ARTIFICIAL OXYGEN CARRIERS

Umetni prenašalci kisika

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Abstract

The artificial oxygen carrying red cell substitutes are currently under development for use in a variety of surgery and trauma-related clinical conditions. The need for such fluids continues to be driven by the shortage of donor blood, the complex logistics of blood banking, the risk of virally transmitted diseases, current transfusion practices, and the projected increased demand for blood products in the future. The effort to develop a replacement for the red cell component has evolved over the last century and has presented a number of significant challenges including safety and efficacy concerns. Recent progress in understanding the fundamental interactions of haemoglobin (Hb) with the body at the molecular, cellular and tissue levels has led to the production of improved red cell substitutes suitable for clinical testing. Currently, few products are being tested for a variety of applications including trauma, surgery, sepsis, cancer and anaemia. Although some of these trials were unsuccessful, the majority of the available products exert no toxicity or only low level side effects. Encouraging results in early clinical trials with oxygen-carrying fluids support further development of these products and have increased the hope that a usable oxygen-carrying fluid will soon be available for clinical use. The purpose of this review is to provide up-to-date information on the status of these products with special emphasis on pre-clinical and clinical experience.

Introduction

The search for a blood substitute began almost simultaneously with the first attempts to establish the transfusion of human blood as a form of medical treatment. Beside animal blood, all manner of solutions have been tried as the blood substitutes, including wine and milk (1). When blood transfusion was recognized as a practicable clinical procedure, efforts to maintain adequate blood supply and it's complex logistics were the main reasons for further investigation of blood substitutes. After the recognition of risks related to transfusion transmitted diseases, especially of HIV infection in the early 1980s, blood safety became the main force that accelerated the research of this field (2). It was additionally supported by a projected increased demand for blood products in the future. Considering all given difficulties related to current transfusion therapy and blood supply, the potential benefits of replacing some or all blood products by substitutes produced on a large scale, are therefore both medical and financial (3). Currently some blood substitutes for erythrocytes and platelets are in clinical trials. In addition, recombinant haematopoietic cytokines (erythropoietin, thrombopoietin, G-CSF...) were introduced in practice recently as a "virtual blood substitutes" that stimulate the production of blood cells in bone marrow.

Artificial oxygen carriers

In this article the term " artificial oxygen carriers" (AOCs) is used for the group of solutions that have the ability to transport oxygen and are developed as red cell substitutes capable to increase oxygen carrying capacity of blood.

There are three main approaches in development of AOCs (4). One is represented by completely artificial substances named perfluorocarbons (PFC) in which

oxygen is dissolved. The second is based on modified haemoglobin (Hb) solutions that transport gases in the same manner as Hb in the erythrocytes. Liposome-encapsulated Hb or "artificial red cells " represents the third approach.

Although safety and supply adequacy have been the primary goals of AOCs, they also must be able to carry, load and unload oxygen and CO2 within the useful intravascular half-life and physiological conditions. Their rheological, osmotic and oncotic properties are also important characteristics (Table1) (5).

Table1. Characteristics for artificial oxygen carriers

Characteristics	Requirement
Efficacy	High capacity for O_2 and CO_2
	Physiological gas exchange
	Suitable intravascular half-life
	Approximately isoncotic and isosmotic
	Favorable rheological properties
Safety	Minimal infectious risk
	Minimal non infectious risk
	Non-toxic
	Limited extraneous physiological effects
Logistics	Stability
	Availability
	Abundance
	Low cost

Perfluorocarbons

The PFC are synthetic organic chemicals developed during the Second World War during a search for an inert fluid for handling with highly reactive uranium isotopes (5). The carbon backbone of this cyclic or linear molecules is extensively substituted with fluorine atoms. They are chemically and biologically inert due to the extremely strong chemical bonds between fluorine and carbon atoms and the relative protection of intra molecular carbon bonds by the water excluding fluorine atoms (Figure 1.).

Additionally, they are capable of disolving large volumes of non polar gases such as O_2 and CO_2. Their oxygen delivering capacity was demonstrated in 1966 by experiment in which a mouse was kept alive while immersed in oxygenated PFC solution (6). The oxygen content in PFCs is linearly proportional to the oxygen tension in its environment so the oxygen "loading" and "unloading" is driven by pressure gradient, diffusing from areas of high oxygen tension to areas where it is low. Therefore, if the pO_2 can be increased, greater amounts of O_2 can be carried.

