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REVIEW

Current perspectives of artificial oxygen carriers as red blood cell substitutes: a review of old to cutting-edge technologies using in vitro and in vivo assessments

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Abstract

Background Several circumstances such as accidents, surgery, traumatic hemorrhagic shock, and other causalities cause major blood loss. Allogenic blood transfusion can be resuscitative for such conditions; however, it has numerous ambivalent effects, including supply shortage, needs for more time, cost for blood grouping, the possibility of spreading an infection, and short shelf-life. Hypoxia or ischemia causes heart failure, neurological problems, and organ damage in many patients. To address this emergent medical need for resuscitation and to treat hypoxic conditions as well as to enhance oxygen transportation, researchers aspire to achieve a robust technology aimed to develop safe and feasible red blood cell substitutes for effective oxygen transport.

Area covered This review article provides an overview of the formulation, storage, shelf-life, clinical application, side effects, and current perspectives of artificial oxygen carriers (AOCs) as red blood cell substitutes. Moreover, the pre-clinical (in vitro and in vivo) assessments for the evaluation of the efficacy and safety of oxygen transport through AOCs are key considerations in this study. With the most significant technologies, hemoglobin- and perfluorocarbon-based oxygen carriers as well as other modern technologies, such as synthetically produced porphyrin-based AOCs and oxygen-carrying micro/nanobubbles, have also been elucidated.

Expert opinion Both hemoglobin- and perfluorocarbon-based oxygen carriers are significant, despite having the latter acting as safeguards; they are cost-effective, facile formulations which penetrate small blood vessels and remove arterial blockages due to their nano-size. They also show better biocompatibility and longer half-life circulation than other similar technologies.

Keywords Red blood cell substitutes · Artificial oxygen carriers · Old to cutting-edge technologies · In vitro and in vivo assessment

Introduction

Blood supplies (O_2) to tissues and organs via red blood cells (RBCs) and removes carbon dioxide (CO_2) from the body; thus, it is an important body fluid in humans and animals. It is impractical to live without blood (Sarkar 2008; Moradi et al. 2016). Accidents, surgeries, and other causalities cause major blood loss. Furthermore, hemorrhagic shock, along with trauma and acute coagulopathy, increases the mortality rate in austere environments, such as battlefields and remote civilian localities (Nosé 2004; Castro and Briceno 2010; Sen Gupta 2019). Allogeneic red blood cell (RBC) transfusions are used to resolve this situation and are the most common method of resuscitation for hospitalized patients (Weiskopf et al. 2017).

Although allogeneic blood transfusion can be resuscitative for injured patients, it involves a few challenges, such as shortage of blood, more time and cost for blood grouping (Castro and Briceno 2010; Bachert et al. 2020), and the possibility of spreading an infection, such as human immunodeficiency virus (HIV), Zika virus and viral hepatitis (hepatitis B and C viruses). Moreover, parasitic diseases, such as babesiosis,

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which is mainly transmitted through a tick bite, may also be spread through blood transfusion (Chen et al. 2009; Moritz et al. 2016; Khan et al. 2020). However, the greatest obstacle to allogeneic transfusion is the short shelf-life of blood, which may pose serious problems in harsh environments, such as in battlefields (Castro and Briceno 2010; Bialas et al. 2019).

Many patients suffer from hypoxia and ischemia secondary to lung and airway injury or obstruction, thereby causing heart failure, neurological problems, and multiple organ damage (Kheir et al. 2012; Fix et al. 2015) as well as leading to increased mortality (Matsuki et al. 2014; Legband et al. 2015; Fix et al. 2015). Mechanical ventilation is usually used in hospitals to treat respiratory insufficiency. However, delayed measurement of inspired oxygen or inadequate oxygen inhalation can lead to further loss of organ or even patient death (Feshitan et al. 2014; Legband et al. 2015).

To address this emergent medical need for resuscitation and to treat hypoxic conditions as well as to enhance oxygen transportation, various therapeutic processes have been developed (Khan et al. 2018a). In the very beginning, William Harvey described blood circulation; many scientists aspired for its artificial replacement (Sarkar 2008; Moradi et al. 2016), which was further highlighted after the origin of HIV in the 1980s (Moradi et al. 2016). It may not be feasible to provide blood to every patient, with so many of them worldwide in need of blood every second. Therefore, artificial oxygen carriers (AOCs) are a workable and protected method that can be safely used in major surgeries and other hospital emergencies (Matton et al. 2018).

AOCs provide various amenities other than allogeneic blood transfusions, which may lead to morbidity and mortality advantages in patients with serious distress (Jahr et al. 2021). In addition to emergency hospitals, some AOCs play an important role in organ preservation during transplantation (Matton et al. 2018), sickle cell crisis (Davis et al. 2018), and oxygen supply to the brain in cases of cardiac arrest (Shellington et al. 2011). This review article provides an overview of the formulation, storage, shelf-life, clinical application, side effects, and current perspectives of AOCs as RBC substitutes. Moreover, this review also includes pre-clinical (in vitro and in vivo) assessments to evaluate the efficacy and safety of oxygen transport through AOCs. With the most significant technologies, hemoglobin- and perfluorocarbon-based oxygen carriers as well as other modern technologies, such as synthetically produced porphyrin-based AOC systems and oxygen-carrying micro/nanobubbles, have also been elucidated.

Oxygen carrier systems

The systemic circulation conveys oxygen and nutrients to the cells as well as simultaneously carries CO_2 and waste products from cells. Oxygenated blood flow starts from the left ventricle and is transported to the tissues of the body through the arteries. Deoxygenated blood comes from the tissue capillaries and enters the right atrium of the heart through the veins (Fig. 1a). In oxygenated blood, oxygen is carried by two pathways: (1) oxygen dissolution in the blood (1.5%) and (2) oxygen binding with hemoglobin (Hb) (98.5%) (Padsalgikar 2017).

Hb is a tetrameric protein molecule that is responsible for carrying oxygen and is contained in RBCs; it contains two alpha and two beta subunit peptides and has a molecular weight of 64,400 Da. Each peptide subunit is enclosed in a globin and an iron-containing central heme group that can bind to one oxygen molecule (four oxygen molecules per Hb) (Fig. 1b) (Sen Gupta 2017). Typically, iron can subsist in a ferrous (Fe^{2+}) redox state, which binds with oxygen. After oxidation (loss of an electron), Fe^{2+} (ferrous) becomes Fe^{3+} (ferric), called methemoglobin or ferrihemoglobin. Methemoglobin cannot bind oxygen (Pittman 2011).

The binding of oxygen with Hb is cooperative, where Hb affinity enhancement for oxygen depends on the increased number of bound oxygen molecules. Deoxygenated Hb is denoted as the tense state (T-state), which has low oxygen affinity (Mihailescu and Russu 2001). After binding to oxygen, Hb can change its shape and prevail in a relaxed state (R-state) with high oxygen affinity (Mihailescu and Russu 2001; Modery-Pawlowski et al. 2013). When oxygenated Hb reaches tissues containing low partial pressure of oxygen (pO_2), it is divided into Hb and oxygen, thereby providing increased local pO_2; if the oxygenated Hb reaches pulmonary circulation containing high pO_2, it results in less pO_2 due to increased oxygenation of Hb and an oxygen binding curve (sigmoidal curve) (Fig. 1b) (Modery-Pawlowski et al. 2013; Sen Gupta 2017).

Factors affecting the oxygen-binding ability

Oxygen-carrying capacity depends on the concentration of oxygen in the blood. Several parameters, such as environmental factors, temperature, effector molecules such as 2,3-diphosphoglycerate (2,3-DPG), and diseases can affect oxygen-binding ability, as defined below (Bialas et al. 2019):

Blood pH and CO_2 levels: For cellular respiration, several biochemical reactions essential causing enhances metabolic activity in tissues results in the CO_2 production as a metabolic byproduct. CO_2 and water discharge from cellular respiration as metabolic waste products through the carbonic anhydrase enzyme and both of them reacts with each other (Benner et al. 2022) and form bicarbonate (HCO_3^-) and H^+ (hydrogen) ions in the blood. When blood CO_2 level

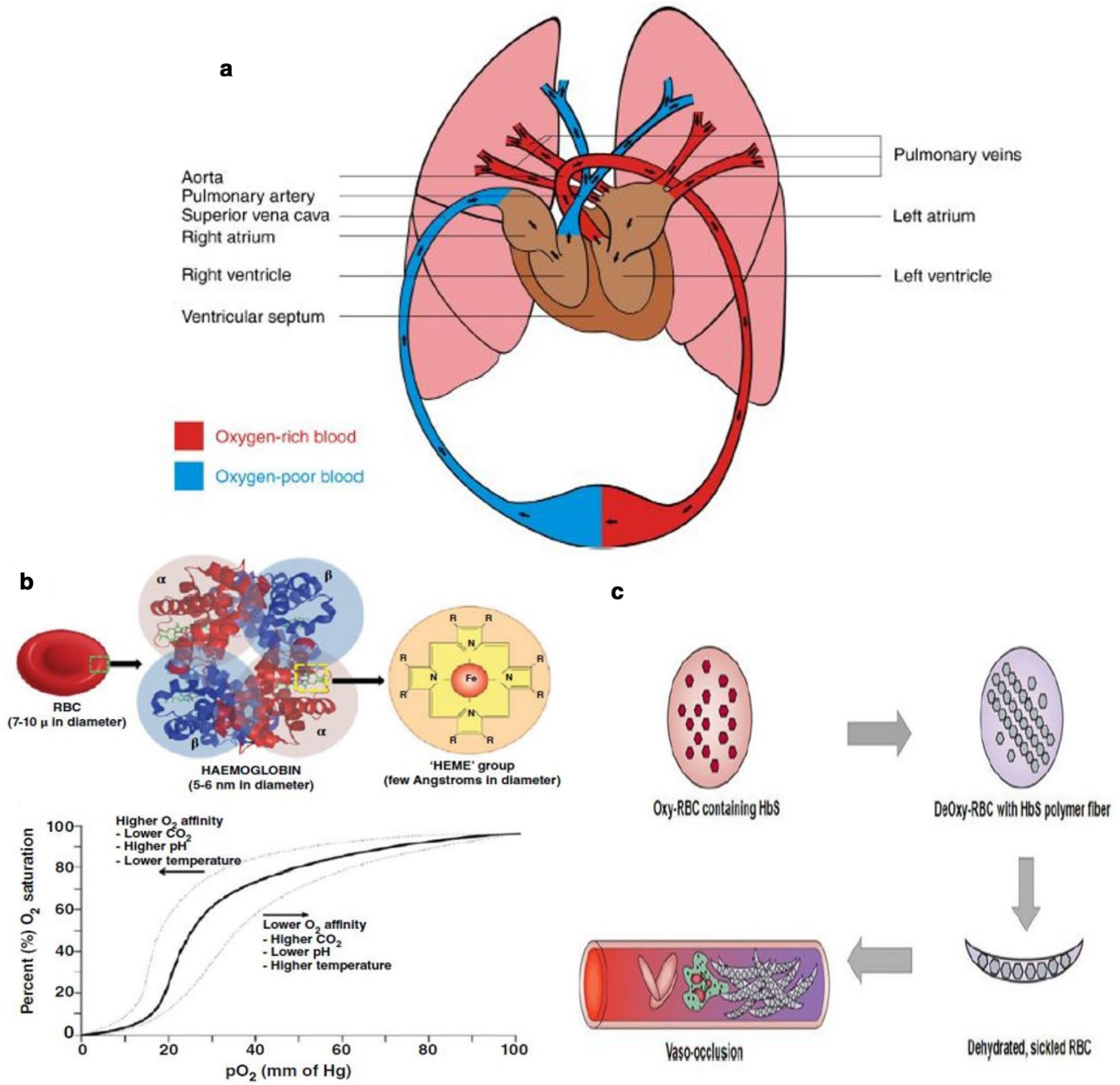

Fig. 1 **a** Blood circulation system [Reprinted with permission from (Padsalgikar 2017). Copyright © 2017, Elsevier Inc.], **b** Top: RBC (Red blood cell), the hemoglobin (Hb) structure within RBC, and the 'Heme' porphyrin structure within Hb; and bottom: oxygen binding curve (sigmoidal curve) for Hb exhibited the cooperative binding nature [Reprinted with permission from (Sen Gupta 2017). Copyright © 2017, Wiley Periodicals, Inc.], **c** Changed RBC shape and vaso-occlusion in sickle cell anemia [Reprinted with permission from (Li et al. 2017). Copyright © 2016, Elsevier Ltd]

increases, H^+ ions are also enhanced, thus resulting in reduced pH of the neighboring peripheral tissue environment in where the desired yield is to discharge O_2 in peripheral tissue and input O_2 in the lungs. But due to decrease pH, Hb acts as a buffering agent by discharging its O_2 and Hb decreased it's affinity for oxygen (Fig. 1b), and vice versa (Modery-Pawlowski et al. 2013; Sen Gupta 2017; Benner et al. 2022). This effect was basically noticed by Bohr and his colleagues in 1904 which is also known as Bohr effect (Malte and Lykkeboe 2018; Benner et al. 2022).

Temperature When body temperature increases in active skeletal muscles, simultaneous heat production also increases, which reduces the affinity of Hb for oxygen. During decreased tissue metabolism, heat production also decreases as a consequences of decrease temperature, which raises the affinity of Hb for oxygen (Bialas et al. 2019).

2,3-diphosphoglycerate (2,3-DPG): Allosteric 2,3-DPG is an intermediate chemical metabolite in the Luebering–Rapoport glycolytic pathway and found within RBCs which is formed from 1,3-diphosphoglycerate (1,3-DPG) in the presence of catalyst diphosphoglycerate mutase (Płoszczyca et al. 2021); it is tied to the β chain of Hb. Under increased levels of 2,3-DPG, it preferentially binds to Hb, thereby reducing the affinity of Hb for oxygen and vice versa (Khan et al. 2020).

Diseases Many diseases, such as sickle cell anemia and thalassemia, affect Hb levels and diminish the delivery capacity of oxygen in the body. In sickle cell anemia, the RBC shape changes from biconcave discoid to stiffened and elongated crescent-shape. This shape cannot pass through the blood capillaries, thus resulting in vaso-occlusion and the inability to transport oxygen (Fig. 1c) (Li et al. 2017). Thalassemia is a genetic disease that produces an elevated number of RBCs; however, these cells have less Hb than normal, so the oxygen-binding and carrying capacities are reduced (Li et al. 2017).

AOCs and their benefits

AOCs play an important role in the management of blood conditions in patients with serious diseases (Spahn 2018). AOCs can be grouped into hemoglobin-based oxygen carriers (HBOCs), in which oxygen and Hb are covalently linked, and perfluorocarbon-based oxygen carriers (PFOCs), in which oxygen is dissolved within a perfluorocarbon (PFC) molecule (Castro and Briceno 2010; Spahn 2018; Bialas et al. 2019; Sen Gupta 2019; Jägers et al. 2021). In addition, synthetically produced porphyrin polymers and oxygen micro/nanobubbles may be effective options for AOCs (Kitagishi et al. 2017; Khan et al. 2018a; Albalawi et al. 2018). Different AOCs is presented in Table 1.

In the early history of HBOCs, Sydney Ringer developed Ringer's solution in 1883, which may not be a perfect blood substitute or oxygen carrier; however, it acts as a plasma volume expander that is still being used (Bialas et al. 2019). The first endeavor to use HBOCs was formed in the 1930s (Sakai et al. 2009). In 1933, Amberson et al. experimented on a cat with renal toxicity; they replaced its blood with cell-free Hb in Ringer's solution, which displayed a sustained life (Amberson et al. 1933). Thus, several patients in the US navy were treated with cell-free Hb in the 1950s, although cardiovascular complications (Sen Gupta 2017) were noted as side effects. Subsequently, Hb was encapsulated with nylon, collodion, and other materials for the first time by Chang in 1957, and later with gelatin and silicone in 1960. A pioneering study on liposome-encapsulated Hb (LEH) was performed in 1977, in which Hb was encapsulated in phospholipids, fatty acids, and cholesterol (Sakai et al. 2009). The first chemically modified HBOCs was HemAssist, licensed in 1985. Polyheme is a polymerized form of Hb that is free from unreacted tetramers, which was clinically developed in 1996 (Gould and Moss 1996). The concept of PFCs as oxygen carriers started in 1966; an human serum albumin -derived PFC-based AOC, which began in 2017, was considered as a cutting-edge technology and was utilized in various in vivo studies (Wrobeln et al. 2017a). Another cutting-edge technology was HemoCD (porphyrin-based AOC), which was also artificially synthesized; an in vivo study is currently ongoing as a carbon monoxide (CO) removal agent (Kitagishi and Minegishi 2017). The current status of the AOCs is shown in Fig. 2, which is briefly described later.

