitations of each substance, including the biochemical and physiologic background are included. “Fancy” ideas such as iron-based substances, O2 microbubbles, cyclo-dextranes, or lugworms are also elucidated. To conclude, this systematic up-to-date review includes all actual achievements and ongoing clinical trials in the field of artificial blood products to pursue the dream of artificial oxygen carrier supply. Research is on the right track, but the task is demanding and challenging.

Introduction

Every day, thousands of patients receive red blood cell (RBC) concentrates to maintain essential functions such as oxygen delivery (Meier et al., 2016). In the recent years, state of the art methods have been developed, such as conservation of blood, anticoagulants, and safety regarding infections. Nevertheless, while blood saves life, RBC concentrate transfusions have important side effects such as immune modulations, acute transfusion reactions, transfusion-related lung injury, volume overload, and hemolytic reactions. RBC concentrates cannot be stored without obtaining side effects called the storage lesion (Bruskin et al., 2015; Tissot et al., 2017). Transfusion-associated bacterial contamination and viral infections have been reported. The incidence of cancer receding and an increase in mortality have been reported for bladder, colon, and gastric cancer (Sun et al., 2015; Velásquez and Cata, 2015; Amri et al., 2017; Furrer et al., 2018), and rare—but still occurring—mistransfusions may lead to severe problems.

To minimize the risks of RBC concentrate transfusions, patient blood management programs have enabled more careful use of blood products (Meybohm et al., 2016). Demographic changes have led to more elderly people who require surgery. Therefore, artificial oxygen carriers (AOCs) may be required to enable surgery in all patients since donated blood has also become a scarce source. The actual
idea in the field of AOCs has shifted during the past 40 years from blood substitution to oxygen therapeutics (Spahn, 2018). Many compounds have been developed, but the ideal, clinical useful product has not yet been developed (Simoni, 2017). The overall aim is to provide an additional tool for physicians in clinical situations, in which blood may not be available, might not be an option (such as antibodies against blood compounds or religious reasons), or oxygen delivery is required (i.e., transplantation).

An overview of the demands of a perfect AOC is given in Fig. 1. Besides high affinity to $O_2$ with easy release at the tissue, high affinity to carbon monoxide (CO)/carbon dioxide (CO$_2$) with easy release during the lung passage are required goals. Major problems associated with AOCs are the induction of the inflammatory reactions of the body, hypotension, and hypertension.

This review gives an overview (please see Fig. 2) about the current preclinically and clinically relevant AOCs, which include hemoglobin (Hgb)-based oxygen carriers (HBOCs), perfluorocarbon-based oxygen carriers (PFOCs), and stem cells (SCs). As shown in Fig. 2A, perfluorocarbons (PFCs)

![Fig. 1. Demands of the perfect artificial oxygen carrier. In Fig. 1, the demands of a perfect AOC are depicted. High affinity to oxygen with an easy release at the tissue is an essential objective. High affinity to CO and CO$_2$ with also an easy release at the lung passage is a second required goal. The major problems of artificial components are the induction of the inflammatory reactions of the body, hypotension, and hypertension.](image1)

![Fig. 2. Overview of different types of AOCs. To date, the following AOC categories can be described: (A) PFOCs, which are halogene-substituted compounds; (B) HBOCs, which have a central ion atom (most frequently iron) that is oxygenated and surrounded by tetrapyroles; (C) SCs, which can develop into different target tissues; and (D) oxygen emulsions, which are useful in increasing oxygen in liquids. Structural formula in part adapted with permission from Ferenz (2019).](image2)
are halogene-substituted compounds, while HBOCs (Fig. 2B) have a central ion (most frequently iron) surrounded by tetrpyrroles. SCs can develop into RBC concentrates or other target tissues (Fig. 2C) and oxygen emulsions are useful in increasing oxygen in liquids (Fig. 2D). The biochemical and physiologic details of HBOCs and PFOCs are given in Tables 1 and 2, respectively. A summary of the important achievements, such as quality improvement prior to transplantation, with AOCs used for organ conservation is shown in Fig. 3.