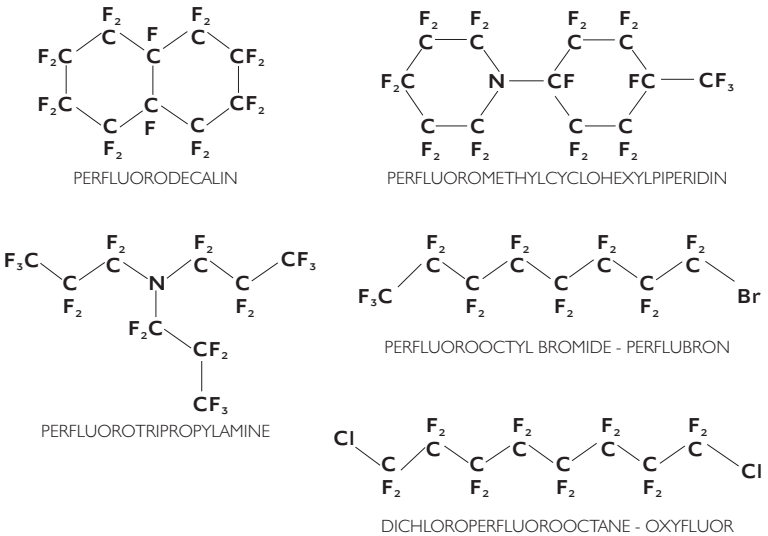

Figure 1. Some perfluorocarbon molecules.

The stable emulsions of PFCs can be formed with the addition of surfactants and stabilizers such as lecithin. The necessity to be emulsified and toxicities considerations limits the maximum concentration of PFC that can be achieved in blood. Consequently, the theoretical oxygen carrying capacity of PFC is practically not achievable, but it is greater than that of plasma or intravenous replacement fluids and lower than that of whole blood (Figure 2) (5).

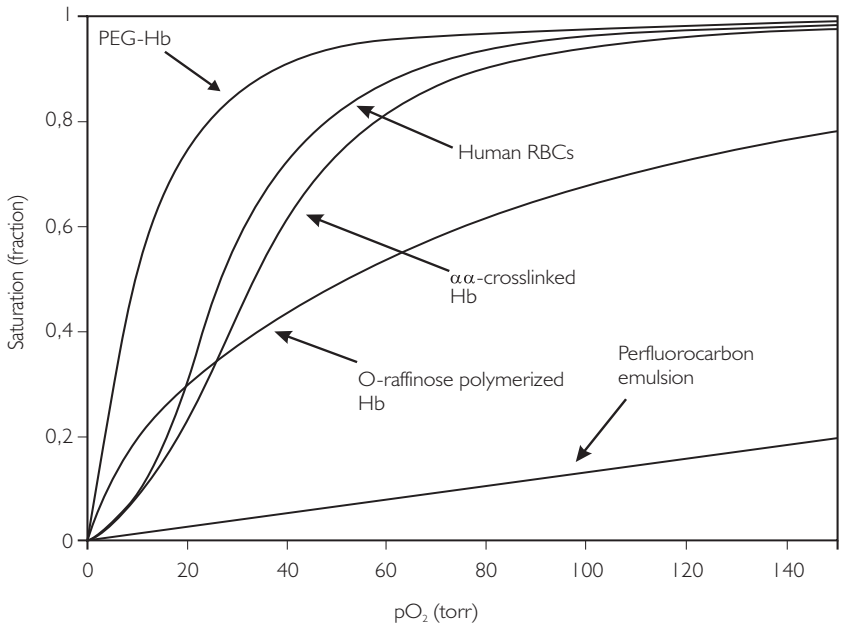

Figure 2. **Oxy haemoglobin dissociation curves for red blood cells and AOCs.**

Following intravenous injection into mammals, the droplets of the emulsion are removed from the circulation by the reticuloendothelial system (RES), usually in a few hours. They are eventually exhaled via lungs, but they may remain in the RES for a prolonged period before excretion. Some of the earlier PFCs studied remained in the RES for months, newer formulations are excreted within several days (7).