The main benefit of these systems is that they immediately provide oxygen through the circulation system to save life without any impairment (Haldar et al. 2019). AOC products are feasible in traumatic conditions as well as in austere environments where blood donation is impossible. In addition, these may be used in medical treatments such as elective and cardiovascular surgeries (Spahn 2018; Haldar et al. 2019); they are effective in alleviating ischemic conditions such as cerebral hypoxia (Kaneda et al. 2014) and fetal ischemia (Ohta et al. 2017). In addition, they are non-perishable, stable to supply, and economically feasible (Bialas et al. 2019). Moreover, AOCs are compatible with Jehovah's Witnesses patients who require blood transfusion. They believe that receiving blood is against God's will; they reject blood transfusion not only from others but also from their own system. Blood management is an inconvenience for rare blood groups, such as the Bombay type (Oh), and highly immunocompromised patients, such as those with sickle cell anemia (Khan et al. 2020). Therefore, AOCs are a useful system for life recovery and act as a safeguard for patients with serious hypoxia.

Hemoglobin based oxygen carriers (HBOCs)

HBOCs are used as universal oxygen carrier systems that can use in several life-threatening conditions, such as hemorrhagic shock, trauma, stroke, myocardial infarction, and acute blood loss (Bedőcs and Szebeni 2020). At first Cell-free Hb was used for oxygen delivery, but it had several problems in carrying oxygen. The first problem was the high affinity of acellular Hb for oxygen. The p50 for intact RBC was 26–28 mmHg, whereas that for cell-free Hb was 10–15 mmHg, thereby resulting in oxygen being more tied with tissues. During the purification process, 2,3-diphosphoglycerate is lost owing to the high affinity of oxygen (Kim and Greenburg 2004). Furthermore, acellular Hb was administered intravenously in some patients who complained of kidney toxicity, hypertension, and cardiovascular complications. Hb tetramers are separated into dimers and monomers, which can easily be secreted into

Table 1 Types of AOCs

Types	Sub-types	Products name/compositions	References
Hemoglobin-based oxygen carriers (HBOCs)	Chemically altered Hb-based HBOCs	Cell-Free Hb	Modery-Pawlowski et al. (2013), Moradi et al. (2016), Sen Gupta (2017, 2019), Ferenz and Steinbicker (2019), Bialas et al. (2019), Jahr et al. (2021)
		HemAssist	
		Optro	
		Hemolink	
		Hemopure (HBOC-201 and HBOC-301)	
		Polyheme	
		PHP (Pyridoxylated Hb) or Hemoximer	
		PolyHb-SOD-CAT-CA	
		PolyHb-Fibrinogen	
		Hemotech	
		Hemospan	
		Sanguinate	
		PEG-Hb	
		SanFlow (PNPH)	
		$HemO_2Life$/ Hemarina-M101	
		OxyVita Hb	
	Encapsulated HBOCs	Hb Corpuscles (artificial)	
		Liposome Encapsulated Hb (LEH)	
		Polymersome Encapsulated Hb (PEH)	
Perfluorocarbon-based oxygen carriers (PFOCs)	–	Fluosol DA	Spiess (2009), Castro and Briceno (2010), Wrobeln et al. (2017a), Sen Gupta (2017), Culp et al. (2019), Hill (2019), Jägers et al. (2021)
		Oxypherol	
		Perftoran	
		Oxygent	
		Oxyfluor	
		Oxycyte	
		Dodecafluoropentane (DDFPe)	
		Albumin derived perfluorocarbon based artificial oxygen carrier (A-AOC)	
Synthetically produced porphyrin-based AOCs	–	'Picket fence' iron porphyrin	Wang et al. (2005), Modery-Pawlowski et al. (2013), Kitagishi et al. (2017), Sen Gupta (2017), Norvaiša et al. (2021)
		LipidHeme porphyrin	
		HSA-heme porphyrin	
		HemoCD porphyrin	
O_2 microbubbles	Lipid shell types	1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC)	Sirsi and Borden (2009); Tao and Ghoroghchian (2014), Fix et al. (2015), Khan et al. (2018a)
		1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N- [amino (polyethylene glycol)] (DSPE-PEG)	
		DSPC, PEG-40-S (9:1)	
		DSPC, BRIJ 100	
		F-PC, DMPC	
		1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)	
		N-(Carbamoyl-methoxypolyethylene glycol 5000)-1,2-dipalmitoyl-cephalin sodium (DPPE-MPEG5000)	
	Protein shell type	Bovine serum albumin	
	Polymer shell types	Chitosan	
		Dextran with or without polyvinylpyrrolidone (PVP)	
	Surfactants stabilized microbubbles	Span 60, D-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS)	

the kidneys and cross through the glomeruli, thus causing renal toxicity (Taguchi et al. 2017; Bachert et al. 2020). Additionally, cell-free Hb scavenges nitric oxide (NO), a vasodilator that may cause heart dysfunction (Sen Gupta 2017; Bialas et al. 2019). In addition, when NO abates the circulation, it actively inhibits platelet aggregation (Bachert et al. 2020).

Hb cannot be used directly as an oxygen-carrying component owing to the disruption and toxicity of Hb during extraction from RBC. To avoid this, outdated human, animal (bovine), and recombinant Hb were used as raw ingredients to prepare HBOCs, which were further chemically modified and microencapsulated (Kim and Greenburg 2004). Hb is extracted through cell lysis, sterile filtration by purification, chromatography, and low-heat sterilization during the use of outdated human or animal (bovine) RBCs. First, Hb is attached to oxygen at a slow speed, thus ascending the attaching relation for the second, third, and fourth oxygen molecules; this has a positive effect on the sigmoidal oxygen equilibrium curve (Alayash 2010; Sen Gupta 2017). HBOCs are divided into two main types: chemically altered HBOCs and encapsulated HBOCs (Hb encapsulated within a defensive shell), as shown in Fig. 3 (Jansman and Hosta-Rigau 2018).

Chemically altered HBOCs

In chemically altered HBOCs, Hb is cross-linked both intra- and inter-molecularly. The first chemically altered HBOC was HemAssist (Baxter, Illinois, USA), in which Hb was cross-linked with diaspirin and produced from outdated donated human blood with a half-life of 24 h. Another altered HBOC was Optro (Somatogen, Boulder, Colorado, USA), which was modified recombinantly and cross-linked with glycine; it had a half-life of 2–19 h. Hemolink (Hemosol, Toronto, Ontario, Canada) is another intramolecular HBOC that is cross-linked with o-raffinose and produced from expired human Hb; it had a half-life of 24 h (Bialas et al. 2019; Sen Gupta 2019).

In this era, polymerization brought about a dramatic change, in which Hb molecules were cross-linked intermolecularly with glutaraldehyde (Hemopure, Biopure; Cambridge, MA, USA and Polyheme, Northfield Labs; Evanston, IL, USA), polyoxyethylene pyridoxylated polymer (PHP (Pyridoxylated Hb), or Hemoximer (Curacyte/Apex Bioscience) to increase their molecular size (Moradi et al. 2016). During polymerization, the PolyHb molecule had four to five Hb molecules instead of one, which also significantly enhanced the pharmacokinetics (Bialas et al. 2019). Hemopure originates from bovine Hb and polymerizes with glutaraldehyde, which is used in cases of hemorrhagic shock, perioperative transfusion, and acute normovolemic hemodilution cardiac surgery (Bialas et al. 2019; Khan et al. 2020). As it was produced from bovine Hb, it was suitable for use in special patients, such as Jehovah's Witness (Rogers and Crookston 2006). The half-life of hemopure is 16–20 h for healthy volunteers and 8.5 h for patients with liver disease (Taguchi et al. 2017). Polyheme is created from human Hb, which resembles hemopure and polymerizes with glutaraldehyde. During trauma, surgery, and in different bleeding disorders, it has a half-life of 24 h. Another polymerized HBOC is PHPoxylated hemoglobin (PHP), or hemoximer, which is sourced from human Hb and surface-modified with a polyoxyethylene pyridoxylated polymer. PolyHb-SOD-CAT-CA originates from the cross-linking between Hb and superoxide dismutase (SOD), carbonic anhydrase (CA), and catalase (CAT). It can be sterilized and preserved at room temperature for 320 days and is used not only as an oxygen carrier but also for the withdrawal of radical oxygen and CO_2 transportation. PolyHb-fibrinogen, produced from bovine Hb and cross-linked with fibrinogen, displays both oxygen and coagulation (platelet-like activity) characteristics (Moradi et al. 2016; Bachert et al. 2020).

Another form of HBOCs is PEGylated modified Hb, including hemospan (MP4; Sangart, San Diego, CA, USA), PEG-Hb (Enzon, Piscataway, NJ, USA), sanguinate (Prolong Pharmaceuticals, South Plainfield, NJ, USA), and SanFlow (PNPH) (Synzyme). Hemospan is composed of human Hb and is modified with maleimide-polyethylene glycol (PEG) with a molecular weight of 96 kDa. The name hemospan was changed to MP4OX, which was used as an oxygen carrier to enhance the supply of oxygen in comparison to blood replacement (Jahr et al. 2012). MP4OX has been used to treat sickle cell anemia to reduce the associated pain and duration (Keipert and MP4CO-SCD-105 Study Investigators 2016). PEG-Hb and SanFlow (PNPH) are bovine and human Hb products modified with polyethylene glycol conjugated (PEGylated) and polynitroxylated polyethylene glycol conjugated (PEGylated) Hb, respectively (Bedőcs and Szebeni 2020). Sanguinate was extracted from bovine Hb and cross-linked with polyethylene glycol-conjugated (PEGylated) carboxyhemoglobin with a molecular weight of 120 kDa. It has anti-apoptotic and anti-inflammatory properties owing to the release of CO. It has a half-life of 13–20 h and is used in vaso-occlusive crises and sickle cell anemia (Ferenz and Steinbicker 2019).

$HemO_2$Life/Hemarina-M101 (Hemarina) was created from lungworms, *Arenicola marina* (invertebrate), which is not attached to Hb or other membranes (Ferenz and Steinbicker 2019; Bedőcs and Szebeni 2020; Varney et al. 2021). Hb (extracellular) has 40 times more oxygen receptivity than vertebrate Hb (Lupon et al. 2021). It is a hexagonal bilayer-linked globin molecule with a molecular weight of 3600 kDa and 2.5-h half-life. It can recover oxygen-related radicals owing to its natural superoxide dismutase characteristics

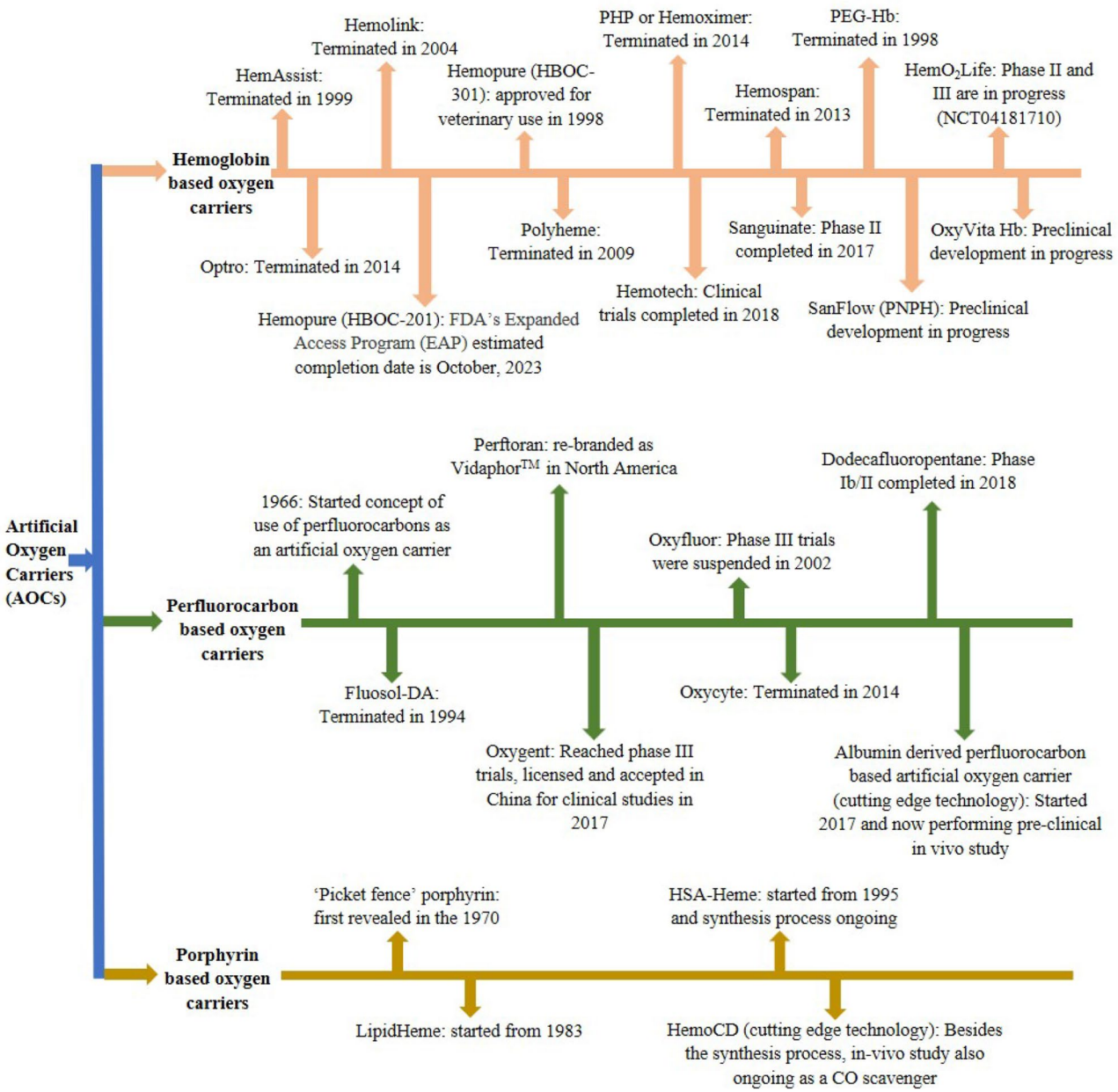

Fig. 2 Current status of AOCs

(Mallet et al. 2014). It should be used in cases of sickle cell anemia, hemorrhagic shock, and organ preservation (Ferenz and Steinbicker 2019). Currently, it is used for oxygen therapy in covid- 19 patients and has shown significant survival improvement, thus avoiding tracheal intubation, delivering rapid oxygen supply, and treating more patients without the use of invasive machines (Lupon et al. 2021). Furthermroe, OxyVita Hb (Oxyvita Inc.), which is inter- and intramolecularly cross-linked, originates from bovine Hb and is stabilized with sebacoly diaspirin. It has two subtypes, OxyVita Hb and OxyVitaHbCO (Ferenz and Steinbicker 2019). Oxyvita Hb has a greater success in controlling severe hemorrhage in a battlefield model than other HBOCs. It has a half-life of 72 h (Bedőcs and Szebeni 2020). A summary of chemically altered HBOCs is presented in Table 2.