For a variety of other substances, please see the Supplemental Material, which includes the following detailed information: recent clinical trials with Hemopure (Supplemental Table 1); clinical studies on other HBOCs (Hemolink, Polyheme, pyridoxalated hemoglobin polyoxyethylene conjugate, and Hemotech) (Supplemental Table 2); trials with hemospan/MP4OX (Sangart Inc.) and MP4CO (Sangart) (Supplemental Table 3); clinical trials with Sanguinate (Supplemental Table 4); sophistically engineered HgbS (including OxyVita, poly-Hb-tempol, Sanflow, VitalHeme, YQ23, antioxidative bromoacethylethyleneglycol-ferulate-linked human hemoglobin (BAEGF-Hb), and polymerized human placenta hemoglobin (PolyPhb)/bovine pegylated hemoglobin (bPEG-Hb)) (Supplemental Table 5); standard Hgb plus engineered envelope (HbVesicles, HbMP-700, ErythroMer, hemoglobin nanoparticles (HbN), polymer encapsulated bovine hemoglobin (HbP), liposome-encapsulated hemoglobin (LEH), antioxidative polydopamine-coated bovine Hgb nanocapsules (Hb-PDA), polydopamine-coated bovine hemoglobin microparticles (PDA-Hb microcapsules), Hemoact, red blood cell-like microgel particles loaded with bovine hemoglobin (RBCM), Mal-PEG-β-xl-Hb and hemoglobin-loaded nanoliposome) (Supplemental Table 6); and other artificial blood products in preclinical stage (HemoCD, pegylated *Lumbricus terrestris* erythrocruorinin (PEGLeC), hemerythrinst-based oxygen carriers (HrBOC), coA1-replaced myoglobin, coA2 porphyrin–based micelles, and Lipid-based oxygen microbubbles (LOM/Polymer hollow microparticles (PHMs)) (Supplemental Table 7). Please note, that per definition, free, unmodified hemoglobins do not belong to AOCs and have, therefore, not been included in this review.

### Methods

Medline was searched up to July 23, 2018 ([https://www.ncbi.nlm.nih.gov/pubmed/?holding=ideudeilib](https://www.ncbi.nlm.nih.gov/pubmed/?holding=ideudeilib)). The following search terms were used: perfluorocarbon-based oxygen carriers, perfluorocarbon based oxygen carriers, hemoglobin-based oxygen carriers, hemoglobin based oxygen carriers, artificial oxygen carriers, artifical AND blood AND substitutes, organ preservation AND perfluorocarbons, organ preservation AND hemoglobin-based artificial oxygen carriers, organ preservation AND artificial oxygen carriers,

<table>
<thead>
<tr>
<th>Table 1 HBOCs at a glance: natural Hgb from an organism</th>
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<tbody>
<tr>
<td><strong>Important Parameter/Compounds</strong></td>
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<tr>
<td>Compounds (abandoned)</td>
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<td>Compounds (recently investigated)</td>
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<tr>
<td>Compounds (recently investigated preclinically)</td>
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<tr>
<td>Misciability with blood</td>
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<tr>
<td>Origin of hemoglobin</td>
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<tr>
<td>High range of oxygen affinity (p50)</td>
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<td>High carbon monoxide affinity</td>
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<tr>
<td>High molecular weight/size</td>
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<tr>
<td>Metabolism</td>
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<tr>
<td>Intravascular half-life</td>
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<td>Recent studies performed in animals</td>
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<td>Recent studies performed in humans</td>
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<tr>
<td>Concerns of regulatory authorities</td>
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<td>Reviews elucidating biochemistry/physiology behind HBOCs</td>
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relevant key articles are cited. Side effects associated with each class of substances important and blood products, point out milestones, and explain typical problems and Oxycyte. In order to introduce the reader into the context of artificial conjugate, Hemospan, MP4OX, MP4CO, Sanguinate, Oxygent, and HemAssist, Polyheme, pyridoxalated hemoglobin polyoxyethylene AND pegylated AND hemoglobin, HbVesicles, Hemoact, Hemotech., Cell, Hemolink, Oxyvita, OxyVita Hb, Vitalheme, polynitroxylated Hbmp-700, Hemopure, Hb-201, Hemoxycarrier, Hemo2life, HEMOX-up to July 23, 2018, using the drug names DCLHb, ErythroMer, New Zealand Clinical Trials Registry (http://anzctr.org.au]) were searched Trials Register (https://www.clinicaltrialsregister.eu/), and Australian Australia [ClinicalTrials.gov (https://clinicaltrials.gov), EU Clinical Trials Register (https://www.clinicaltrialsregister.eu/), and Australian New Zealand Clinical Trials Registry (http://anzctr.org.au)] were searched and stored in the 1930s using free Hgb extracted from human blood (Amberg et al., 1933) resulted in undesirable side effects, e.g., nephrotoxicity (Chang, 1988; Elmer et al., 2012; Cardenas (Amberson et al., 1933) resulted in undesirable side effects, in the 1930s using free Hgb extracted from human blood (erythrocytes). Unfortunately, the pioneering work performed in the 1930s using free Hgb extracted from human blood (Amberg et al., 1933) resulted in undesirable side effects, e.g., nephrotoxicity (Chang, 1988; Elmer et al., 2012; Cardenas et al., 2017). Twenty-seven years later, the first description of AOCs in the strict sense, namely, nano-bio-technologically engineered Hgb and other synthetic compounds surrounded by an artificial membrane or otherwise chemically engineered Hgb, evolved (Chang, 2012). Until now, three classes of AOCs have been defined: • HBOCs; • PFOCs; and • AOCs derived from SCs.