Table2. Perfluorocarbon emulsions in clinical trials

Product (manufacturer)	Perfluorocarbon	Trial level	Application
Fluosol-DA (GreenCross/Alpha)	Perfluorodecalin Perfluoropropylamine	Phase II (discontinued) Approved (withdrawn)	Acute blood loss PTCA
Oxygent (Alliance)	Perflubron	Phase II	CABG-ANH Surgery-acute blood loss
Imagent (Alliance)	Perflubron	Phase III (approved)	Surgery-ANH GI-imaginig
Liquivent (Alliance)	Perflubron (neat)	Phase Ib/II PhaseII/III (discontinued)	Liquid ventilation -IRDS Liquid ventilation -pedi and adult ARDS
Oxyfluor (HemaGen/PFC)	Perfluorodichlorooctane	Phase II (discontinued)	Surgery, neuroprotectant/bypass

PTCA- percutaneous translumminal coronary angioplasty; CABG – coronary artery bypass graft; ANH – acute normovolemic haemodilution; GI – gastrointestinal; IRDS – infant respiratory distress syndrome; ARDS – adult respiratory distress syndrome.

The synthetic source permits the large scale production of PFCs with relatively low manufacturing costs. The shelf life of current preparations is prolonged to 24 hours. Because of their non biological origin they have minimal infectious risks and immunogenecity. However, the requirement for emulsification/stabilisation and heterogeneous particle size are disadvantages that can affects their clearance by the RES (secretion of cytokines and a "flu like" syndrome) and cause the complement activation (8). The need for elevated oxygen tension in order to load these preparations with the useful amount of oxygen together with the rapid plasma clearance also imposes limitations.

Some of the PFC emulsions that are in clinical trials are listed in Table 2. The first PFC that reached clinical trial was Fluosol-DA (a mixture of two PFCs). It was approved and licensed for the percutaneous transluminal coronary angioplasty (PTCA) for oxygenation of the distal vascular bed (9). However, it was withdrawn from the market in 1994 because of poor product sales due to the difficult preparing of emulsion before administration, short product stability after reconstitution and because of improvements of the catheter technology that permitted blood to perfuse through the catheter lumen during balloon inflation. Additionally, the clinical trials did not show improvement in clinical outcomes of patients with acute blood loss after treatment with Fluosol-DA (9).

The perfluorooctyl bromide (perflubron) emulsions are stabilized with lecithin and can be stored at room temperature. The formulation of perflubron named Oxygent is in phase II/II clinical trials for use in the acute normovolaemic haemodilution (ANH) where its administration is intended to permit more extreme haemodilution and for use in acute intra-operative blood loss as the "bridge" to preserve O_2 transport until conventional erythrocytes transfusion can be given. Oxygent was also used to sensitise solid tumours to irradiation. It is known that O_2 potentiates the effect of irradiation and chemotherapy on malignant cells and its absence in hypo-perfused or hypoxic areas of a tumour limits the effectiveness of these therapies. The effect can be augmented by improving O_2 delivery to the tumour with PFCs (3). Bromine in the perflubron emulsions renders it radioopaque so a preparation named Imagent was approved in gastrointestinal radiography (10). The Imagent US is a formulation of perflubron for use as an ultrasound contrast medium (11) .

One of the most novel applications of the PFCs is in the partial liquid ventilation where the PFC emulsion is instilled neat into the patient's lungs, partially filling the alveoli. There it serves as a source of the readily available oxygen and as a surfactant helping to expand the alveoli and improve gas diffusion. The partial liquid ventilation with the perflubron emulsion Liquivent was investigated in the acute respiratory distress syndrome but because of high mortality in the treatment group, the trial was discontinued (12).

Perfluorodichlorooctane named Oxyfluor showed good oxygen delivering capabilities in the treatment of shock and surgical bleeding in animal models. It was also efficient in removing - dissolving the micro bubbles that form in the patients undergoing cardiopulmonary by-pass and preventing the micro embolization in the brain with the neuropsychiatric consequences (13).

The PFC emulsions may be used in the future in the concept of augmented ANH with low preoperative Hb levels where PFC emulsion is given to maintain oxygen delivery during surgery and the autologous blood is subsequently retransfused in the postoperative period. The ability to expose PFCs to 100% O_2 through oxygenator also affords a ready means of loading it with the large amounts of O_2 and use it during open hearth surgery. Additional uses of PFC emulsions include treatments of diseases with compromised tissue oxygenation, such as cerebral or myocardial ischaemia, air embolism and also in trauma surgery to maintain tissue oxygenation as long as the allogeneic blood is not available.