Clinical trials Few HBOCs have reached safety studies and attained phase III trials; some of them have been accepted, while a few others were terminated due to their failure. HemAssist reached phase III clinical trial for use during cardiac surgery and trauma/stroke, but was terminated in 1999 due to increased mortality rate (Chen et al. 2009; Jahr et al. 2012). Optro reached a phase II trial for elective surgery and showed fewer adverse effects, although

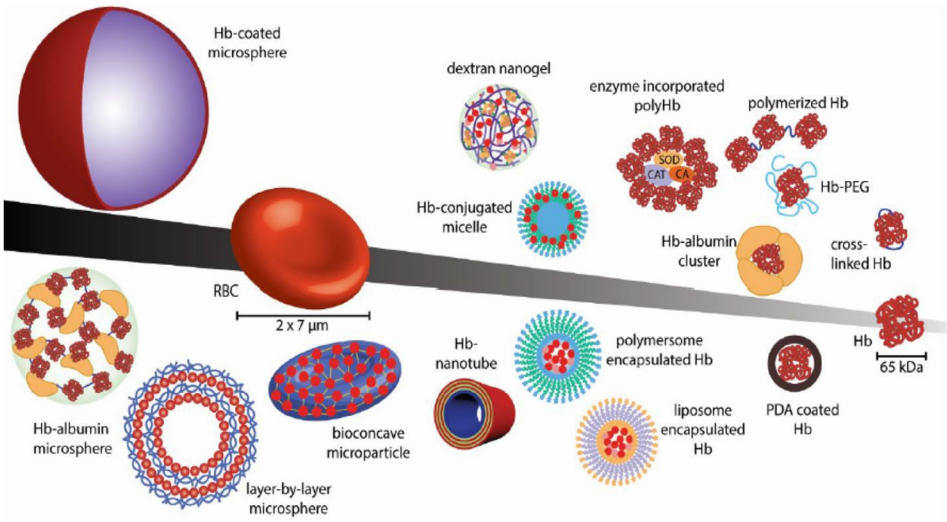

Fig. 3 Two main HBOCs. Top: chemically altered HBOCs and bottom: encapsulated HBOCs [Reprinted with permission from (Jansman and Hosta-Rigau 2018)]. Copyright © 2018, Elsevier B.V

it was discontinued in 2014 due to NO scavenging panic (Bedőcs and Szebeni 2020). Hemolink was halted in 2003 after a phase II trial and subsequently terminated in 2004. Hemopure faced many phases I–III safety studies; during a phase III study, HBOC-201 was accepted as an oxygen carrier in South Africa in 2001 and in Russia in 2012. Subsequently, oxyglobin (HBOC-301) was accepted for veterinary use. The Food and Drug Administration (FDA) permitted its emergency use when no option is available to save the patient's life (Jahr et al. 2021). Although Hemopure has not been FDA approved for human use for any indication in the United States, it is obtainable for use in life-threatening anemic patients in clinical trials and in the FDA's expanded access program (EAP). This expanded study started in October 2013, with an estimated completion date on October 2023 (NCT number: NCT01881503) (Englewood Hospital and Medical Center 2021). Polyheme completed phase III trials in 2007, but failed its biologics license application (BLA) in 2009 due to its adverse effects, and was discontinued (Carmichael et al. 2000; Jahr et al. 2012). PHP (Pyridoxylated Hb) or Hemoximer failed phase III trials due to the associated increased mortality and was terminated in 2014 (Yabuki et al. 1990; Bedőcs and Szebeni 2020). Hemotech is composed of purified bovine Hb, which is cross-linked with ATP, adenosine, and glutathione, and has been used in patients with acute blood loss; it has also completed phase I trials (Simoni et al. 2012; Bedőcs and Szebeni 2020). Hemospan, a PEGylated modified Hb, completed phases IIa and IIb in 2012, although phase IIc was dismissed and finally discontinued in 2013. Sanguinate completed phase II in 2017 (NCT02411708) (Prolong Pharmaceuticals 2018). In 2020, $HemO_2$Life/ Hemarina-M101 was used for oxygen therapy in life-threatening COVID-19 patients with severe respiratory problems; it is currently an ongoing study (Lupon et al. 2021). $HemO_2$Life completed phase I study in 2018; phases II and III (NCT04181710) are currently in progress to determine its efficacy in renal transplantation (Lupon et al. 2021) because of its high oxygen carrying capability with minimal side effects (Bedőcs and Szebeni 2020; Varney et al. 2021, p. 101). The clinical trials of chemically altered HBOCs are summarized in Table 3.

Encapsulated HBOC systems

Encapsulated Hb products are produced in a way to share maximum similarities with RBCs, which do not cause vasoactivation due to NO scavenging. These systems were developed in 1950 and 1960 by Chang et al. (Bialas et al. 2019). Encapsulation using different effector molecules or reductive enzymes was carried out to recapitulate natural RBCs. The encapsulated Hb products showed more amenities, such as mitigation of hypertension, increased half-life, and longer shelf-life than acellular Hb products (Moradi et al. 2016). Hb is mainly encapsulated by polymeric membranes produced from collodions (cellulose nitrate), PEG-polylactate polymer, cholesterol with phospholipids, etc. Furthermore, the artificial Hb corpuscle maintains the activity of erythrocyte-related enzymes, such as CAT, carbonic anhydrase, and 2,3-DPG (Sen Gupta 2019). Compared to the size of erythrocytes, liposome-encapsulated Hb particle size is much smaller (1:30), which enables it to enter the body where RBCs cannot. Therefore, it provides more oxygen during trauma, shock, and stroke due to its ability to pass blockages (Moradi et al. 2016).

Hb vesicles are composed of cholesterol, neo-red cells, neohemocytes, or phospholipids (liposomes). Liposomes are deliberated first-generation nanoparticles, which are lipid-based (Hegde et al. 2022) and developed to produce nano-size liposome-encapsulated Hb. Moreover, sub-micron-sized

Table 2 Summary about chemically altered HBOCs

HBOCs	Products name	Sponsors name	Sources	Altered Hb	Functions	References
Cell-free Hb	–	–	Human, bovine and recombinant (*E. coli*) sources	–	Oxygen carrier	Sen Gupta (2017), Bialas et al. (2019)
Cross-linked Hb (Intra molecularly)	HemAssist	Baxter	Human	Cross-linked with diaspirin		Chen et al. (2009), Moradi et al. (2016), Bialas et al. (2019)
	Optro	Somatogen	Recombinant	Cross-linked with glycine		
	Hemolink	Hemosol	Human	Cross-linked with O-raffinose		
Polymerized and/or tethered Hb	Hemopure (HBOC-201 and HBOC-301)	Biopure (afterward called OPKbiotech, now HbO_2 Therapeutics)	Bovine	Polymerization with glutaraldehyde		Wong and Chang (2007), Simoni et al. (2012), Modery-Pawlowski et al. (2013), Moradi et al. (2016), Sen Gupta (2017), Haldar et al. (2019), Jahr et al. (2021)
	Polyheme	Northfield lab	Human	Polymerization with glutaraldehyde		
	PHP (Pyridoxylated Hb) or Hemoximer	Curacyte/ Apex Bioscience	Human	Polyoxyethylene pyridoxylated polymer (Surface-modified)		
	PolyHb-SOD-CAT-CA	–	Bovine	Cross-linked or polymer tethered with different enzymes like SOD (Superoxide dismutase), CAT (Catalase) and CA (Carbonic anhydrase)	Oxygen carrier, withdrawal of oxygen radical, CO_2 transportation	
	PolyHb-Fibrinogen	–	Bovine	crosslinking fibrinogen to hemoglobin	Oxygen carrier and coagulation (platelet-like activity)	
	Hemotech	HemoBiotech	Bovine	Cross-linked with ATP (Intra molecularly) and adenosine, glutathione (Inter molecularly)	Oxygen carrier	

Table 2 (continued)

HBOCs	Products name	Sponsors name	Sources	Altered Hb	Functions	References
PEGylated modified Hb	Hemospan	Sangart	Human	Maleimide-polyethylene Glycol (PEG)-modified Hb	Oxygen carrier	Bialas et al. (2019), Bedőcs and Szebeni (2020), Jahr et al. (2021)
	Sanguinate	Prolong Pharmaceuticals	Bovine	Polyethylene glycol conjugated (PEGylated) carboxyhemoglobin		
	PEG-Hb	Enzon	Bovine	Polyethylene glycol conjugated (PEGylated) Hb		
	SanFlow (PNPH)	Synzyme	Human	Polynitroxylated Polyethylene glycol conjugated (PEGylated) Hb		
Natural extracellular biopolymer Hb	$HemO_2$ Life/Hemarina-M101	Hemarina	*Arenicola marina* (Invertebrate)	Hexagonal-bilayer linked globin molecules		Bedőcs and Szebeni (2020), Varney et al. (2021)
Zerolink polymer Hb	OxyVita Hb	Oxyvita Inc	Bovine	Hb stabilized with sebacoly diaspirin		Ferenz and Steinbicker (2019), Bedőcs and Szebeni (2020)

(100–200 nm diameter) liposomes were surface modified with PEG to decline opsonization and increase its circulation lifetime to 60 h, thus resulting in shortened scavenging of NO due to an additional encapsulate component. PEG not only increases their half-life but also reduces antigenicity, thus expanding specific site targeting and generating water-soluble properties (Kaneda et al. 2009; Haldar et al. 2019). They are also adaptable to the body's immunity. Furthermore, biodegradable polymeric vesicles, such as poly (L-lactic acid)/poly(ε-caprolactone), poly(L-lysine), poly(lactic-coglycolic acid), and PEG copolymers, produced polymersome-encapsulated hemoglobin (PEH), which was spherical in structure and smaller in size (80–200 μm in diameter), thus resulting in increased bioavailability (Rameez et al. 2008; Haldar et al. 2019). Polymersomes attached to different effector molecules and enzymes provide similar environmental and indigenous biophysical characteristics to erythrocytes (Bialas et al. 2019). In human RBCs, the Hb loading concentration is approximately 150 mg/mL, whereas it is 1–2 mg/mL in the PEH systems. PEH originates from both human and bovine Hb and resembles the biophysical characteristics and oxygen equilibrium kinetics of RBCs (Rameez et al. 2008). The source, encapsulated components, function, and features of the encapsulated HBOCs are shown in Table 4. A regular RBC is a biconcave disc, which is slender in the middle and is extremely flexible with modified flow dynamics based on the size of the blood vessel and saturation of oxygen. Generally, erythrocyte flow is distributed in the middle of large arteries and veins, whereas PEH is equally divided in the microcirculation for high oxygen delivery, which facilitates development of new replacements with similar characteristics as that of RBCs (Bialas et al. 2019).

Preclinical assessment (in vitro and in vivo) for evaluation of efficacy and safety of oxygen transport through HBOCs

HBOCs have been investigated using cell lines (in vitro) and animal models (in vivo) to evaluate the efficacy and safety of HBOCs products (Table 5). HBOCs are being promoted for oxygen- and plasma-expanded therapeutics to ameliorate NO scavenging and vasoconstriction linked with oxidative tissue injury (Bäumler et al. 2014; Kao et al. 2018, p. 700). Hb microparticles (HbMP-700) showed high oxygen affinity that impeded premature oversupply of oxygen and vasoconstriction in blood vessels in vitro; no toxicity or clinical signs were observed during in vivo experiments in mice (Kao et al. 2018, p. 700). In addition, 700-nM Hb particle (HbPs) limits NO scavenging, which resulted in increased tissue oxygenation in mice and rats (Xiong et al. 2013; Bäumler et al. 2014). PEGylated bovine carboxyhemoglobin, SANGUINATE®, showed a better heart function and mitral competence after myocardial infarction in rats (Kawaguchi

Table 3 Summary about status of clinical trials of chemically altered HBOCs

Products name	Half-life	Indications	Trials description	Current status	References
HemAssist	Healthy volunteer: 2.5–3.3 h Hemodialysis patient: 2.1–4.3 h Cardiac surgery patient: 24 h	Hemorrhagic shock	In phase III trials during cardiac surgery, trauma/stroke, it increased mortality rate	Terminated in 1999	Chen et al. (2009), Jahr et al. (2012), Khan et al. (2020)
Optro	2–19 h	Cardiac surgery	It failed in phase II trials due to excessive vasoconstriction	Terminated in 2014	Bedőcs and Szebeni (2020)
Hemolink	Healthy volunteer: 18–20 h	Cardiothoracic surgery, acute normovolemic hemodilution	Halted after Phase II trials due to indemnity purpose	Terminated in 2004	Carmichael et al. (2000) Jahr et al. (2021)
Hemopure (HBOC-201 and HBOC-301)	Healthy volunteer: 16–20 h Patient undergoing a liver section: 8.5 h	Severe anemia, hemorrhagic Shock, perioperative transfusion, acute coronary syndrome, coronary occlusion, myocardial infarction	Not approved by FDA for human use in the United States, hence HBOC-201 is used in clinical trials and FDA's Expanded (compassionate use) Access Program (EAP) for life-threatening anemic patients, for them whom blood is not an option	FDA's Expanded (compassionate use) Access Program (EAP) estimated completion date is October, 2023 (NCT01881503)	Taguchi et al. (2017), Bedőcs and Szebeni (2020), Englewood Hospital and Medical Center (2021)
Polyheme	24 h	Trauma, surgery, bleeding disorder	In 2007, phase III was completed, but failed BLA in 2009 due to adverse effects	Terminated in 2009	Taguchi et al. (2017), Jahr et al. (2021)
PHP (Pyridoxylated Hb) or Hemoximer	Healthy volunteer: 24 h	Systemic inflammatory response syndrome with shock	Phase III failed due to increased mortality	Terminated in 2014	Yabuki et al. (1990), Bedőcs and Szebeni (2020)
Hemotech	24 h	Acute blood loss	Completed phase I	Completed phase I	Simoni et al. (2012), Taguchi et al. (2017), Bedőcs and Szebeni (2020)
Hemospan	Healthy volunteer: 42.8–66.2 h Patient with orthopedic surgery: 14–23 h	Critical limb ischemia, hemorrhagic Shock, Ischemia, Hypotension	Phase III study was used to impede hypotension (NCT00421200)	Terminated in 2013	Taguchi et al. (2017)
Sanguinate	13–20 h	Vaso-occlusive crisis, sickle cell anemia, cerebral ischemia, renal insufficiency	Phase II completed in 2017 (NCT02411708)	In progress	Prolong Pharmaceuticals (2018)
PEG-Hb	15.0 ± 2.3 h rat models (No human clinical study)	To increase tumor oxygenation, radiation, and chemotherapy	Completed phase I	Terminated in 1998	Bedőcs and Szebeni (2020)
SanFlow (PNPH)	Not available	Hemorrhagic Shock, brain injury, stroke	Preclinical development in progress	In progress	Bedőcs and Szebeni (2020)
$HemO_2Life$	2.5 days	For COVID-19 patients, sickle cell anemia, hemorrhagic Shock, organ preservation	Phase I is completed in 2018	Phase II and III in progress (NCT04181710)	Lupon et al. (2021), Varney et al. (2021)

Table 3 (continued)

Products name	Half-life	Indications	Trials description	Current status	References
OxyVita Hb	72 h	Hemorrhagic shock, severe hemorrhage	Preclinical development in progress	In progress	Harrington and Wollocko (2011), Ferenz and Steinbicker (2019)

et al. 2018). Furthermore, OxyVita C improved systemic blood pressure, which also prevented pial arterioles and cerebral vasoconstriction in rat brains (Abutarboush et al. 2014). Moreover, liposome-encapsulated hemoglobin (LEH) can fix oxygen deficiency, which prevents hemorrhagic shock and sustains vital organ perfusion. In *Cynomolgus* monkeys, LEH showed high oxygen affinity, which reduced histological damage in the cerebral cortex and protected the cerebral metabolic rate of oxygen (Kawaguchi et al. 2017). In addition, a newly formed LEH conjugated with polyethylene glycol (PEG2K) and non-phospholipid hexadecylcarbamoyl methyl hexadecanoate (HDAS-PEG2K-LEH) is immune-neutral and well-tolerated in repeated doses (Yadav et al. 2014). HBOCs showed higher oxygen affinity with better circulatory response and low oxidation; some also helped to control systemic blood pressure and impede vasoconstriction.

Perfluorocarbon-based oxygen carriers (PFOCs)

Researchers developed a biocompatible synthetic oxygen carrier, namely perfluorocarbons (PFCs), which have a huge ability to dissolve gases. In 1966, Clark and Gollan conducted a new experiment on the utilization of oxygen-carrying agents; they submerged mice in fluorobutyltetrahydrofuran (FX- 80) equilibrated with 100% oxygen (Clark and Gollan 1966). PFCs are chemically stable and inert molecules that are structurally similar to hydrocarbons, where fluorine replaces these hydrogen groups. It is a nanoparticle that is 100 times smaller than erythrocytes (Haldar et al. 2019). Hydrocarbons in PFC contain hydrogen atoms that are substituted by fluorine atoms or halogens, in which fluorine can extract electrons from other atoms and toughen its bonds with the carbon backbone of fluorine compounds (Jägers et al. 2021). Within PFCs, oxygen can be dissolved via the Van der Waals interactions. The oxygen transport process follows Henry's law, which is controlled by the partial pressure of oxygen. Oxygen transport through perfluorodecalin (PFD) is much faster than that through water because oxygen finds more space to move freely in PFD molecules (Jägers et al. 2021). However, PFC emulsions are made of hydrophobic PFCs with surfactants, such as fluorinated compounds or lipids, which can be made miscible with water through high-pressure homogenization (Haldar et al. 2019; Jahr et al. 2021).