Table 2: PFOCs at a glance

<table>
<thead>
<tr>
<th>Important Parameter/Compound</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compounds (abandoned)</td>
<td>Flusisol-Da, Oxyyte: no more in trials since 2014</td>
</tr>
<tr>
<td>Compounds (recently investigated in clinical trials)</td>
<td>Perftoran (Perftec): approved for human clinical use in Russia, Mexico, Kazakhstan, Kyrgyzstan, and Ukraine (Castro and Briceno (2010)), e.g., resuscitation from hemorrhagic shock, cardiopulmg or Oxycyte: produced, licensed, and approved for clinical studies in China (Liu (2017)), e.g., patients undergoing orthopedic and non-cardiac surgery after hemodilution to hgb of 9g/dl</td>
</tr>
<tr>
<td>Compounds (recently investigated in pre-clinical trials)</td>
<td>Albumin-derived perfluorodecalin-filled nanocapsules</td>
</tr>
<tr>
<td>Miscibility with blood</td>
<td>To provide compatibility with the aqueous medium blood, PFCs have to be emulsified or encapsulated.</td>
</tr>
<tr>
<td>High oxygen (ρO2) and carbon monoxide affinity</td>
<td>No saturation of O2 and CO2 occurs, solubility dependent on gas partial pressure, in addition to respiratory gases PFCs also dissolve CO and N2, which are relevant in the treatment of flue-gas poisonings or gas embolism and decompression sickness (Spiess BD (2009)).</td>
</tr>
<tr>
<td>High molecular weight/size</td>
<td>Emulsified or encapsulated displaying a droplet size of 100–300 nm</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Fully halogenated, mainly fluorinated, molecules. Strong carbon-fluorine bond, no toxic metabolites are formed. Elimination: first, uptake into macrophages, and then diffusion into the blood, association to lipoproteins, and transport to the lung, where they can be exhaled (if vapor pressure is favorable, e.g., perfluorocarbon or perfluorocytoglobin) (Lowe (2003), 2006).</td>
</tr>
<tr>
<td>Easy release of O2, CO, and CO2</td>
<td>Oxygen loading and unloading is two times faster than in erythrocytes and the oxygen extraction rate is 5-fold higher since PFCs release more than 90% of the loaded oxygen to the tissue (Faithfull NS, 1992; Keipert PE, Faithfull NS, Roth DJ, Bradley JD, Batra S, Jochelson P, Flaim KE, 1996).</td>
</tr>
<tr>
<td>Intravascular half-life</td>
<td>158 min till 8 days</td>
</tr>
<tr>
<td>Current-to-future intent</td>
<td>1) Introduce Perftoran (Perftec) as Vidaphor to the markets in the United States and Europe; 2) resume development of Oxycyte in China.</td>
</tr>
</tbody>
</table>

Development of Artificial Blood Products

The development of blood transfusions started decades ago: In 1667 blood was transfused from a dog to a human (Roux et al., 2007), and in 1692 from lambs to humans. On September 1, 1818, the first blood transfusion from human to human was performed by Blundell (1818). However, only with the discovery of the ABO blood type by Landsteiner and Decastello survival improved (Greenwalk, 2005).

In parallel, the development of AOCs started with the aim of 1) eliminating whole blood–associated side effects and 2) providing unrestricted disposal of blood, or at least parts of it (erythrocytes). Unfortunately, the pioneering work performed in the 1930s using free Hgb extracted from human blood (Amberg et al., 1933) resulted in undesirable side effects, e.g., nephrotoxicity (Chang, 1988; Elmer et al., 2012; Cardenas et al., 2017). Twenty-seven years later, the first description of AOCs in the strict sense, namely, nano-bio-technologically engineered Hgb and other synthetic compounds surrounded by an artificial membrane or otherwise chemically engineered Hgb, evolved (Chang, 2012). Until now, three classes of AOCs have been defined:
The foci of this review are new preclinical and clinical developments within the last 5 years.