Haemoglobin based artificial oxygen carriers

The concept of developing a Hb solution as the oxygen carrying red cell substitute is based on the capability of Hb to bind and release oxygen and to survive outside of the red cells. Additionally, so called "stroma free" Hb is without membrane antigens, therefore no compatibility testing is necessary before its application. The efficacy of the Hb solution was demonstrated in 1934 by Amberson et al. in the experiment where the cats survived after their blood was gradually removed and replaced with the crude red cell lysate (14). The clinical trials have demonstrated significant toxicities of early Hb solutions so further efforts were made how to harvest the Hb protein and prepare it in a form that would be safe and useful in a clinical setting (15).

The starting material for Hb solutions can be obtained from haemolysed human or bovine red cells or generated by recombinant or transgenic technology (Table 3)(15).

Table 3. Hemoglobin sources

-Human
-Bovine
-Recombinant
-Transgenic

Inside the red blood cells, the Hb molecules are linked in tetramers comprising four subunits: two α subunits and two β subunits. When removed from the red cell, Hb tetramer molecules dissociate into dimers and are quickly filtered from the circulation by the kidneys where they can directly damage renal tubular cells. By the modification of Hb molecules the dissociation of the tetramers can be prevented that results in the prolonged half –lives of Hb solutions to 18-58 hours and in averted renal toxicity. Free Hb dimers and tetramers can diffuse in the vascular wall where they can bind nitric oxide (NO) and consequently release its constitutive relaxing effect and produce vasoconstriction with hypertension (16). Their diffusion through the vascular wall was also solved by polymerisation and by the increase of molecular weight of Hb molecules. High oxygen affinity occurs because 2,3 DPG is lost from the Hb tetramers outside of erythrocyte. The dissociation curve of such Hb molecules is shifted to the left and a greater degree of tissue hypoxia is required to induce oxygen unloading. This was prevented with the modification of Hb molecules by chemical methods or site-directed mutagenesis of the recombinant Hbs as well as by the usage of bovine Hb solutions which Hb dissociation curve resembles that of the intraerythrocytic Hb (17,18). Cell free unmodified Hb auto-oxidizes to methaemoglobin that has no ability to carry oxygen. Oxygen free radicals formed in the auto-oxydation process can damage endothelial cells. Auto-oxidation can also be prevented by site-directed mutagenesis in recombinant preparations or by Hb polymers that have chemically linked methemoglobin reductase to tetramers. The observed low immunogenicity of animal and human Hb solutions is under clinical investigations (19). The murine studies also showed that Hb binds to bacterial endotoxin and potentiated its lethal effect so the safety of the use of Hb solutions for patients with bacterial sepsis or endotoxemia has to be evaluated (20).

The efforts to eliminate toxicities of Hb solutions were concentrated on attempts to stabilize the simple unmodified tetramer by chemical modifications (Table 4)(21).

Table 4. Hemoglobin preparations

-Unmodified tetramer
-Conjugated tetramer
-Cross-linked tetramer
-Polymer

A conjugated tetramer involves the binding of a macromolecules such as polyethylene glycol (PEG), dextran or polyoxyethylene to Hb tetramers and formation of a large molecules (22). A cross-linked tetramer has intra molecular chemical links between $\alpha\beta$ globin dimers that stabilize the native Hb molecule in the tetrameric

structure. The linkage can be formed between two β or two α globin proteins at the highly reactive sites in the central part of the Hb molecule (23,24). The polymerization results in a variety of polymers composed of different number of tetramers linked together (Figure 3).