To date, different PFC-based oxygen carriers (Fig. 4a) including formulation and properties have been investigated (Table 6). Fluosol-DA (Green Cross Corp., Japan) was made from 14% PFD and 6% perfluorotripropylamine (20% W/10.6% volume), with surfactants such as Pluronic F-68, egg yolk phospholipid, and potassium oleate, which was the first PFC system approved by the FDA in 1989. Unfortunately, it was discontinued in 1994 due to short

Table 4 Summary about encapsulated HBOCs system

Products name	Sources	Encapsulated components	Functions	Features/ Characteristics	References
Hb corpuscle (artificial)	Hemoglobin	(i) Collodion (cellulose nitrate) (ii) PEG-polylactate polymer (iii) Cholesterol with phospholipid	Oxygen carrier	More oxygen transport performance, easily circulates due to less viscosity	Moradi et al. (2016), Sen Gupta (2019)
Liposome encapsulated hemoglobin (LEH)	Bovine hemoglobin	Sub-micron size liposomes and PEGylated liposomes		Increase circulation half-life, reduce antigenicity, expand the specific site targeting and generate water-soluble	Kaneda et al. (2009), Haldar et al. (2019)
Polymersome-encapsulated hemoglobin (PEH)	Human and bovine hemoglobin	Polymeric vesicles such as Poly (L-lactic acid)/ poly(ε-caprolactone) and poly(L-lysine), poly(lactic-coglycolicacid)/PEG copolymers	Oxygen carrier, used as drug delivery for cancer treatment	Resemblance to human RBC, made in huge quantities, more Hb loading capability	Rameez et al. (2008), Haldar et al. (2019)

shelf-life, pulmonary complications, and reduced platelet, increased WBC, and decreased neutrophil counts (Ohyanagi et al. 1984; Police et al. 1985; Riess 2001; Castro and Briceno 2010; Jägers et al. 2021). The reason for short shelf-life related to their stability are flocculation or coalescence and Ostwald ripening leading to reversible droplet growth which is the prevailing way of colloidal instability and destabilization of AOCs (Jägers et al. 2021). In coalescence, two nanodroplets merged together on their surfaces and form larger droplets. By establishing zeta-potential surface charge, repulsion of droplets can minimize coalescence (Grapentin et al. 2015). Furthermore, to prevent flocculation and coalescence, surfactants like lipids or proteins need to use to create a high surface charge density leading to the droplet's repulsion (Dichiarante et al. 2018). Ostwald ripening destabilization is dominated by molar volume, solubility, and diffusion coefficient of the scattered phase material (Lambert and Janjic 2021). By this way, smaller droplets vanish away, then forming bigger droplets due to the larger curvature of small particles directed to an enhanced capillary pressure ensuing the Kelvin effect. It can prevent by mixing a small amount of higher homologue of the prime dispersed ingredients (Grapentin et al. 2015).

Furthermore, Perftoran (14% PFD and 6% perfluoromethyl-cyclohexylpiperidin) was used to treat severe blood loss and was approved for clinical use in Russia, Kazakhstan, Kyrgyzstan, Ukraine, and Mexico from 2005 to 2010. Recently, Perftoran was produced by Good Manufacturing Practice (GMP) standards, re-branded as Vidaphor™ in North America and Europe, and safely administered as an allogeneic blood transfusion to over 35,000 patients, with only mild complications (Castro and Briceno 2010; Ferenz and Steinbicker 2019; Krafft and Riess 2021). Subsequently, new-generation products, such as Oxygent (58% perfluorooctyl bromide and 2% perfluorodecyl bromide) (Alliance Pharmaceutical Corp., USA) and Oxyfluor (78% perfluoro-dichlorooctane) (HemaGen, St. Louis, USA), were used to resolve the side effects of Fluosol-DA. Oxygent is used in orthopedic surgery, cardiovascular surgery, non-cardiac surgery, coronary bypass, and coagulation procedures; it reached phase III trials and was approved in China for clinical studies (Castro and Briceno 2010; Ferenz and Steinbicker 2019; Jägers et al. 2021; Krafft and Riess 2021). In contrast, phase III trials of Oxyfluor were suspended owing to several side effects, such as stroke and thrombocytopenia. Oxycytes (60% tert-butylperfluorocyclohexane) (Oxygen Biotherapeutics Inc., North Carolina, USA) have been investigated in different animal models as well as in some clinical trials in patients with traumatic brain and spinal cord injuries (Castro and Briceno 2010; Hill 2019). Oxycytes completed phase II trials in 2008, but was discontinued in 2014 when several indemnity concerns were proposed (Castro and Briceno 2010; Sen Gupta

Table 5 Preclinical assessment (in vitro and in vivo) of HBOCs

Products	Purposes	Animal model/cell line	Administration (Route and dose)	Results	References
Hemoglobin microparticles (HbMP-700)	To investigate the influence of newly introduced HbMP-700 on vasoconstriction along with genetic toxicity	Mouse (*Mus musculus*, strain NMRI, young healthy adults)	Intravenous; 10 mL/kg body weight	No toxicity and clinical signs were observed during the experiment It impeded premature oversupply of oxygen and vasoconstriction and also gives high oxygen affinity	Kao et al. (2018, p. 700)
Hemoglobin particles (HbPs)-700 nm	To solve the vasoconstriction problem caused by NO scavenging and checking oversupply of oxygen	Wistar rats (3-month-old)	Intravenous; animal's blood (approximately 20%) was replaced by HbPs in 0.9% NaCl-2% HSA	No oversupply of oxygen NO scavenging limited	Bäumler et al. (2014)
	New Hb particles content assembled to 80% of local Hb content of RBC	Male C57BL/6 adult mice	–	Non-vasoconstrictive behavior No oversupply of oxygen NO scavenging limited	Xiong et al. (2013)
PEGylated carboxyhemoglobin bovine (SANGUINATE®)	To investigate the effect of sanguinate after myocardial infarction (MI)	Lewis rats (5 weeks)	In the left anterior descending artery, underwent ligation (MI) in rats and was treated with 10 mL/kg	Preserve the myocardium, heart function, and mitral competence after MI	Kawaguchi et al. (2018)
SanguinateTM (SG), PEGylated carboxyhemoglobin (COHb)	To inquire the effect of SanguinateTM (SG) in collateral & reperfusion cerebral blood flow (CBF) and brain injury during middle cerebral artery occlusion (MCAO)	Male spontaneous hypertensive rats	Sanguinate infused after 30 min (early treatment) and 90 min (delayed treatment) of MCAO	Preventing declination of reperfusion CBF Increasing collateral flow and sustaining it for 1.5 h of ischemia	Cipolla et al. (2018)
MP4OX (PEGylated HBOC with the high affinity of oxygen) and ααHb (αα-cross-linked HBOC with low affinity of oxygen)	To evaluate the properties of Hb that accord apoptosis in rat brain and if like this signs aid cryoprotection or damage	Sprague–Dawley rats (14 weeks)	Intravenous; MP4OX (4.3 g/dL) and purified αα Hb (4.4 g/dL)	MP4OX showed high levels of hypoxia-inducible factor (HIF-1α) than ααHb indicating MP4OX showed low levels of apoptosis than ααHb	Vandegriff et al. (2014)
Polynitroxylated PEGylated hemoglobin (PNPH, aka SanFlow)	To determine the effect of small amount transfusion of PNPH in traumatic brain injury (TBI) plus hemorrhagic shock model guinea pigs	Male Hartley guinea pigs (650 ± 110 g)	Intravenous; 10 mL/kg body weight	It gives neuroprotection to the guinea pig brain from secondary neurodegeneration	Seno et al. (2020)
OxyVita C	To evaluate effects (vasoactive) of OxyVita C on cerebral pial arteriole diameters and systemic blood pressures	Rats	Intravenous; 2 mL/kg body weight	In small and medium-sized pial arterioles, no vasoconstriction was observed In addition, no cerebral vasoconstriction was observed	Abutarboush et al. (2014)

Table 5 (continued)

Products	Purposes	Animal model/cell line	Administration (Route and dose)	Results	References
Liposome-encapsulated hemoglobin (LEH) with high O_2 affinity	To investigate the effect of LEH during skin wound healing in diabetic mice	Male dB/dB mice	Intravenous; 2 mL/kg	LEH quickly healed the wound in dB/dB mice In addition, decreased hypoxia, inflammation and raised surface perfusion, in situ cell proliferation	Fukui et al. (2017)
Liposome-encapsulated hemoglobin (LEH)	To investigate the capability of LEH to prevent hemorrhagic shock and to supply more oxygen	Male Sprague Dawley rats (250–300 g)	Intravenous; 1 mL/min	Reduce hemorrhagic shock-related pro-inflammatory cytokines and injury markers to the critical organs Decline the plasma levels of corticosterone (stress hormone)	Yadav et al. (2016)
	To evaluate the effect of LEH on resuscitation with 45% hypovolemic shock	Male Sprague Dawley rats (250–300 g, Age: 9–10 months)	Intravenous; 1 mL/min	Correct oxygen deficits Recuperate the cerebral metabolism and build a pro-survival phenotype	Rao et al. (2015)
	To assess the high O_2 affinity of LEH is better than the low O_2 affinity of LEH using positron emission tomography (PET) during middle cerebral artery occlusion (MCAO) and reperfusion	Cynomolgus monkeys (*Macaca fascicularis*)	Intravenous; 10 mL/kg body weight	High O_2 affinity of LEH reduces histological damage in the cerebral cortex and protected the cerebral metabolic rate of O_2	Kawaguchi et al. (2017)
LEH arisen on the use of polyethylene glycol connected with non-phospholipid hexadecylcarbamoylmethyl-hexadecanoate	To assess the effect of HDAS-PEG2K-LEH on the immunity of mice	Male Sprague Dawley rats (250–300 g)	Intravenous; 10 mL/kg body weight	The modified LEH is immune-neutral It is also well tolerable even it is used in repeated dose	Yadav et al. (2014)
Polymerized bovine Hbs (PolybHbs) (ratios: glutaraldehyde to bovine Hb is 10:1, 20:1, 30:1, 40:1)	To investigate the selection of Polymerized bovine Hbs from different ratios as oxygen therapeutics	Male Hartley guinea pigs (350–450 g)	Intravenous	30:1 preparation showed better circulatory response with low oxidation Also, less elevation of blood pressure, less iron is deposited in the liver	Baek et al. (2012)
Hemoglobin microparticles (HbMP-700)	To investigate oxidative pressure, vasoconstriction effect, and genetic toxicity	Mouse lymphoma L5178Y cells	–	The high affinity of oxygen, obstructs the oversupply of premature oxygen and avoid vasoconstriction of small blood vessel	Kao et al. (2018)

Table 5 (continued)

Products	Purposes	Animal model/cell line	Administration (Route and dose)	Results	References
$HemO_2Life$ (M101)	To investigate the toxicity of the liver	Progenitor HepaRG cells	–	Reducing amanitin-induced hepatotoxicity	Le Daré et al. (2021)

2017; Lambert et al. 2019; Jägers et al. 2021). Dodecafluoropentane (DDFPe) (2% DDFPe with 5% human serum albumin) completed phase Ib/II trials in 2019; intravenous administration in animal studies showed 3–7 times more oxygenation than other PFCs and 9–15 times more transport of oxygen than that via blood, with mild adverse effects; the first 3 h of infusion alleviated stroke complications (Culp et al. 2019; Graham et al. 2019).

Albumin-derived PFC-based AOC (A-AOC) (17% Perfluorodecalin with 5% human serum albumin) are cutting-edge technologies that have been used in animal models; researchers are attempting to improve on this development. A-AOC is a nanocapsule technology with high biocompatibility. In rats, A-AOC was well-tolerated during intravenous administration without changing other parameters of tissue injury (Wrobeln et al. 2017a). Furthermore, it is assumed that the A-AOC nanocapsules coat the surface area of nitrogen bubbles; hence, it can obstruct the mass collection of bubbles and thus qualify for successful transportation in blood plasma. It can also eliminate nitrogen bubbles, which depend not only on the nanocapsule shell permeability but also on the interchangeability of PFCs. Nitrogen bubbles are encapsulated by nanocapsules in the crescent site, which is further stabilized in an aqueous solution; Fig. 4b shows the capability of PFC-containing nanocapsules to bind with nitrogen bubbles attached to the wall of the endothelium and to transport it to the lungs for excretion (Mayer and Ferenz 2019).

In addition, erythrocytes are situated in the middle of the blood vessel, enclosed by a cell-free plasma layer due to the Fåhræus-Lindqvist effect, which occurs in < 0.3-mm diameter blood vessels; this effect proposes two outcomes for increasing oxygen transport by PFOCs. In Fig. 4c, skimming of plasma in the bifurcations of the blood vessel is the first outcome, in which the RBC amount is higher in the larger vessel, whereas this condition is alleviated in the microcirculation. During shock or other pathological conditions, it causes tissue hypoxia and vasoconstriction, which is an obstacle for the movement of RBCs. Nevertheless, PFOCs can penetrate and pass through narrow blood vessels under such conditions, owing to their nano-sized droplets and sustained oxygen supply (Culp et al. 2012; Ryzhkov et al. 2016; Jägers et al. 2021). Another outcome was the diffusion distance, which was enhanced by the plasma layer. PFOCs are capable of decreasing the diffusion distance because of their close connection to the endothelia, thus acting as stepping stones for O_2 (Fig. 4c) (Spiess 2009; Wrobeln et al. 2017b; Jägers et al. 2021).

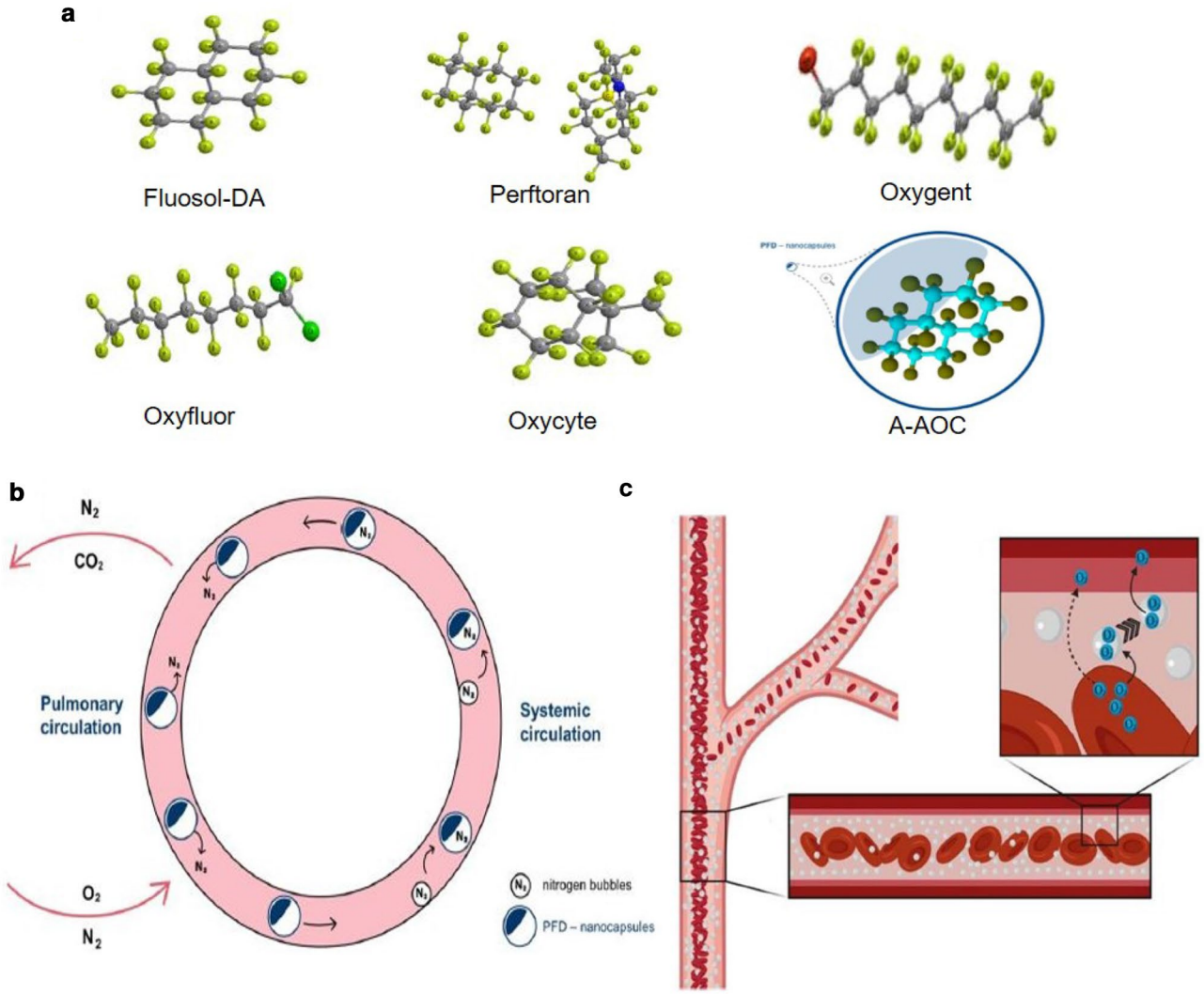

Fig. 4 **a** Fluosol-DA (perfluorodecalin), Perftoran (perfluorodecalin+perfluoromethyl-cyclohexylpiperidine), Oxygent (perfluorooctyl bromide), Oxyfluor (perfluorodichlorooctane), Oxycyte (tertbutylperfluorocyclohexane) (Reprinted with permission from (Modery-Pawlowski et al. 2013). Copyright © 2013, American Chemical Society), Albumin-derived perfluorocarbon based artificial oxygen carrier (A-AOC) [Reprinted with permission from (Mayer and Ferenz 2019). Copyright © 2019, The Author(s), Springer]. **b** This figure exhibited the capability of PFC containing nanocapsules bind with nitrogen from bubbles attached with the wall of endothelium and transportation to the lungs for excretion [Reprinted with permission from (Mayer and Ferenz 2019). Copyright © 2019, The Author(s), Springer] **c** Increase number of RBCs are located in the middle area of the blood vessel which is surrounded by the plasma layer. RBC number is decreased in the bifurcation of the vessel (plasma skimming), the nanosized PFC droplets (light grey) can penetrate and uniformly distribute in the blood vessel without plasma skimming and the O_2 uptake into the PFC droplet occurs rapidly which decrease the diffusion distance between RBCs and endothelium and act as stepping-stones for O_2 [Reprinted with permission from (Jägers et al. 2021) Copyright © 2020, The Author(s), Springer Nature]