**General Requirements of Artificial Blood Products**

Clinicians and researchers have physiologic, biochemical, and technical demands on the perfect artificial blood product (Fig. 1). Of course, supplying tissue with O2 in combination with evacuation of CO2 from the periphery are the most important ones (Fig. 1). The partial pressure of O2 at which Hgb is saturated to 50% is given by the $P_{50}$ value. In a healthy adult, 26.6 mm Hg (3.5 kPa) is normal. If the $P_{50}$ value is higher, the affinity to O2 decreases, and the standard curve shifts rightwards. A lower $P_{50}$ value indicates higher affinity to O2 with a left shift of the O2 affinity curve. The $P_{50}$ values are listed in the text and Table 1.

**Classes of AOCs**

**HBOCs: Specifications, Peculiarities, and Limitations**

HBOCs are compounds consisting of natural Hgb from different organisms (Table 1). HBOCs are attractive AOCs since they are able to deliver O2 to the tissue without an increased inspiratory O2 concentration.

The half-life of 18–23 hours is much shorter than the half-life of erythrocytes, which is 120 days; therefore, repetitive doses of HBOCs would be required to maintain O2 delivery for days (which was the original reason for the development of HBOCs as blood substitutes). More recently, HBOCs have been designed to bridge patients safely in clinics in order to gain time until a RBC concentrate transfusion is available. In the presence of flue gases, they do not remain functional since CN$^-$ or CO displaces O2 from its binding sites in the Hgb molecule, and additionally components of flue gas oxidize Hgb into methemoglobin (Met-Hgb).

HBOCs always require natural Hgb, either from outdated human RBC concentrates, extracted from animal blood or bacteria/yeast/plants. Therefore, the availability of HBOCs is still dependent on people’s willingness to donate blood and the risk of infections remains (e.g., prions). In contrast, disposability of bovine blood is nearly unlimited. Instead of using *Escherichia coli* or *Saccharomyces cerevisiae*, there are now efforts to obtain recombinant Hgb (Varnado et al., 2013) from plants, e.g., from *Nicotiana benthamiana* (Eriksson and Bülow, 2017), and furthermore researchers are focusing more and more on fetal Hgb, which is more stable than adult Hgb (Ratanasopa et al., 2016; Simons et al., 2018).

**Free, unprocessed mammal Hgb that is not encased with any type of membrane is associated with typical problems (Table 3) (Cardenas et al., 2017). Encasing in any type of membrane by crosslinking between monomers (to obtain stable tetramers) as well as tetramers (to affect O2 affinity or size) (Centis and Vermette, 2009), can reduce these side effects. Furthermore increasing knowledge on the**

**TABLE 3**

Typical side effects of unprocessed mammal Hgb

<table>
<thead>
<tr>
<th>Problem</th>
<th>Complication</th>
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<tbody>
<tr>
<td>Dissociation into dimers (Chang, 1988; Elmer et al., 2012)</td>
<td>Overloading the renal tubular cells (renal failure)</td>
</tr>
<tr>
<td>Nitric oxide (NO) stealing property mainly from the endothelial cell layer (Deherty et al., 1998; Olson et al., 2004; Cabreras and Friedman, 2013; Alayash, 2014)</td>
<td>Systemic and pulmonary vasoconstriction (myocardial damage pulmonary hypertension)</td>
</tr>
<tr>
<td>Lack of mediator of thrombocyte aggregation and adhesion (impaired clotting)</td>
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</tr>
<tr>
<td>Local hyperoxia due to decreased oxygen affinity (no diffusion barrier existent) (McCarthy et al., 2001; Alayash, 2014)</td>
<td>Gastrointestinal side effects</td>
</tr>
<tr>
<td>Auto-oxidation (Buohler et al., 2010; Scurtu et al., 2013; Alayash, 2014)</td>
<td>Systemic hypertension</td>
</tr>
<tr>
<td>Nonfunctional hemoglobin</td>
<td>Formation of superoxide ions</td>
</tr>
<tr>
<td>Altering transcriptional activity of heme oxygenase and other antioxidant enzymes</td>
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</tr>
</tbody>
</table>
influence of the size and surrounding shell on the pharmacokinetic properties has helped to decrease side effects (Taguchi et al., 2017). Of note, crosslinking agents and shell material may also cause immunoreactions or increase Met-Hgb formation (Centis and Vermette, 2009).