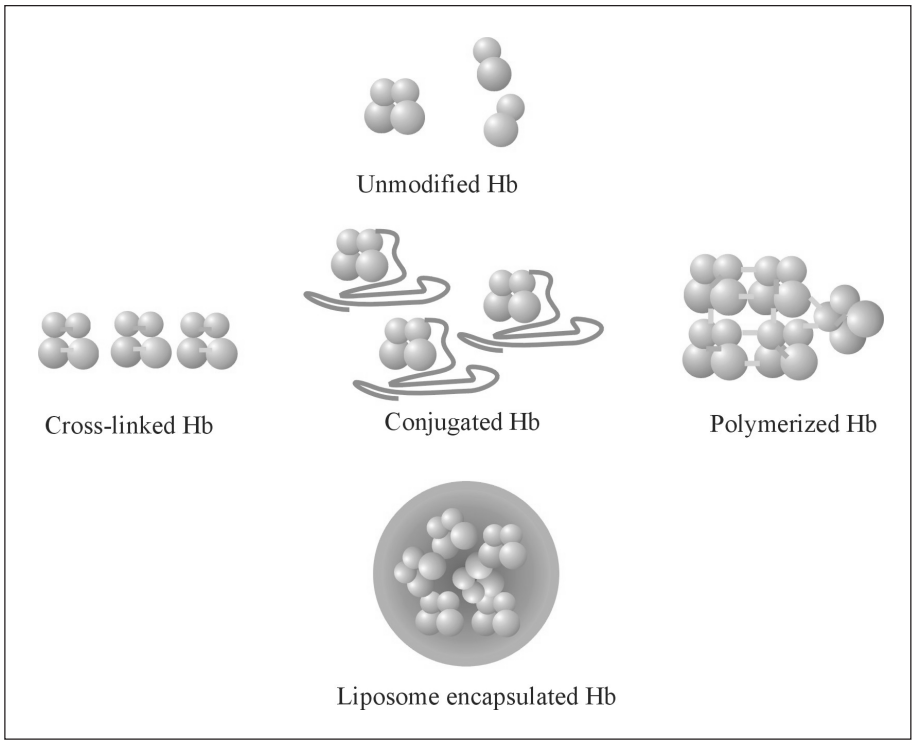

Figure 3. Haemoglobin based artificial oxygen carriers

The Hb based artificial oxygen carriers have many potential advantages as well as disadvantages. The obvious advantage is the good oxygen carrying capacity at the physiological pO_2 levels. The effects of low viscosity of Hb solutions on the oxygenation low flow or obstructed areas not accessible to erythrocytes and high oncotic pressure on increase of cardiac output are under investigation (25). In the absence of erythrocyte antigens for application of Hb solutions, compatibility testing is not necessary. The logistic problems are also reduced by stability and the long shelf-life of Hb solutions. Sterilization and virus inactivation of Hb solutions can be done by the same procedures as for plasma derivatives.

During the last seven years four products received approval for Phase III trials (PolyHeme, HemAssist, Hemopure and Hemolin) and Oxyglobin (Biopure) was approved for veterinary use in 1998 (Table 5) (25). The Phase III clinical investigations of HemAssist preparations were discontinued because their infusions were a statistically significant predictor of worse outcome in the patients with acute ishaemic stroke or traumatic haemorrhage (26). Although the outcome of these pioneering efforts were disappointing it gave us insight into the pathophysiology underlying these events that may enable development of a formulation that will be safe and effective.

Table 5. Haemoglobin based oxygen carriers in clinicla trials

Product (manufacturer)	Perfluorocarbon	Trial level	Application
PolyHeme (Northfield)	Polymerized human Hb (glutaraldehyde)	III	Trauma, surgery
Hemopure (Biopure)	Polymerizied bovine Hb (glutaraldehyde)	III II	Orthopedic and cardiac surgery Surgery Sickle cell crisis Haemodilution Trauma erythropoiesis
Oxyglobin (Biopure)	Polymerizied bovine Hb (glutaraldehyde)	approved	veterinary
Hemolink (Hemosol)	Polymerizied human Hb (oxidized O-raffinose)	III II	Cardiac surgery Surgery Hemodilution dialysis
PHP (Apex Bioscience)	Surface-modified (PEG) Human Hb	III	NO induced hypotension
PEG-Hb (Enzon)	Surface-modified (PEG) Bovine Hb	Ib	Solid tumour radiosenzitization
Optro (Somatogen Baxter)	Recombinant	I II (all terminated)	Erythropoiesis - end stage renal disease ANH, acute blood loss-surgery

PolyHeme (polymerized human Hb) was investigated in acute blood loss in trauma and surgery where large amounts up to 20 units (50g of Hb each) and the transfusion of blood was reduced in patients randomly selected to receive PolyHeme (27). Hemopure (glutaraldehyde polymerized bovine Hb) and Hemolink (oxidized O- raffinose polymerized human Hb) are in Phase III clinical trials in cardiac surgery and orthopedy. In a recent case report was described a patient with acute autoimmune hemolitic anemia (AIHA) who required extensive RBC support but was refractory to therapy. The patient received 11 units of Hemopure (each containing 30 g of Hb) over the course of several days, until the AIHA episode remitted in response to cyclosporine administration (28). PHP (PEG conjugated Hb) was studied in the therapy of NO induced hypotension and other bovine PEG conjugated Hb- PEG-Hb showed first good results in the solid tumour radiosensitization. Three preparations (Hemolink, Hemopure and Optro) have been observed to have an erythropoietic effect in vivo (29).