Preclinical assessment (in vitro and in vivo) for evaluation of efficacy and safety of oxygen transport through PFOCs

PFC emulsions are used for oxygen delivery owing to their nano-sized particles, which range from 0.2 µm in diameter and is able to perfuse microcapillaries, where RBCs cannot enter and flow due to their larger size (7 µm). It has been used in cell lines (in vitro) and animal models (in vivo) to evaluate the efficacy and safety of PFC (Table 7). A new development of A-AOC showed better biocompatibility and longer half-life circulation, thus resulting in good oxygen transportation in different animal models (Wrobeln et al. 2017b; Ferenz 2017). It was well-tolerated by intravenous administration and gave higher oxygen transport capacity in rats compared to Perftoran® (Wrobeln et al. 2017a). In addition, A-AOC displayed stable body temperature, pH, higher partial pressure of oxygen, and lower partial pressure of CO_2, which was better for improved oxygenation. It can

Table 6 Formulation and properties of different Perfluorocarbon-based oxygen carriers (PFOCs)

Products name	Formulations (%W/V PFC)	Surfactants (%W/V)	Other Substances	Storage condition and shelf-life	Clinical uses	Major side effects	Current status	References
Fluosol-DA (Green Cross Corp.)	14% Perfluorodecalin and 6% Perfluorotripropylamine (20% W/10.6% volume)	2.7% pluronic F-68, 0.4% egg yolk phospholipid, 0.03% potassium oleate	Sodium chloride, potassium chloride, calcium chloride, calcium bicarbonate, magnesium chloride, glycerol, and dextrose	Frozen condition	Hemorrhage, carbon monoxide poisoning, cerebral hypoxia, anemia, angioplasty	Pulmonary complication, pneumonia, reducing platelet counts, increased white blood cell (WBC) count, and decreased neutrophils and platelets	Clinical trials finished in the 1980s; Approved in 1989 by FDA and discontinued in 1994 due to side effects	Ohyanagi et al. (1984), Police et al. (1985), Riess (2001), Castro and Briceno (2010), Jägers et al. (2021)
Oxypherol (Formerly Fluosol 43) (Green cross crop. And Alpha therapertic)	20% perfluorotributylamine	Pluronic F-68	Krebs–Ringer bicarbonate solution	Not available	Perfusion of isolated organs, vasodilation, inflammation	Unacceptable longer half-life in body tissues approximately 500 days	Not available	Castro and Briceno (2010), Jägers et al. (2021)
Perftoran (Perftoran, Russia)	14% Perfluorodecalin and 6% perfluoromethylcyclohexylpiperidin	6.5% proxanol 268, egg yolk phospholipid	Sodium chloride, potassium chloride, magnesium chloride, baking soda, monosubstituted sodium phosphate, glucose, and water	Frozen: − 18 °C to − 4 °C for 3 years or under refrigeration: 4 °C for 2 weeks	Perfusion of organs and hemorrhagic shock	Pulmonary complications and hypotension	Approved for clinical use in Russia, Kazakhstan, Kyrgyzstan, Ukraine, and Mexico from 2005 to 2010. Recently, re-branded as Vidaphor™ in North America	Spahn et al. (2002), Castro and Briceno (2010), Jägers et al. (2021), Krafft and Riess (2021)
Oxygent (Alliance Pharmaceutical Corp., USA)	58% Perfluorooctyl bromide and 2% perfluorodecyl bromide	3.6% egg yolk phospholipid	α-Tocopherol and EDTA, sodium chloride and phosphate buffer	Under refrigeration: 5–10 °C for 1–2 years	Orthopedic surgery, cardiovascular surgery, non-cardiac surgery, coronary bypass, and coagulation	Stroke and flu-like symptoms	Reached phase III trials, licensed and accepted in China for clinical studies in 2017	Castro and Briceno (2010), Ferenz and Steinbicker (2019), Jägers et al. (2021), Krafft and Riess (2021)
Oxyfluor (HemaGen, St. Louis, USA)	78% Perfluorodichlorooctane	Egg yolk phospholipid and Safflower oil	–	Room temperature: 1 year	Hemorrhagic shock, Cardiopulmonary bypass	Stroke	Phase III trials were suspended	Spahn et al. (2002), Castro and Briceno (2010), Hill (2019) Mayer and Ferenz (2019), Jägers et al. (2021)

Table 6 (continued)

Products name	Formulations (%W/V PFC)	Surfactants (%W/V)	Other Substances	Storage condition and shelf-life	Clinical uses	Major side effects	Current status	References
Oxycyte (Oxygen Biotherapeutics Inc., North Carolina, USA)	60% tertbutylperfluorocyclohexane	Egg yolk phospholipid	–	–	Traumatic brain injury, acute ischemic stroke	Ischemic brain damage	Phase II was completed in 2008, but terminated in September, 2014 due to lack of patient assignation	Castro and Briceno (2010), Lambert et al. (2019), Jägers et al. (2021)
Dodecafluoropentane (DDFPe) (also known as perfluoropentane) (NuvOx Pharma, LLC, Tucson, Arizona)	2% DDFPe	5% human serum albumin	Buffered sucrose solution	Room temperature: 1 year, at 4 °C: 2 years and shorten shelf-life at high temperature	Hemorrhagic shock, traumatic brain injury (TBI), ischemia–reperfusion injury	Increase blood pressure and coughing	Phase Ib/II completed in 2018 with acute ischemic stroke	Culp et al. (2019), Graham et al. (2019), Jägers et al. (2021)
Albumin derived perfluorocarbon based artificial oxygen carrier (A-AOC) (New development)	17% Perfluorodecalin	5% human serum albumin (HSA)	–	Shelf-life: 1 year	Not yet available	–	Performing preclinical in vivo study	Wrobeln et al. (2017a), Ferenz (2017), Ferenz and Steinbicker (2019), Jägers et al. (2021), Lambert and Janjic (2021)

impede hypoxic tissue damage, although it shows higher arterial blood pressure and lower blood glucose levels in treated rats (Wrobeln et al. 2020). Moreover, it significantly decreased decompression sickness (DCS) lesions and mortality rates in a rat model (Mayer et al. 2020). Recently, A-AOC has been analyzed in animal models, wherein scientists have tried to improve and establish its implementation owing to its fewer side effects. Oxycytes improves oxygen transport in the blood and lungs (Haque et al. 2016). It improved the prognosis of spinal cord injuries (Mahon et al. 2013a), but was discontinued due to indemnity concerns in 2014 (Castro and Briceno 2010; Lambert et al. 2019; Jägers et al. 2021). Dodecafluoropentane (DDFPe) alone neither enhances the survival rate nor improves oxygen transport compared to fresh whole blood (FWB) after resuscitation in swine (Bonanno et al. 2018); however, it provided more oxygenation and increased oxygen transport in rat brain tissue (Moon-Massat et al. 2014). Therefore, DDFPe requires further pre-clinical evaluation, although it has completed phase Ib/II trials for acute ischemic stroke (Culp et al. 2019; Graham et al. 2019; Jägers et al. 2021). Usually, PFCs are administered intravenously; hence, PLGA-PEG/PFC emulsion was delivered via pulmonary delivery in rats to investigate oxygen transport, which showed increased oxygen transport with improved lung ventilation (Yao et al. 2015).

Advantages of PFOCs

PFCs have emerged as effective materials because of their physicochemical properties, which physically dissolve significant quantities of gaseous species along with respiratory gases, such as oxygen, CO, CO_2, and NO (Lowe 2001). When PFC and RBC are present together in the circulation, the oxygen release of PFC firstly acts as a safeguard for Hb-bound oxygen until its arrival in hypoxic tissues (Cabrales et al. 2007). PFCs are resistant to physical parameters such as pH, and are not adversely affected by temperature changes as compared to AOCs. The oxygen carrier function of PFCs is not significantly influenced by pharmacological, environmental, and chemical factors. Moreover, they are chemically resistant to heat and do not undergo metabolic transformation in vivo. Hence, PFOCs are a secure choice as AOCs as compared to HBOCs, which exhibit side effects such as immune reactions (except some modified LEH conjugated with PEG2K and non-phospholipid hexadecyl-carbamoyl methyl hexadecanoate which is immune neutral and well tolerated in repeated dose), high blood pressure, and short half-life (Lambert and Janjic 2021). Furthermore, PFCs allow optimal oxygenation in the human body because they do not interact with oxygen; thus, oxygen supply increases at the plasma level.

The most beneficial effect of PFCs is that they can be preserved at room temperature for more than 1 year and can penetrate small blood vessels and arterial blockages for oxygen transport (Haldar et al. 2019; Lambert and Janjic 2021). PFOCs has higher storage stability compared to other oxygen carriers because of they are functionally resistant to temperature and pH influence and also chemically heat resistant due to their covalent carbon–fluorine bond (Lambert and Janjic 2021). Newly developed albumin-derived PFC-based nanoparticles act as novel AOCs and exhibit higher oxygen transportation capacity without many undesirable effects in rat animal models (Wrobeln et al. 2017a). In addition, these nanoparticles can also protect tissues from hypoxic damage; however, they have not yet been tested in clinical trials (Wrobeln et al. 2020). The study of PFOCs was successful in non-cardiac surgery without major safety concerns, and reduced the need for allogeneic RBC transfusion (Spahn 2018).

Synthetically produced porphyrin-based AOCs

Chemically similar structures of natural Hb and myoglobin contain porphyrin groups in cyclic form with four pyrrole rings attached by methine bridges. In the porphyrin rings, the pyrrole nitrogen groups approve ferrous ion chelation to protoporphyrin, which takes part in oxidative metabolism and iron chelation (ferrous) for protoporphyrin to generate 'heme', which is the active site of oxygen transfer (Themes 2017). Therefore, many researchers have investigated Fe (II)-containing porphyrin systems for oxygen transfer in the body (Table 8). These synthetically produced porphyrins unite the heme molecule with interchanging chemicals to express an interrupted hydrophobic matrix (Themes 2017; Bialas et al. 2019). Information on the in vitro and in vivo applications of porphyrin-based oxygen carrier systems is limited; further pre-clinical assessment is needed in proper cell lines and animal models. Owing to the lack of this information, we included several experiments on different porphyrin (synthetic)-based oxygen carriers, which showed the efficiency of this system.

Previously, "picket fence" Fe^{2+} porphyrin molecules were revealed by Collman et al. in the 1970s to narrate the *α4*-atropisomer of [5,10,15,20-tetrakis(2-pivalamidophenyl) porphyrinato] iron (II), or FeTpivPP (Fig. 5a), which exhibited the reversible oxygenation of Hb and myoglobin (Modery-Pawlowski et al. 2013; Norvaiša et al. 2021). Picket fence porphyrins have four pivalamide groups at the ortho-positions of the phenyl groups. This is the first instance of a myoglobin model that envisages both prosthetic group and apoprotein functions (Kitagishi and Kano 2021). Previously, Gottwald and Ullman successfully identified four separate 5,10,15,20-tetrakis(*o*-hydroxyphenyl) porphyrin atropisomers. In addition, the former model produced the μ-oxo ferric dimer, which was capable of irreversible iron oxidation, although it showed a delusion of reversible oxidation. The

Table 7 Preclinical assessment (in vitro and in vivo) of PFOCs

Products Name	Purposes	Animal model/Cell line	Administration (Route and dose)	Results	References
Albumin-derived perfluorocarbon-based artificial oxygen carrier (A-AOC)	To prove the function of albumin-derived perfluorocarbon as novel AOCs in a rat Langendorff-heart perfusion model	Female wistar rats (*Rattus norvegicus*, 225–275 g, age-4 months)	Several concentrations of capsules (2, 4, and 6 vol%)	It preserved the rat heart function because of good oxygen transportation	Wrobeln et al. (2017b)
	To evaluate the physicochemical characteristics and pharmacological performance of albumin-derived perfluorocarbon-based AOCs	Male Wistar rats (*Rattus norvegicus*, 400–450 g)	32 vol% or 64 vol% capsules (20 mL/kg body weight X h); Intravenous	In healthy rats, intravenous administration is well tolerated. Except for the spleen (some minor tissue damage, maybe due to effects of dose), any objectionable effects were not observed	Wrobeln et al. (2017a)
	To re-tested the improvement of in-vivo evaluation of biocompatibility of new nanocapsule (A-AOCs)	Male Wistar rats (*Rattus norvegicus*)	20 mL/kg body weight x h; Intravenous	This new nanocapsule showed better biocompatibility and longer half-life circulation	Ferenz (2017)
	To evaluate the albumin derived capsule function in a normovolemic hemodilution rat-model	Male Wistar rats (Rattus norvegicus, 430–460 g)	5% HSA solution together with 10 mM glucose comprising 12 vol% capsules; Intravenous	After being treated with this capsule, animals showed high arterial blood pressure, stable body temperature and pH, higher partial pressure of oxygen and as well as lower partial pressure of CO_2. Finally, this capsule impeded hypoxic tissue damage	Wrobeln et al. (2020)
	To prevent decompression sickness (DCS)	Male Wistar rats (318–430 g; 11 weeks old)	Albumin nanocapsules filled with neutral oil; Intravenous	A-AOC decreased DCS lesions and mortality significantly	Mayer et al. (2020)
Perftoran	To evaluate the properties (potential vasoactive) of perftoran by measurement of pial arteriolar diameters in a healthy rat brain	Rat (Sprague–Dawley, Male, 300–450 g)	Perftoran 10 mL/kg/h per infusion	It does not increase vasoconstriction in the pial arterioles of the brain. Additionally, it does not elevate systemic blood pressure compared with the control group	Abutarboush et al. (2016)
Oxygent (w/v 60% PFC) or Perftoran (w/v 20% PFC)	To know the extent effects of platelet like mechanism, inflammation after PFC treatment	Sheep (juvenile female Dorser (Dorper); 3–4 months old, 18–32 kg)	5 mL/kg of Oxygent (w/v 60% PFC) or Perftoran (w/v 20% PFC); Intravenous	After PFC infusion, there were no changes in inflammatory cell lines. After oxygent infusion, decreased no of platelet found on day 4 which was corrected on day 7. In the case of perftoran infusion, no platelet effect was found	Zhu et al. (2021)

Table 7 (continued)

Products Name	Purposes	Animal model/Cell line	Administration (Route and dose)	Results	References
Dodecafluoropentane (DDFPe)	To evaluate the dodecafluoropentane efficacy compared with fresh whole blood (FWB) after resuscitation	Male Yorkshire swine (78 ± 5 kg)	2 mL/min of DDFPe; Intravenous	DDFPe administration with fresh frozen plasma (FFP) does not enhance survival rate or ameliorate oxygen transport	Bonanno et al. (2018)
Oxycyte	To evaluate the seizure latency (oxygen toxicity of CNS) and duration after PFC administration with 6 ATA of oxygen in swine	Yorkshire swine	5 ml/kg of the PFC Oxycyte; Intravenous	The result exhibited safety during the use of PFC to treat DCS	Mahon et al. (2013b)
	To know the effect of oxycyte after a lateral fluid percussion injury (LFPI) on cognitive recovery and mitochondrial oxygen consumption treatment	Rats (Adult male Sprague–Dawley)	A lower dose of Oxycyte (4.5 mL/kg); a higher dose of Oxycyte (9.0 mL/kg); Intravenous	It showed improvement of cognitive recovery and abated the loss of CA3 neuronal cell	Zhou et al. (2008)
	To assess the effect of PFC oxtcyte™ in severe decompression sickness (DCS) in ovine model	Juvenile male sheep (weight 24.4 ± 2.10 kg)	5 ml/kg of the PFC Oxycyte; Intravenous	After the onset of DCS, oxtcyte™ reduce injury of the spinal cord although did not decrease the mortality rate	Cronin et al. (2021)
	To know the effect of decreasing the dose of oxycyte (3 cc/kg) in swine model of DCS	Yorkshire swine	3 cc/kg of the PFC Oxycyte; Intravenous	It improves injury of the spinal cord, but it didn't significantly increase the survival benefit	Mahon et al. (2013a)
	As PFCs showed 50 times more oxygenation than human plasma, so they wanted to evaluate the intravenous dose of the PFC emulsion whether it ameliorate the tissue oxygenation and alleviate the oleic acid lung injury (OALI) effects	Yorkshire swine	5 mL/kg of the PFC Oxycyte; Intravenous	After treatment of OALI, it ameliorates oxygen transport in the blood and histology of lung	Haque et al. (2016)
NVX-108 (Perfluorocarbon Dodecafluoropentane)	To know the effects of NVX-108 on cerebral microvasculature in rat	Male Sprague–Dawley rats	Intravenous infusion (High dose: 1.0 mL/kg; low dose: 0.25 ml/kg)	It provides more oxygen and increases oxygen transport in brain tissue except for vasoactivity (systemic or cerebral)	Moon-Massat et al. (2014)