The novel lugworm Hgb lacks the typical side effects of immunoreaction and inflammation (Rousselot et al., 2006). Other relevant worms are oligochaetes (e.g., earthworms) containing erythrocrurin (Jani et al., 2017; Zimmerman et al., 2017) or worm-like animals such as sipunculans containing hemerythrin (Toma et al., 2018). Erythrocrurin contains 144 globin chains and heme-molecules. The mechanism of oxygen binding is the same as in mammal Hgb (formation of a dioxygen complex). Hemerythrin that does not contain any heme group forms hydroperoxides. HBOC compounds recently under clinical investigation (up to 2017) are reviewed in Njoku et al. (2015) and Gupta (2017).

**Hemopure.** Hemopure is glutaraldehyde-polymerized bovine Hgb. It has a molecular weight of 250 kDa and a $p_{50}$ value of 38 mm Hg (Jahr et al., 2008). The Food and Drug Administration terminated clinical trials in 2008 because of safety concerns (Keipert, 2017). Nevertheless, many phase I–III studies have been performed with Hemopure (Van Hemelrijk et al., 2014; reviewed in Chen et al., 2009; Jahr et al., 2012), but are no longer listed in the clinical trials registry. In other countries, clinical studies have been completed or are ongoing (Supplemental Table 1). Despite the safety concerns, the substance was clinically approved in South Africa in 2001 (Hemopure, 2014; Mer et al., 2016) and Russia in 2012 (Ortiz et al., 2014), and it has been provided to patients with life-threatening anemia in the United States for whom allogeneic blood transfusion was not an option (studies that allogeneic blood transfusion was not an option (studies NCT01262196). Relevant clinical trials with Hemopure/MP4OX and MP4CO are listed in Supplemental Table 3. A retrospective phase II b study (NCT01262196) was criticized, since the authors reported a numerically higher percentage of patients treated with MP4OX were alive and discharged from hospital at day 28 (primary efficacy endpoint) versus controls ($57\%$ vs. $50\%$ $P = 0.18$) (Keipert, 2017), although the study was underpowered (Keipert, 2017).

**Sanguinate.** Sanguinate is bovine Hgb crosslinked to poly(ethylene)glycol to enlarge the molecule and hide it from the immune system. It has a molecular weight of 120 kDa and a $p_{50}$ value of 7–16 mm Hg (Abuchowski, 2016). Sanguinate releases CO to provide antiapoptotic and anti-inflammatory properties (see MP4CO); CO additionally reduces auto-oxidation of Hgb (Abuchowski, 201). Clinical trials with Sanguinate are listed in Supplemental Table 4. Sanguinate is available under an emergency investigational new drug protocol. Thus far, it has enabled survival of only a few patients refusing transfusion fusion due to religious reasons (Posluszny and Napolitano, 2014; Abuchowski, 2016; Resar et al., 2016). The basis for suspending clinical trials [see the meta-
analysis by Natanson et al. (2008)] has been subsequently questioned and reevaluated by many researchers, revealing many methodological flaws and basically pointing out that there is no evidence of any NO-related toxic class effect applicable for every HBOC (Mackenzie et al., 2015, 2017; Dubé et al., 2017). However, Hemopure-induced vasoconstriction is undisputed, and meanwhile the underlying mechanisms (NO scavenging and upregulated endothelin production) have been elucidated (Cabralas and Friedman, 2013; Taverne et al., 2017).

Other HBOCs such as Hemolink or Polyheme have been developed for indications similar to Hemopure (Supplemental Table 2) (Jahr et al., 2012). To date, none of these therapeutic products has entered the clinics due to increased 30-day mortality, hypertension, and myocardial infarction (Njoku et al., 2015).