Liposome-encapsulated Hb

The promise of encapsulation systems for the sequestration of Hb has been the long-held belief that encapsulation more closely mimics nature's strategy for circulating Hb, and could alleviate Hb based toxicities and increase persistence in circulation. The first idea of using encapsulated Hb as a artificial oxygen carrier was proposed in 1957 by Chang (30). Various polymers have been proposed to deliver Hb. One approach toward the encapsulation of Hb has been to employ biodegradable, biocompatible vehicles such as phospholipid vesicles, or liposomes. The majority of encapsulation work with Hb over recent years was focused on liposome encapsulated Hb with demonstrations of efficacy and safety in the isovolemic and hypovolemic exchange models, hemodynamics, circulation persistence and distribution in

organs, processing methods, long term storage through freeze-drying, and serum changes as well as histopathological consequences following administration in small animals. The data collected thus far indicate that encapsulation of Hb does significantly alter many of the traditionally observed effects following the administration of cell free Hb solutions. Liposome encapsulated Hb circulates for 20-24 hours in small animals and principally distributes to the liver and spleen. The significant accumulation of liposome encapsulated Hb in these organs poses new questions for short and long-term effects on the reticuloendothelial system and macrophage function which are currently under investigation. In addition, transient haemodynamic and serum changes have been observed following the administration of liposome encapsulated Hb. Many of these are similar to the effects observed following the administration of liposomes without intravesicular Hb and are dictated by liposome parameters such as surface charge and character, size, and lipid composition. Finally, fundamental large scale production issues such as encapsulation efficiency and particle size distribution must be optimized to facilitate the commercial development of encapsulated haemoglobin. So the liposome-encapsulated Hb preparations has not yet reached the clinical trial level.

Present and future of the artificial oxygen carriers

It is now becoming apparent that beside the artirficial oxygen carriers can be used not only in the case of acute anaemia or extreme normovolemic haemodilution but their unique characteristics suit them for a new applications beyond the scope of erythrocytes. Because of their origin, some of AOCs are acceptable for people who refuse blood transfusion because of religious beliefs or can be potentially used to increase blood supply in the population with endemic infection. The absence of red cell antigens makes them suitable for use in patients with the multiple red cell antibodies. The potential applications of artificial oxygen carriers are listed in the Table 6.

The animal and human studies confirmed the ability of AOCs to deliver usefull quantities of oxygen to the tissues but their efficacy is not easy to demonstrate because of the absence of generally accepted means of assessing the efficacy of the erythrocytes transfusions. The clinical outcomes of patients receiving AOCs compared to those receiving conventional transfusions could be the relevant endpoints (ie. avoidance of erythrocytes transfusions or reduction of mortality) (31). In the safety assessment of the artificial oxygen carriers besides their lower infectious risks, the non-infectious risks must also be included.

Table 6. Potential applications of artificial oxygen carriers

- Acute blood loss - trauma, surgery
- Acute blood loss - Jehovah's Witnesses, multiple red cell antibodies/rare blood type, endemic infection in donor blood supply
- Extender in acute normovolemic hemodilution
- Septic shock
- Anti-ischaemic-sickle cell crisis, PTCA, MI, cardiopulmonary by-pass, vaso-oclusive stroke
- Ex vivo organ/tissue preservation
- Neuroprotectant - cardiopulmonary by-pass
- Sensitizer for chemo-and radiotherapy, imaging
- Partial liquid ventilation
- Erythropoiesis

Conclusion

The development of AOCs has made rapid progress in recent years. All of them are capable of transporting and delivering useful quantities of oxygen. However, they have not reached approval for routine use, primarily because of difficulties in the

validation of their efficacy and occurrence of unpredictable adverse effects after application in humans. Nevertheless, it is likely that some of them will be licensed for clinical use in the near future, either as the red blood cell substitute or for some other application. The knowledge of their practical and theoretical basis as well as difficulties with their clinical implementation can help us to be prepared for their potential role in the future.

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