Table 7 (continued)

Products Name	Purposes	Animal model/Cell line	Administration (Route and dose)	Results	References
PLGA-PEG/PFC emulsion	(i) To assess the PLGA-PEG/PFOB emulsion effect on HCT 116 cell viability, intracellular ROS production, and for detection of the hypoxic condition, reoxygenation by expression of HIF-1α	HCT 116 cells (in vitro); Rat (in vivo)	cell viability assay for HCT 116 cells;	Cell viability and intracellular ROS exposed hypoxia-reoxygenation injury in HCT 116 cells which were sub-lethal and HIF-1α contributed to cell viability;	Yao et al. (2015)
	(ii) To assess oxygen transport through new administration way "pulmonary delivery" in rats		0.3 mL of the emulsion through pulmonary delivery (from trachea)	PLGA-PEG/PFC emulsion increased oxygen transport which improved lung ventilation in rats	
PFC nano emulsion (Perfluorodecalin; perfluorotributylamine; perfluorooctylbromide)	To optimize the nanoscale perfluoro emulsion through evaluation of different critical factors like materials, emulsification time, and particle size with stability	Mouse insulinoma beta cells (MIN-6, passages 30–40)	300 µL of pure PFC inoculate per well in 12 well plate	It demonstrates particle size affecting transportation of oxygen and enhanced micelle size decrease diffusion of oxygen	Fraker et al. (2012)

main objective of picket fence porphyrin was to build a protective pocket for dioxygen binding similar to natural Hb, but were amendable to irreversible oxidation in aqueous solutions (Modery-Pawlowski et al. 2013; Norvaiša et al. 2021). Li et al. examined three different iron-based picket fence porphyrins, namely Fe (TpivPP)(1-EtIm) (O_2), Fe (TpivPP) (1-MeIm) (O_2), and Fe (TpivPP)(2-MeHIm) (O_2), to determine the rotation of Fe-oxygen and tert-butyl motion using multitemperature X-ray structural studies and Mössbauer spectroscopy. The results indicated that the Fe-oxygen bond was temperature-dependent, and not orientational (Li et al. 2013). The experimental conditions of the picket fence porphyrin system are listed in Table 9.

To avoid irreversible oxidation in a picket aqueous solution, scientists constructed an iron porphyrin, which was attached to the phospholipid liposome bilayer (Fig. 5b) (Tsuchida et al. 2009). Liposomes are spherical aqueous inner core vesicles surrounded by a lipid bilayer consisting of phospholipids (natural or synthetic) and sterols (Noh et al. 2022); reversible oxidation is possible due to their hydrophobic and non-polar environment. Lipid-heme showed high consistency with phospholipids, forming an immensely stable lipid-heme liposome that could reversibly bind oxygen (Tsuchida et al. 2009). A few in vivo experiments were conducted to check this lipid-heme porphyrin, which is summarized in Table 10 along with other experiments.

Another synthetic heme model has been developed, the human serum albumin (HSA) incorporated with iron porphyrin systems (Fig. 5c), which showed oxygen-carrying abilities similar to that of Hb and myoglobin. HSA is the most abundant protein found in blood plasma (Komatsu et al. 2005b; Nakagawa et al. 2008; Watanabe et al. 2012). Recombinant human serum albumin (rHSA) with iron porphyrin showed good blood compatibility and longer half-life, with similar oxygen distribution to the tissues. In addition to HSA and iron porphyrin, polyethylene glycol (PEG) has also been used, thus resulting in an increased circulation time and reduced oxidation (Nakagawa et al. 2007). The experimental conditions of the HSA-heme synthetic system are presented in Table 11.

Another cutting-edge technology is HemoCD (iron porphyrin complex), which is composed of a 1:1 complex of 5,10,15,20-tetrakis (4-sulfonatophenyl)porphinatoiron(II) (Fe[II]TPPS) and a per-*O*-methylated b-cyclodextrin dimer with a pyridine linker (Py3CD) (Fig. 5d) (Kano and Kitagishi 2009). Cyclodextrin dimers are toroidal in shape and consist of oligosaccharides attached to D-glucopyranose units (Kim et al. 2020) and encapsulated Fe[II]TPPS, which is necessary for oxygen binding and has a longer half-life (Kano and Kitagishi 2009). In addition, another new 1:1 complex, Fe^{II}PImCD (5,10,15,20-tetrakis- (4-sulfonatophenyl) porphinatoiron(II) (FeIIP) and an O-methylated b-cyclodextrin dimer with an imidazole linker, (ImCD),

Table 8 Synthetically produced porphyrin-based AOCs

Synthetically produced porphyrin-based AOCs	Used components	References
'Picket fence' porphyrin	Iron (II) containing heme group interchanging of the molecule and attached in a hydrophobic matrix (eg- polymer, albumin)	Kitagishi and Kano (2021)
LipidHeme	Iron (II) porphyrin attached to the phospholipid liposome bilayer	Komatsu et al. (1994)
HSA-heme	Iron (II) containing porphyrin systems attached within HSA microsphere structures	Tsuchida et al. (1999, p. 2)
HemoCD	Iron (II) porphyrin systems attached within the middle of the cyclodextrin (hydrophobic pockets)	Kitagishi et al. (2017)

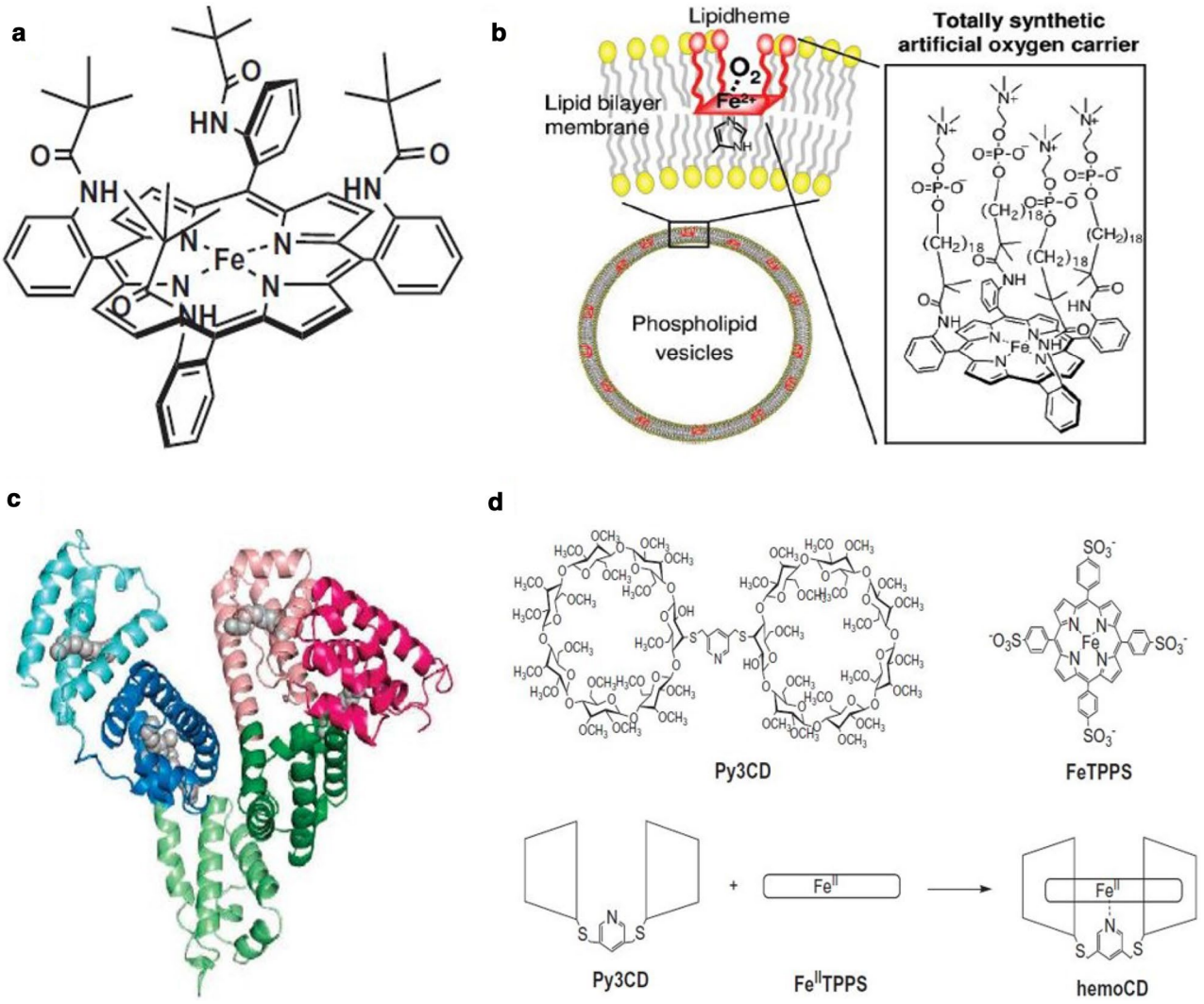

Fig. 5 **a** Structure of picket-fence porphyrin [Reprinted with permission from (Kano and Kitagishi 2009). Copyright © 2009, The Authors. Journal compilation © 2009, International Center for Artificial Organs and Transplantation and Wiley Periodicals, Inc.], **b** LipidHeme porphyrin vesicles as an artificial O_2 carrier [Reprinted with permission from (Tsuchida et al. 2009). Copyright © 2009, American Chemical Society] **c** HSA-heme porphyrin as an artificial O_2 carrier (crystal structure) [Reprinted with permission from (Tsuchida et al. 2009). Copyright © 2009, American Chemical Society], **d** Structures of Py3CD, FeTPPS, and hemoCD [Reprinted (adapted) with permission from (Kano and Kitagishi 2009). Copyright © 2009, The Authors. Journal compilation © 2009, International Center for Artificial Organs and Transplantation and Wiley Periodicals, Inc.]

exhibited 10-times increased dioxygen affinity than that of HemoCD (Kano et al. 2006). HemoCDs were modified with PEGylated dendrons to enhance their circulation time in the blood, which was more effective in protecting HemoCDs from opsonization by the reticuloendothelial system (Kano et al. 2011; Karasugi et al. 2012). Several experiments were conducted on HemoCD, as presented in Table 12. In addition, HemoCD depleted CO under in vivo conditions (Kitagishi and Minegishi 2017) (Table 12). These nanotechnologies require pre-clinical assessment in animal models as well as clinical evaluation for further improvement.

O_2 micro/nanobubbles

Micro/nanobubble-mediated oxygen transport is another method of oxygen delivery under hypoxic conditions. It can be used to transport oxygen directly to deoxygenated erythrocytes, hypoxic tissues, and blood vessels (Bialas et al. 2019). Microbubbles and nanobubbles (MNBs) are spherical vesicles made by encapsulating different shells, such as phospholipids, proteins, and polymers, with a core-containing gas. Microbubbles have been investigated for non-invasive molecular imaging process, called the "photoacoustic imaging method", whereas nanobubbles have been used for therapeutics and diagnosis (Khan et al. 2018a). MNBs are usually used to reverse hypoxia, which is a typical feature of solid tumors resulting in decreased therapeutic reaction and malignancy. Hypoxic cells are more resistant to chemo- or radiotherapy (Eisenbrey et al. 2015; Fix et al. 2015). Using MNBs, it is possible to improve the oxygen enhancement ratio (OER), which increases the sensitivity of tumor radiation therapy (Kwan et al. 2012; Khan et al. 2018a). The MNBs are smaller (0.1–20 µm) and can enter into major and minor blood vessels; their stability is controlled by Laplace pressure (inside and outside shell pressure difference), coalescence, and Ostwald ripening (Hernot and Klibanov 2008; Lee et al. 2015; Fix et al. 2015). The common structure of MNBs is shown in Fig. 6a, where the core gas is surrounded by a hydrophilic shell or amphiphilic biomaterial that has been used in different areas, such as drug delivery, oxygen transport, molecular imaging, and gene therapy (Khan et al. 2018a).

Different shells (lipids, proteins, polymers, and surfactants) exhibit numerous significant functions, including the mechanical stability of bubbles, protection of bubbles, and increasing oxygen transport safety, by decrementing the relationship between gas and neighboring blood and tissues (Fix et al. 2015). Lipid shell materials are the most common microbubbles, which are approximately 3 nm in thickness and are composed of phospholipids that are frozen by block copolymers, thus forming lipid shell microbubble emulsions (2–4 µm in diameter) (Fig. 6b: left panel) (Fix et al. 2015; Khan et al. 2018a). Figure 6b (right panel) shows the rapid transport of encapsulated oxygen to deoxygenated RBCs (Tao and Ghoroghchian 2014). The permeability of oxygen ranges from 10^{-4} cm/s to 10^{-3} cm/s in lipid shell microbubbles (Fix et al. 2015). The compositions and characteristics of different phospholipid microbubbles are listed in Table 13.

Protein-shelled MNBs are synthesized by protein denaturation and emulsification, which form a monolayer shell around the core gas. These shells are rigid and beneficial for their stability, half-life, and amphiphilicity. Protein shell-type microbubble formulations, such as Albunex (commercial products), have been accepted by the FDA for commercial use. The thickness of the protein shell was 15 nm, whereas the diameter of the bubble was 1–15 µm (Sirsi and Borden 2009; Swanson and Borden 2010; Khan et al. 2018a). Polymer shells are tenacious and thicker than other shells. They was able to improve stability; however, owing to oscillation defense until the shell cracked, echogenicity was decreased (Fix et al. 2015). Their thickness is 150–200 nm; during ultrasound, they are more resistant to expansion and compression. MNBs are synthesized using several processes, including sonication, microfluidic devices, agitation, inkjet processes, and laser ablation (Hernot and Klibanov 2008; Sirsi and Borden 2009; Khan et al. 2018a).