**Hemospans/MMP4X.** Hemospans is human Hgb conjugated with maleimide-poly (ethylene)glycol. It has a molecular weight of 96 kDa and a $p_{50}$ value of 6 mm Hg (Winslow, 2006). Its Hgb content of 4.2 g/dl is too low to solely supply an organism with oxygen. Therefore, Hemospans, later named MP4OX, was developed as an O$_2$ therapeutic to improve the oxygen supply rather than to fully replace blood (Jahr et al., 2012). Injection of MP4OX caused a low antioxidant response and a tendency to extravasation into tissue in a rat model (Terraneo et al., 2017). In recent years, MP4OX was refined as a therapeutic treatment for special occasions such as in the treatment of sickle cell anemia. CO prevents and reverses polymerization of hemoglobin-S and thus distortion of sickled erythrocytes (Keipert et al., 2016). By using MP4OX, the pain, severity, and duration of a sickle cell anemia crisis can be reduced. Additionally, low-dose CO also acts as a signaling molecule to reduce inflammation and O$_2$ requirement as well as to prevent apoptosis in patients (Keipert et al., 2016). The scientific and medical underlying mechanisms have been studied. MP4OX leads to induction of nuclear factor-erythroid 2 p45-related factor-2 and hepatic hemeoxygenase-1 as well as inhibition of the nuclear factor kappa-light-chain enhancer of activated B-cells observed in an animal model (Belcher et al., 2013), and has been reviewed in Simoni (2017). After unloading CO, the compound is oxygenated in the lungs and thereby transforms into MP4OX. The effects of MP4OX have been further investigated in animal models of sickle cell anemia (Tsai et al., 2015). Relevant clinical trials with Hemospans/MP4OX and MP4CO are listed in Supplemental Table 3. A further investigated in animal models of sickle cell anemia (Tsai et al., 2015). Relevant clinical trials with Hemospans/MP4OX and MP4CO are listed in Supplemental Table 3. A retrospective phase II b study (NCT01262196) was criticized, since the authors reported a numerically higher percentage of patients treated with MP4OX were alive and discharged from hospital at day 28 (primary efficacy endpoint) versus controls ($57\%$ vs. $50\%$ $P = 0.18$) (Keipert, 2017), although the study was underpowered (Keipert, 2017).

**Hemo2life.** Hemo2life is Hgb extracted from lugworms, which is not packed into erythrocytes or any other membrane. It has a molecular weight of 3600 kDa and a $p_{50}$ value of 7 mm Hg (Mallet et al., 2014). One molecule transports up to 156 molecules of O$_2$, which is 38$\%$ more than mammal Hgb. Hemo2life has natural superoxide-dismutase–like activity that compensates for oxygen-related radicals (Mallet et al., 2014). A clinical phase I open label trial in kidney transplantation [cold storage in Belzer (University of Wisconsin) versus hypothermic machine perfusion with Belzer (University of Wisconsin) + Hemo2life before transplantation] was completed in February 2018 (NCT02652520). Furthermore, Hemo2life improved static storage of donor hearts prior to transplantation in a preclinical animal model (Teh et al., 2017) and early graft function after hypothermic static preservation after prolonged cold ischemia of a pig lung (Glorion et al., 2018).

**In the Pipeline/Preclinical Development**

Many new research approaches have evolved within the last few years, all of which still are in preclinical status. The four most advanced compounds are OxyVita, HbVesicles, ErythroMer, and HemoAct.
OxyVita (OxyVitaHb). OxyVita is bovine Hgb that is inter- and intramolecularly crosslinked, leading to a homogenous globular-like molecule. It has a molecular weight of 17 kDa. OxyVita has two subtypes: OxyVita Hb and OxyVita HbCO (Wollocko et al., 2017). Importantly, the Hgb tetramers are linked to each other via amide bonds without any linker molecule (Wollocko et al., 2017). Normally, toxic linker molecules such as glutaraldehyde are necessary for these linking reactions.

The release of free heme-iron into the circulation is low so that toxic side effects are minimized (Wollocko et al., 2017). OxyVita was tested in different preclinical studies, among them a pre-hospital setting (mimicking initial medical treatment of severely injured patients prior to hospital) of hemorrhagic shock in rats (Jahr et al., 2012). No other HBOC developed thus far has provided success in a battle-field model of severe hemorrhage (Jahr et al., 2012).

HbVesicles. HbVesicles are human Hgb encapsulated by a biocompatible liposome (Azuma et al., 2017). They are 250–280 nm in diameter, decorated with PEG5000 and have a $P_50$ value between 9 and 30 mm Hg that is adjusted with pyridoxal phosphate (Sakai, 2017). Depending on the Hgb core (Hb-CO or HbO2) HbVesicles can be used as a CO or O2 carrier.

ErythroMer. ErythroMer is human Hgb surrounded by a NO-attenuating polymer shell (mainly 3, Hb-HSA3) (Haruki et al., 2015). The intravascular exchange and a rat hemorrhagic shock model (Pan et al., 2016; Sakai, 2017) has been studied in a murine hemodilution model of 70% blood volume (Riess, 2001). Compared with water, PFCs exhibit high fluorine bond, no toxic metabolites in the body are formed (turned to markets in the United States and Europe (Latson, 2010). Perftoran is now produced under standard manufacturing practice (branch name Vidaphor) with the aim of introducing it to markets in the United States and Europe (Latson, 2017; Vidaphor, 2017).