Preclinical assessment (in vivo) for evaluation of efficacy and safety of oxygen transport through O_2 microbubbles

Researchers have established different therapeutic methods for artificial transportation of oxygen. Microbubbles, which showed more oxygenation during hypoxic conditions, have been applied in several animal models to assess their efficacy (Table 14). Instead of intravenous administration, some MNBs are used for peritoneal oxygenation in animal models with a large peritoneal surface area. The main advantage is their smooth penetration for catheterization in the peritoneal cavity, wherein oxygen circulates safely and the mesothelium acts as a gas permeable barrier (Feshitan et al. 2014). Furthermore, DSPC, PEG-40-S (9:1) was administered peritoneally in lung-injured rats, which showed higher oxygen-carrying capacity (Feshitan et al. 2014). These results suggested a probable benefit of MNBs for hypoxic patients (Legband et al. 2015). Surfactant-stabilized microbubble, $SE61_{O2}$, was more workable for oxygenation in hypoxic tissues in mice (Eisenbrey et al. 2015). DSPC, (DSPE-PEG-2000Amine), and (DSPE-PEG-2000-Biotin) was applied to MDA-MB-231 breast cancer cells to assess the performance

Table 9 Experiments about picket fence porphyrin

Name	Purposes	Used porphyrin	Results	References
Iron-based 'Picket fence' porphyrin	To determine the rotation of Fe-oxygen and also tert-butyl motion of three different iron-based picket fence porphyrins (Fe (TpivPP)(1-EtIm) (O_2), Fe (TpivPP)(1-MeIm) (O_2), Fe (TpivPP) (2-MeHIm) (O_2))	(i) (Fe (TpivPP)(1-EtIm) (O_2) (ii) Fe (TpivPP)(1-MeIm) (O_2) (iii) Fe (TpivPP)(2-MeHIm) (O_2))	The results indicate the bond of Fe-oxygen was temperature-dependent, not orientational preference	Li et al. (2013)
	Hemoglobin showed cooperative binding of O_2 which was exhibited high affinity at high pO_2 and low affinity at low pO_2 in the lungs and tissues respectively. In this study, they reported such cooperativity in synthetic ferrous porphyrins quantitatively	(i) Meso-tetra(a,a,a,a-o-pivalamidophenyl) porphyrinato Iron(II)-2-methylimidazole (FeTpivPP(2MeIm)) (ii) Meso-tetra(a,a,a,a-o-pivalamidophenyl)porphyrinato Iron (II)1,2-dimethylimidazole (FeTpivPP(Me_2Im))	The results indicate the O_2 binding process was similar for Hb and that model	Collman et al. (1978)
Synthetic iron (II) porphyrin (FeP) with heat resistant recombinant enzymes	To examine physicochemical characteristics, O_2 binding properties, and enzymatic features of recombinant enzymes *Thermotoga maritima* xylanase B and *Dictyoglomus thermophilum* xylanase B	(i) 2-[8-(2-Methylimidazolyl) octanoyloxymethyl]-5,10,15,20-tetrakis [(R, R, R, R-*o*-pivalamido) phenyl] porphinatoiron(III) bromide [FeP Br-] (ii) *Thermotoga maritima* xylanase B (iii) *Dictyoglomus thermophilum* xylanase B	The heat resistant recombinant enzymes were incorporated with synthetic iron (II) porphyrin which can make O_2 complexes up to 90 °C temperature. It was the first synthetic heat-resistant O_2-carrying enzyme	Komatsu et al. (2005a)
Picket-fence cobalt porphyrin	To examine the oxygen transport facilitation in membranes by Picket-fence cobalt porphyrin (CoP) with four polymer matrixes	(i) *meso*-R, R, R, R-tetrakis(*o*-pivalamidophenyl) porphyrinatocobalt (II) (ii) Poly (octyl methacrylate-*co*-vinylimidazole) (Olm-CoP) (iii) Poly (lauryl methacrylate-*co*-vinylimidazole) (Llm-CoP) (iv) Poly (vinylidene dichloride-*co*-vinylimidazole*co*-methyl methacrylate) (Clm-CoP) (v) Poly(1-trimethylsilyl-1-propyne) (SP/Blm-CoP)	The polymer matrix with the CoP membrane was bound with oxygen and exhibited oxygen transport facilitation. SP/Blm-CoP membrane showed high oxygen permeability with lowest separation factor, but CIm-CoP membrane showed highest separation factor, although it has a low oxygen permeability	Shentu and Nishide (2003)

Table 10 Experiments about LipidHeme porphyrin system

Name	Purposes	Used components	Results	References
Liposome-embedded-heme (L/H)	To examine the oxygen-carrying capacity by interchange transfusion in beagles	Liposome-embedded-heme (L/H) solution; 15 ml/kg of blood; Intravenously	The Liposome-embedded-heme (L/H) was able to unite with oxygen and transport and release to the tissue	Kobayashi et al. (1991)
Liposome-embedded-heme/microsphere (LH-M)	To synthesize new synthetic oxygen carrier made by microsphere (coating oil droplets) with synthetic lipidheme in dogs	Liposome-embedded-heme (L/H) solution; 30 ml/kg of blood; Intravenously	The Liposome-embedded-heme (L/H) conducted oxygen and exempted it to the tissue	Kakizaki et al. (1994)
	To examine the structure, characteristics of solution, and oxygen-binding capability of lipidheme- microsphere	(i) Triglyceride microsphere (ii) Heme phospholipid derivative (5,10,15,20-tetrakis [α, α, α, α -o-[2,2-dimethyl-20- [2-(trimethylammonioethoxy) phosphonatoxy]eicosanamido]phenyl]porphinatoiron(II)	Solubility of oxygen was greater than heme concentration in human blood	Komatsu et al. (1994)
	To compare the oxygen-carrying capability between red blood cells and heme/lipid microsphere	(i)Lipophilic heme (1-laurylimidazole-ligated 5,10,15,20-tetrakis (α, α, α, α -o-pivalamidophenyl) porphinatoiron(II) complex)	The oxygen transport was similar to oxyhemoglobin	Tsuchida et al. (1992)
		(ii) Triglyceride microsphere [Prepared a red color lipid microspheres suspension (approx. 250 nm in diameter)]	The lipid heme microspheres release oxygen at 37 °C in the aqueous solution	
		(iii)Intravenous injection in rabbits		
Liposome-embedded-heme (L/H) and Liposome-embedded-heme/microsphere (LH-M)	To evaluate the capability of oxygen transport of two types of oxygen carrier in beagles followed by hemorrhagic shock	(i) Liposome-embedded-heme (L/H)	In the case of L/H, 12.7–24.4% oxygen consumed of the total volume	Kobayashi et al. (1994)
		(ii) Liposome-embedded-heme/ triglyceride microsphere (LH- M)	In the case of (LH- M), 13.1–16.4% of oxygen consumed of the total volume	

Table 11 Experiments about of HSA-Heme porphyrin system

Name	Purpose	Used components	Results	References
Human serum albumin (HSA) incorporating with 5,10,15,20-tetrakis{R,R,R,R-o-(1′-methylcyclohexanamido)phenyl} porphinatoiron(II) and covalently attached 1-methyl-L-histidine or 3-methyl-L-histidine (HSA-FeP(1-MHis), HSA-FeP(3-MHis))	To assess the binding capacity of oxygen of human serum albumin (HSA) attached with iron porphyrin with 1-methyl-L-histidine or 3-methyl-L-histidine linking	(i) Human serum albumin (HSA) (ii) 2-[{4-methoxycarbonyl(1-methyl) histidinamidobutanoyloxy}methyl]-5,10,15,20-tetrakis{R,R,R,R-o-(1′-methylcyclohexanamido)phenyl} porphinatoiron; (iii) 2-[{4-methoxycarbonyl(3-methyl) histidinamidobutanoyloxy}methyl]-5,10,15,20-tetrakis{R,R,R,R-o-(1′-methylcyclohexanamido)phenyl} por-phinatoiron	The complex of HSA with iron porphyrin linked with methyl-L-histidine isomer showed greater oxygen affinity by a factor of 90 in toluene	Nakagawa et al. (2008)
Human serum albumin (HSA) based peroxidase with iron protoporphyrin IX	To examine the peroxidase activity of mutant HSA complex, incorporate with iron porphyrin IX in a heme pocket	(i) rHSA (mutant)–heme complex (rHSA(wt)–heme, rHSA(I142H/Y161F)–heme, rHSA(I142H/Y161L/L182H)–heme, rHSA(I142H/Y161F/L185H)–heme, rHSA(I142H/Y161F/R186H)–heme) (ii) Iron protoporphyrin IX (heme)	Genetically engineered HSA heme complex showed higher magnitude peroxidase activity than naturally found rHSA heme complex	Watanabe et al. (2012)
Poly (ethylene glycol) (PEG)-conjugated human serum albumin (HSA)	To know the structure and oxygen binding behavior of PEG-conjugated artificial hemoprotein	(i) Tetrakis(R,R,R,R-*o*amidophenyl) porphinatoiron(II) derivative (FeP) [PEG(HSA-FeP)] (ii)Poly (ethylene glycol) (PEG)	The oxygen binding affinity of PEG-conjugated artificial hemoprotein was lower than aqueous media The attachment of hyaluronic acid gave thin film which can bind oxygen and release it well	Nakagawa et al. (2007)
Recombinant human serum albumin (rHSA)	To examine the physicochemical character of human serum albumin (rHSA) with its albumin-heme hybrid as oxygen carrier	(i) Tetrakis{(1-methylcyclohexanamido) phenyl} porphinatoiron(II) derivative (FecycP) (ii) Recombinant human serum albumin (rHSA) (iii) A thiol group of Cys-34 with 1,6-bis(maleimido)hexane	It showed good blood compatibility, longer half-life in the blood, and oxygen was distributed similarly in tissues	Komatsu et al. (2004)
Human serum albumin (HSA) with Tetrakis(*o*-pivalamido) phenylporphinatoiron(II) (rHSA-FeP)	To produce a synthetic oxygen carrying hemoprotein	(i) 2-[8-{*N*-(2-Methylimidazolyl)} octanoyloxymethyl]-5,10,15,20-tetrakis(*o*-pivalamido) phenylporphinatoiron(II)s (FePs) (ii) Recombinant human serum albumin (rHSA)	The rHSA-FeP showed satisfied oxygen binding affinity and association and dissociation rate	Tsuchida et al. (1999, p. 2)
Tetrakis{(α, α, α, α -*o*-pivalamido) phenyl}porphinatoiron(II) and a protein attachable succinimidyl (glutamyl) group (rHSA(FeP-Glu)) (new albumin-heme conjugate)	To examine the binding and releasing capacity of oxygen by rHSA(FeP-Glu)	(i) Tetrakis{(α, α, α, α -*o*-pivalamido) phenyl}porphinatoiron(II) (ii) rHSA(FeP-Glu) (new albumin-heme conjugate)	This rHSA (FeP-Glu) showed similar oxygen-binding ability and releasing capacity (37 °C, pH 7.3) with hemoglobin and myoglobin	Wang et al. (2005)

Table 12 Experiments about HemoCD porphyrin system

Name	Purposes	Used components	Results	References
Experimental study				
HemoCD	To examine the oxygen-binding ability of HemoCD in an aqueous solution	(i) 1:1 complex (hemoCD) of 5,10,15,20-tetrakis (4-sulfonatophenyl) porphinatoiron(II) (Fe[II]TPPS)	Fe(II)TPPS encapsulated by two cyclodextrins is necessary for oxygen binding	Kano and Kitagishi (2009)
		(ii) Per-*O*-methylated b-cyclodextrin dimer	It acts as a good artificial oxygen carrier	
	To know the benefits of hemoCD synthesis and its O_2/CO selectivity	(i) 5,10,15,20-tetrakis(4-sulfonatophenyl) porphinatoiron(II) (Fe(II)TPPS)	The synthesis of CD dimer was successive to gain it's in gram quantities	Kitagishi et al. (2017)
		(ii) a cyclodextrin (CD) dimer having a pyridine linker	HemoCD was the appropriate compound for oxygen carrier due to its adequate affinity of O_2 and long half-life	
	To evaluate the oxygen-carrying capacity of hemoCD3 with Poly (acrylic acid) (PAA)	(i) Poly (acrylic acid) (PAA) is changed by 5-(4-b-alanylaminophenyl)-10,15,20-tris(4-sulfonatophenyl) porphinatoiron to prepare iron porphyrin- containing PAAs (FeP(n)s)	HemoCD3 with Poly (acrylic acid) (PAA) kept a long time in the blood which also exhibited adequate affinity of oxygen, high stability of the oxygen in the blood	Kano et al. (2011)
	To increase the circulation time in the bloodstream by modification of hemoCD with PEGylated dendrons	(i) 1:1 inclusion complex with Py3CD, a per-O-methylated β-cyclodextrin dimer with a pyridine linker	It increased the circulation time in the bloodstream which was useful to protect hemoCD from opsonization by the reticuloendothelial system	Karasugi et al. (2012)
		(ii) 4-oxo-4-[[4-(10,15,20-tris(4-sulfonatophenyl)-21H,23H-porphin-5-yl) phenyl] amino] butanoic acid (Por-COOH) (PEGylated dendrons)		
	To examine the hemoCD complex as a carbon monoxide depleting agent	(i) Meso-tetrakis(4-sulfonatophenyl) porphinatoiron(II)	This hemoCD system was able to depletion of carbon monoxide	Kitagishi and Minegishi (2017)
		(ii) Per-*O*-methylated *β*-cyclodextrin (TMe-*β*-CD)		
Mal-hemoCD	To increase the oxygen carrier circulation time by conjugation of maleimide group (Mal-hemoCD) and Cys residue of serum albumin through a Michael addition	(i) Maleimide group (Mal-hemoCD)	Mal-hemoCD with serum albumin increased the circulation time of an artificial oxygen carrier	Kitagishi et al. (2015)
		(ii) HemoCD: per-O-methylated b-cyclodextrin dimer and an iron (II) porphyrin		
		(iii) Cys residue of serum albumin		
Fe^{II}PImCD	To examine ability (higher and lower) to bind dioxygen and carbon monoxide in aqueous media than the pyridine analog respectively by preparing a new 1:1 inclusion complex (Fe^{II}PImCD)	(i) 1:1 inclusion complex (Fe^{II}PImCD) of 5,10,15,20-tetrakis-(4-sulfonatophenyl) porphinatoiron(II) (Fe^{II}P)	Fe^{II}PImCD showed a similar function like myoglobin that binds dioxygen and carbon monoxide in aqueous media which was 10 times higher than HemoCD	Kano et al. (2006)
		(ii) O-methylated b-cyclodextrin dimer with an imidazole linker (ImCD)		

Table 12 (continued)

Name	Purposes	Used components	Results	References
HemoCD -AuNPs (Gold nanoparticles)	To evaluate the circulation time in the bloodstream of PEGylated AuNPs (20 nm in diameter)	(i) Tris(4-sulfonatophenyl)-porphinatoiron (III) ($Fe^{III}P2$) (ii) Poly- (ethylene glycol) with thiolated arms (PEG-SH) (iii) Gold nanoparticles (AuNPs) (iv) Aurochloric acid (HAuCl4) (v) 1:1 complex of 5,10,15,20-tetrakis (4-sulfonatophenyl) porphinatoiron(II) and Py3CD	HemoCD-AuNPs used as oxygen and carbon monoxide carrier of diatomic molecules	Karasugi et al. (2011)
In vivo study				
HemoCD1	To develop a new technique to detect and quantify CO using hemoCD1	(i) 5,10,15,20-Tetrakis (4 sulfonatopheyl) porphinatoiron(III) (FeIIITPPS) (ii) Py3CD Animal preparation: Lewis and Sprague–Dawley rats (5 weeks)	HemoCD1 acts as significant adjuvant to oxygenation for elimination of excess CO from organs, even the brain	Mao et al. (2021)
Oxy-HemoCD	To study the effect of oxy-hemoCD as a CO depleting agent	(i) 1:1 inclusion complex of meso-tetrakis(4-sulfonatophenyl) porphinatoiron- (II) (ii) per-O-methylated β-cyclodextrin dimers Animal preparation: Male C57BL/6 N mice (20–22 g) Administration: HemoCD (0.15 mL); Intraperitoneally	The oxy-hemoCD able to expel CO from cell-free CO-Hb	Kitagishi et al. (2016)
HemoCD	To study the effect of HemoCD as a CO removal agent from living body	(i) 5,10,15,20- tetrakis(4- sulfonatophenyl) porphyrinato iron(II) (FeIITPPS) (ii) per-O-methylated b-cyclodextrin dimer with a pyridine linker (Py3CD) Animal preparation: Wistar male rat (270–350 g); Intravenously	The CO was successfully detected and expelled by hemoCD	Kitagishi et al. (2010)