Compounds under Clinical Investigation

Currently there is only one PFOC, Perftoran (Perftec) that has been approved for human clinical use in Russia, Mexico, Kazakhstan, Kyrgyzstan, and Ukraine (Castro and Briceno, 2010). Perftoran is now produced under standard manufacturing practice (brand name Vidaphor) with the aim of introducing it in the treatment of flue-gas poisonings or gas embolism/decompression sickness (Spiess, 2009). Typical side effects of PFOCs are a decrease in the mean arterial pressure, lung damage, thrombocytopenia, and blued-like symptoms, in addition to poor emulsion stability and long organ retention time (Lowe, 2003; Hosgood and Nicholson, 2010).

PFOCs were also used in the context of organ preservation such as autologous blood, or conventional colloid when reaching a partial pressure exceeded. The use of PFOCs allows for RBC concentrate-free normothermic perfusion, and thus for organ regeneration prior to transplantation (Fig. 3).

Compounds under Clinical Investigation

Currently there is only one compound that has been investigated in clinical trials: Oxygent. Oxygent is a 60% PFC emulsion (58% perfluorooctylbromide, 2% perfluorodecylbromide and egg-yolk phospholipids). Oxygent has been known since the 1990s and has been studied in several clinical studies (Castro and Briceno, 2010; Spahn and Keipert, 2017). Among these studies, especially in two phase III studies, the potential of Oxygent was successfully shown. The first study investigated patients undergoing orthopedic surgery, who were preoperatively normovolemic hemodiluted with colloid to a target Hgb of 9 g/dl. The normovolemic hemodilution was followed by either treatment with Oxygent, autologous blood, or conventional colloid when reaching a predefined transfusion trigger. Patients in the Oxygent group showed the longest duration of transfusion-trigger reversal, thus Oxygent was more effective than blood or colloid in stabilizing the patients and avoiding additional transfusions (Spahn et al., 1999). These results were
confirmed in a second clinical trial in patients undergoing noncardiac surgery. Preoperative hemodilution was followed by two doses of Oxygen. Oxygen reduced the need for blood transfusions compared with the standard care (no hemodilution; intraoperative transfusion of RBC concentrates if indicated) (Spahn et al., 2002).

However, in 2002, Oxygen was abandoned because of safety issues in a phase III coronary artery bypass grafting trial (Keipert, 2006). In 2017, Oxygen was reproduced by Double Chrane, licensed and approved for clinical studies in China (J. Liu, personal communication).

With the promising PFOC Oxycyte a successful phase II study was completed in 2008 in patients with traumatic brain injury (NCT00174980) (Fabian, 2011). Another phase II study on safety and efficacy of Oxycyte was started in 2009 (NCT00908063), but was terminated by the sponsor in 2014 due to lack of patient enrollment (Oxygen Biotherapeutics Announces Halt of Oxycyte Phase IIb Traumatic Brain Injury Trial, 2014). The sponsor abandoned the substance.

In the Pipeline/Preclinical Development

Albumin-derived perfluorodecalin-filled nanocapsules showed promising results in a first in vivo toxicity study (Wroblen et al., 2017a) and protected a Langendorff heart (rat) during massive ischemia (Wroblen et al., 2017b). Similarly, a novel PFC emulsion increased myocardial O2 delivery, improved cardiac function, and generated a more physiologic redox state in a Langendorff heart (rabbit) compared with perfusion without the PFC emulsion (Kuzmiak-Glancy et al., 2018).

Other Products

Besides engineering Hgb or the surrounding shell, there are other ideas such as simply using the O2 binding porphyrin structure of Hgb [e.g., embedded in cyclodextranes (HemoCD)] (Kitagishi et al., 2017) or completely using other materials such as cobalt porphyrins (where the central iron ion in the O2-binding structure of Hgb is replaced by cobalt) (Neya et al., 2014; Shen et al., 2016). Furthermore, there have been attempts to directly introduce O2 into particles, thus for the first time permitting for a safe and effective intravenous injection of O2 gas, which locally increases pO2 very rapidly (Seekell et al., 2016; Black et al., 2017). All of these substances (Supplemental Table 7) are still in the preclinical stage.

SCs: Specifications, Peculiarities, and Limitations

In 2006, a new cell source, which was able to differentiate into all cell types of the endo-, ecto-, or mesodermal lineages, was found by Takahashi and Yamanaka (2006). Induced pluripotent SCs can be generated from different somatic cell sources by overexpression of specific transcription factors, e.g., HOXB4 (Takahashi and Yamanaka, 2006; Schiedlmeier et al., 2007; Yu et al., 2007). There are two approaches to using SCs within the context of AOCs (see Fig. 2C): 1) differentiation of SCs into RBC concentrates, and 2) differentiation of SCs into various target cells in an oxygenated environment. These two approaches will be described in the following sections.

Differentiation of SCs into RBC Concentrates. The major challenges to using SCs are the low retention and engraftment of transplanted cells and the adverse effects of inflammation and immunoreactions when allogeneic or xenogeneic cells are used (Van Veen and Hunt, 2015). Giarratana et al. (2011) elucidated the quality of donated hematopoietic SCs from human donors that were developed in culture into RBC concentrates in a proof-of-concept study. The difficulty was not only the viability and cell deformability, but the requirement of an improved production protocol of cultured RBC concentrates without feeder cells at reasonable costs (Giarratana et al., 2013). To provide the required huge amount of RBC concentrates, upscaling has to be improved. In addition, artificial RBC generation has to result in nucleus-free erythrocytes that contain only adult Hgb and no fetal Hgb. An advantage of this technique is that cells can be produced in a patient-specific manner according to the blood phenotype of the recipient.

Differentiation of SCs into Various Target Cells in an Oxygenated Environment. To improve SC differentiation, the O2 supply to hypoxic areas should be high and the O2 gradient formation should be reduced. The differentiation potential and cell viability should be preserved and the extracellular matrix microenvironment should be kept intact. Oxidative stress and the generation of reactive oxygen species are not desired, since hematopoetic SCs might die (Jung and Choi, 2014). Therefore, the support of AOCs in SC exploitation displays an ideal combination.

Le Pape et al. (2017) evaluated the ability of HEMOXCell, a HBOC, to carry O2 in culturing human bone marrow mesenchymal SCs in vitro for three-dimensional culture applications in human platelet lysate–supplemented media. HEMOXCell provoked a cell growth rate induction of 25%, while the mesenchymal SC phenotype was preserved and typical differentiation properties were maintained. In a subsequent study, Le Pape et al. (2018) developed a perfusion culture method to provide a similar distribution of nutrient and O2 throughout the artificially engineered tissue, specifically for the setting of osseointegration in dental implant surgery. HEMOXCell was beneficial in the development of mesenchymal stem cells into allogenic bone substitute (Le Pape et al., 2018).

Similar effects were observed using the PFOC Fluosol-DA. The slopes of the single-dose radiation survival curves for intestinal epithelial cells and spermatogenic SCs in mice breathing air or O2 were not significantly altered by the administration of Fluosol-DA 10 minutes before irradiation, and the doses to achieve an isoeffect were altered by 1.03 or less. When mice were challenged with intravenously injected Fluosol-DA tumor cells, 24 hours after treatment with Fluosol-DA no increase in the number of artificial pulmonary metastases was observed (Mason et al., 1985).

Furthermore, Tang et al. (2017) created an oxygenated environment using a nanogel structure: they encapsulated human cardiac SCs in thermosensitive poly(N-isopropylacrylamide-co-acrylic acid) nanogel in murine and pig models of myocardial infarction. In contrast to conventional SCs, encapsulated human cardiac SCs did not induce an inflammatory reaction or T-cell infiltration in immunocompetent mice, whereas xenogeneic human cardiac SCs injected in saline induced the immune response. The cardiac function was maintained and the scar sizes were reduced. The authors concluded that “thermosensitive nanogels can be used as a carrier: the porous and convoluted inner structure allows nutrient, O2 and secretion diffusion, but can prevent SCs from being attacked by immune cells” (Tang et al., 2017).
A recent publication by Cantaluppi et al. (2018) reported that the addition of PFCs to viable renal tubular epithelial cells in a renal-assist device led to the differentiation of those cells toward renal progenitor cells.

Discussion—Outlook—the Future of AOCs

Looking at the research studies conducted over the years, it can be seen that many improvements have been made in the development of AOCs (HBOCs, PFOCs, and SCs). However, these studies have also revealed the major challenges in relation to inflammatory reactions, conservation, and O2 affinity that have to be managed in order to fully provide a clinically useful AOC. Economic aspects have often played a role and may have caused a delay or even stopped the further development of initially successful compounds. Recently, the safety profile of the HBOC Hemopure was reevaluated since new, very promising HBOCs such as Sanguinate and Hemo2Life have emerged from laboratories. Additionally, the biochemical and physiologic properties of different HBOCs have been compared in a recent study by the US Food and Drug Administration in order to facilitate reevaluation since new, very promising HBOCs such as Sanguinate and Hemo2Life have emerged from laboratories.

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Performed data analysis: Ferenz, Steinbicker.
Wrote or contributed to the writing of the manuscript: Ferenz, Steinbicker.

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