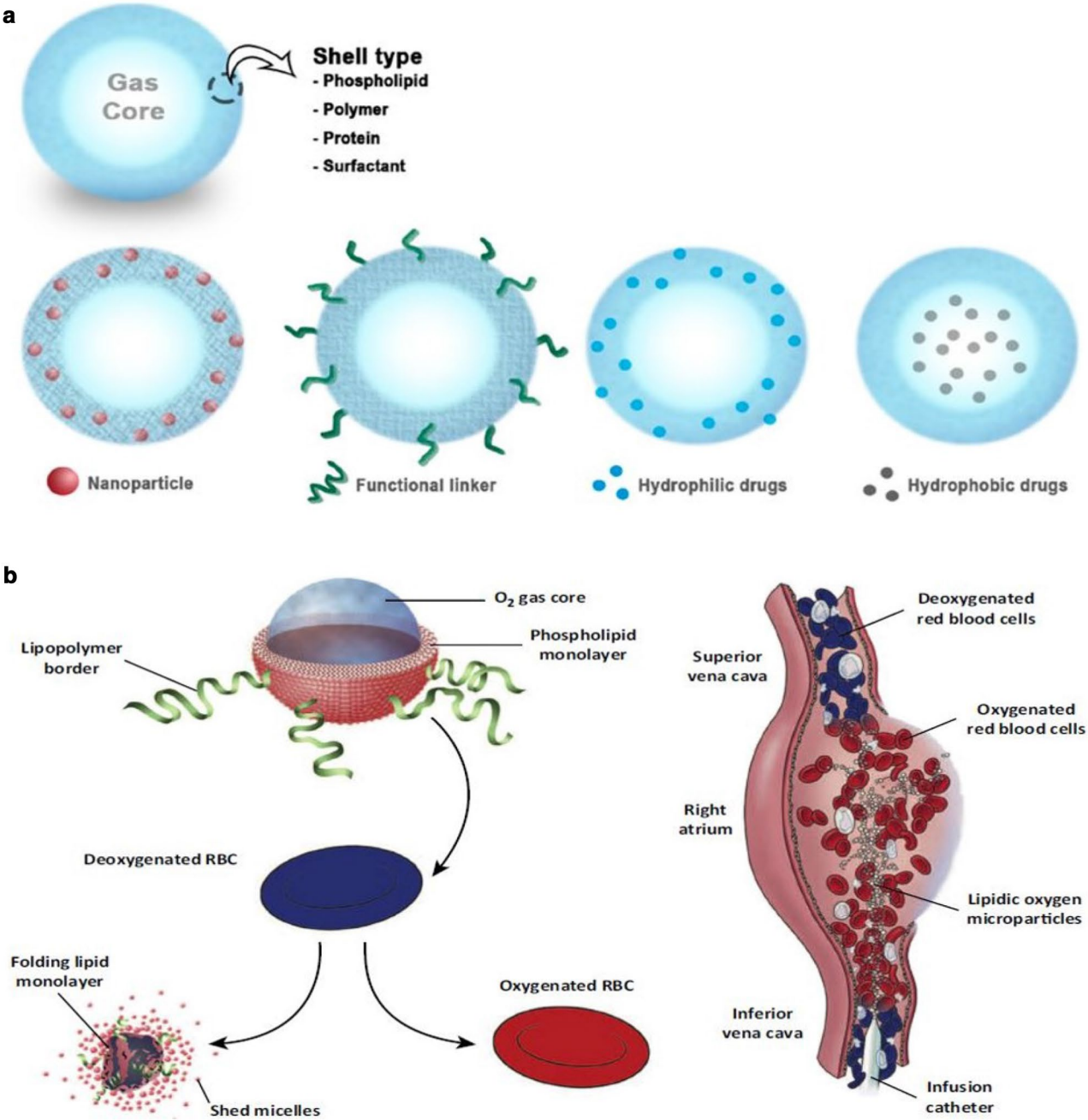

Fig. 6 **a** Structure of different shell-type MNBs [Reprinted with permission from (Khan et al. 2018a). Copyright © 2018, The Authors. MDPI], **b** Transport of oxygen from lipid shell microbubbles to deoxygenated RBCs. Left panel: oxygen gas core is placed within 2 nm phospholipid monolayer, right panel: oxygen delivery in the blood vessel [Reprinted with permission from (Tao and Ghoroghchian 2014) Copyright © 2014, Elsevier Ltd]

of oxygen nanobubbles in a customized hypoxic chamber; results showed improved cell conditions (Khan et al. 2018b). Phospholipid oxygen microbubbles have been used in injured rats to evaluate the effect of peritoneal microbubble oxygenation on acute respiratory distress syndrome; OMBs were able to increase oxygen supplementation (Fiala et al. 2020).

Conclusion

The necessity of RBC substitutes as well as AOCs is increasing with the increase in demand for blood transfusion in patients with life-threatening anemia. Therefore, researchers aspire to amplify biocompatible AOC implementation. However, the US FDA has not yet approved any

Table 13 Properties of different O_2 micro/nanobubbles

Micro/nano bubbles	Compositions	Size of particle	Delivery of gas	Synthesis method	References
Lipid shell types	(i) 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) (ii) 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethyleneglycol)] (DSPE-PEG)	– 4 µm	95% oxygen, 5% perfluorobutane	Sonication	Kwan et al. (2012), McEwan et al. (2015), Fix et al. (2015), Khan et al. (2018a)
	DSPC, PEG-40-S (9:1)	Approximately 3 nm	70 vol% oxygen	Sonication	Feshitan et al. (2014), Khan et al. (2018a)
	DSPC, BRIJ 100	2–4 µm (polydisperse)	From microparticles (70%), oxygen transport within 4 secs	Sonication	Kheir et al. (2012)
	F-PC, DMPC	3 µm, 4 µm	Oxygen	Agitation, sonication	Gerber et al. (2007), Khan et al. (2018a)
	DSPC or 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), PEG 40S	3 µm (polydisperse)	> 50 vol % gas(oxygen)	Sonication	Swanson et al. (2010)
	DSPC, N-(Carbamoyl-methoxypolyethylene glycol 5000)-1,2-dipalmitoyl-cephalin sodium (DPPE-MPEG5000)	1033 ± 72 nm (1 day) 1069 ± 53 nm (3 days) 1055 ± 89 nm (7 days)	Increase sixfold of pO_2 levels in 1 min	Mechanical agitation	Yang et al. (2018)
Protein shell type	Bovine serum albumin	Multi-size	Oxygen	Sonication	Swanson and Borden (2010)
Polymer shell types	Chitosan	708 ± 51.3 nm	Oxygen; Perfluoropentane	High shear mixer	Fix et al. (2015), Khan et al. (2018a)
	Dextran with or without polyvinylpyrrolidone (PVP)	With PVP: 410 ± 5 nm Without PVP: 550 ± 30 nm	Oxygen; Perfluoropentane	Sonication	Fix et al. (2015), Khan et al. (2018a)
Surfactants stabilized microbubbles	Span 60, D-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS)	3.1 ± 0.1 µm (polydisperse)	Oxygen	–	Fix et al. (2015)

oxygen-carrying RBC substitutes due to several of their side effects. Thus, scientists are expanding their research areas, which may contribute significantly to this field. Both HBOCs and PFOCs are the most significant AOC systems; PFOCs are more cost-effective than HBOCs because of their synthetic characteristics and ease of formulation. Additionally, they can be preserved at room temperature for more than 1 year, and can easily penetrate small blood vessels and arterial blockages for oxygen transport. Furthermore, PFOCs have shown better performance and acted as a safeguard for Hb-bound oxygen in the circulation until it reached hypoxic tissues. They are chemically resistant to heat and do not undergo metabolic transformation in vivo. Hence, PFOCs are a secure choice as AOCs as compared to HBOCs, which exhibit side effects such as immune reactions (except some modified LEH conjugated with PEG2K and non-phospholipid hexadecyl-carbamoyl methyl hexadecanoate which is immune neutral and well tolerated in repeated dose), high blood pressure, and short half-life. Furthermore, A-AOC may illustrate a new aspect in this field because they showed better biocompatibility and longer half-life circulation. They also demonstrated tissue protection from hypoxic conditions in animal models. In addition, the synthetically produced porphyrin system is another potential perspective that requires more research. Finally, oxygen-carrying MNBs are used to cure tumor hypoxia and hypoxemic conditions to increase the partial pressure of oxygen in the affected area, although accurately delivered concentrations of therapeutic oxygen are a significant concern during human use. Further research is needed to assess the utility of AOC in improving these substitutes.

Table 14 Preclinical assessment (in vivo) of O_2 microbubbles

Name	Purposes	Animal model/cell line	Administration	Results	References
DSPC, PEG-40S (9:1)	To determine the effect of peritoneal microbubble oxygenation (PMO) method with OMBs (phospholipid-coated oxygen microbubbles) in lung injured (right pneumothorax) rats	Male Wistar rats (430 ± 15 g)	40 mL/min for 1 min, after that for 8 mL/min	(i) PMO method with OMBs was safe and feasible and it needs fewer materials and technical support (ii) It showed higher oxygen-carrying capacity (0.88 mg-O_2 mL^{-1}) and rapid diffusion capability ($k_La = 45\ s^{-1}$)	Feshitan et al. (2014)
1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), polyoxyethylene-40 stearate (PEG40S) (9:1)	(i) To determine the effect of the PMO method (ii) To create a new method OMBs for circulation in the peritoneal cavity	Rabbit (male New Zealand white rabbit, 2.23 ± 0.18 kg)	80 mL/min for 4 min, and then 12.6 mL/min/kg	(i) PMO therapy increase survival time double in rabbits than control (ii) This technology was beneficial for the improvement of hypoxic patients due to extrapulmonary ventilation	Legband et al. (2015)
DSPC, DPPE-MPEG 5000 (9:1)	To determine the effect of microbubbles as an artificial oxygen carrier in the tumor-related hypoxic tissue	Rabbit	0.5 mL Microbubbles; Intravenous	It demonstrated better oxygen transport in the hypoxic tissue	Yang et al. (2018)
$SE61_{O2}$ (mixture of Span 60 and vitamin E (water-soluble))	To determine the capability of $SE61_{O2}$ microbubbles to raise concentration of oxygen to overcome hypoxic condition in tumor case	Mice	0.05 ml injection of SE61O2 followed by 0.1 ml saline; Intravenous	$SE61_{O2}$ microbubbles more workable for oxygen transport to hypoxic cells in solid tumors	Eisenbrey et al. (2015)
DSPC, (DSPE-PEG-2000Amine), (DSPE-PEG-2000-Biotin)	To know the effect of oxygen nanobubbles (ONBs) on the customized hypoxic chamber made by hypoxic cell	MDA-MB-231 breast cancer cells; HIF-1 α assay	–	ONBs deteriorate the HIF-1α which indicated hypoxia was reversed and improved cell conditions	Khan et al. (2018b)
Phospholipid oxygen microbubbles (OMBs)	To evaluate the effect of PMO on injured rats (acute respiratory distress syndrome (ARDS))	Male Wistar rats (490.0 ± 26.2 g)	100 mL/kg bolus of OMBs; Intraperitoneal catheter	OMBs are able to increase oxygen supplementation in injured rats through the PMO method	Fiala et al. (2020)

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Declarations

Conflict of interest All authors (N Mohanto, YJ Park, JP Jee) declare that they have no conflict of interest.

Research involving human and animal rights This article does not contain any studies with human and animal subjects performed by any of the authors.

References

Abutarboush R, Aligbe C, Pappas G et al (2014) Effects of the oxygen-carrying solution OxyVita C on the cerebral microcirculation and systemic blood pressures in healthy rats. J Funct Biomater 5:246–258. https://doi.org/10.3390/jfb5040246

Abutarboush R, Saha B, Mullah S et al (2016) Cerebral microvascular and systemic effects following intravenous administration of the perfluorocarbon emulsion perftoran. J Funct Biomater 7:29–29. https://doi.org/10.3390/jfb7040029

Alayash AI (2010) Setbacks in blood substitutes research and development: a biochemical perspective. Clin Lab Med 30:381–389. https://doi.org/10.1016/j.cll.2010.02.009

Albalawi A, Ciborowski S, Dolloff S, Ojadi E (2018) Porphyrins: super-molecules of the future: porphyrin polymers for artificial blood. Int J Chem Eng Appl 9:163–166. https://doi.org/10.18178/ijcea.2018.9.5.719

Amberson WR, Mulder AG, Steggerda FR et al (1933) Mammalian life without red blood corpuscles. Science 78:106–107. https://doi.org/10.1126/science.78.2014.106

Bachert SE, Dogra P, Boral LI (2020) Alternatives to transfusion. Am J Clin Pathol 153:287–293. https://doi.org/10.1093/ajcp/aqz175

Baek JH, Zhou Y, Harris DR et al (2012) Down selection of polymerized bovine hemoglobins for use as oxygen releasing therapeutics in a Guinea pig model. Toxicol Sci 127:567–581. https://doi.org/10.1093/toxsci/kfs109

Bäumler H, Xiong Y, Liu ZZ et al (2014) Novel hemoglobin particles–promising new-generation hemoglobin-based oxygen carriers. Artif Organs 38:708–714. https://doi.org/10.1111/aor.12331

Bedőcs P, Szebeni J (2020) The critical choice of animal models in nanomedicine safety assessment: a lesson learned from hemoglobin-based oxygen carriers. Front Immunol 11:584966. https://doi.org/10.3389/fimmu.2020.584966

Benner A, Patel AK, Singh K, Dua A (2022) Physiology, Bohr effect. StatPearls Publishing, Treasure Island

Bialas C, Moser C, Sims CA (2019) Artificial oxygen carriers and red blood cell substitutes: a historic overview and recent developments toward military and clinical relevance. J Trauma Acute Care Surg 87:S48–S58. https://doi.org/10.1097/TA.0000000000002250

Bonanno AM, Graham TL, Wilson LN et al (2018) Efficacy of the perfluorocarbon dodecafluoropentane as an adjunct to pre-hospital resuscitation. PLoS ONE. https://doi.org/10.1371/journal.pone.0207197

Cabrales P, Vázquez BYS, Negrete AC, Intaglietta M (2007) Perfluorocarbons as gas transporters for O2, NO, CO and volatile anesthetics. Transfus Altern Transfus Med 9:294–303. https://doi.org/10.1111/j.1778-428X.2007.00085.x

Carmichael FJL, Ali ACY, Campbell JA et al (2000) A phase I study of oxidized raffinose cross-linked human hemoglobin. Crit Care Med 28:2283–2292

Castro CI, Briceno JC (2010) Perfluorocarbon-based oxygen carriers: review of products and trials. Artif Organs 34:622–634. https://doi.org/10.1111/j.1525-1594.2009.00944.x

Chen J-Y, Scerbo M, Kramer G (2009) A review of blood substitutes: examining the history, clinical trial results, and ethics of hemoglobin-based oxygen carriers. Clin Sao Paulo Braz 64:803–813. https://doi.org/10.1590/S1807-59322009000800016

Cipolla MJ, Linfante I, Abuchowski A et al (2018) Pharmacologically increasing collateral perfusion during acute stroke using a carboxyhemoglobin gas transfer agent (Sanguinate™) in spontaneously hypertensive rats. J Cereb Blood Flow Metab 38:755–766. https://doi.org/10.1177/0271678X17705567

Clark LC, Gollan F (1966) Survival of mammals breathing organic liquids equilibrated with oxygen at atmospheric pressure. Science 152:1755–1756. https://doi.org/10.1126/SCIENCE.152.3730.1755

Collman JP, Brauman JI, Rose E, Suslick KS (1978) Cooperativity in 02 binding to iron porphyrins. Proc Natl Acad Sci 75(3):1052–1055. https://doi.org/10.1073/pnas.75.3.1052

Cronin WA, Khan K, Hall AA et al (2021) The effect of the perfluorocarbon emulsion Oxycyte™ in an ovine model of severe decompression illness. Undersea Hyperb Med 48:25–31

Culp WC, Woods SD, Skinner RD et al (2012) Dodecafluoropentane emulsion decreases infarct volume in a rabbit ischemic stroke model. J Vasc Interv Radiol 23:116–121. https://doi.org/10.1016/j.jvir.2011.10.001

Culp WC, Onteddu SS, Brown A et al (2019) Dodecafluoropentane emulsion in acute ischemic stroke: a phase ib/ii randomized and controlled dose-escalation trial. J Vasc Interv Radiol JVIR 30:1244-1250.e1. https://doi.org/10.1016/j.jvir.2019.04.020

Davis JM, El-Haj N, Shah NN et al (2018) Use of the blood substitute HBOC-201 in critically ill patients during sickle crisis: a three-case series. Transfusion (paris) 58:132–137. https://doi.org/10.1111/trf.14386

Dichiarante V, Milani R, Metrangolo P (2018) Natural surfactants towards a more sustainable fluorine chemistry. Green Chem 20:13–27. https://doi.org/10.1039/C7GC03081A

Eisenbrey JR, Albala L, Kramer MR et al (2015) Development of an ultrasound sensitive oxygen carrier for oxygen delivery to hypoxic tissue. Int J Pharm 478:361–367. https://doi.org/10.1016/j.ijpharm.2014.11.023

Englewood Hospital and Medical Center (2021) Expanded access protocol for the treatment use of HBOC-201. clinicaltrials.gov

Ferenz KB (2017) Improved albumin-derived per fluorocarbon-based artificial oxygen carriers: in-vivo evaluation of biocompatibility. Adv Biotechnol Microbiol. https://doi.org/10.19080/aibm.2017.07.555714

Ferenz KB, Steinbicker AU (2019) Artificial oxygen carriers-past, present, and future—a review of the most innovative and clinically relevant concepts. J Pharmacol Exp Ther 369:300–310. https://doi.org/10.1124/jpet.118.254664

Feshitan JA, Legband ND, Borden MA, Terry BS (2014) Systemic oxygen delivery by peritoneal perfusion of oxygen microbubbles. Biomaterials 35:2600–2606. https://doi.org/10.1016/j.biomaterials.2013.12.070

Fiala A, Slagle C, Legband N et al (2020) Treatment of a rat model of LPS-induced ARDS via peritoneal perfusion of oxygen microbubbles. J Surg Res 246:450–456. https://doi.org/10.1016/J.JSS.2019.09.017

Fix SM, Borden MA, Dayton PA (2015) Therapeutic gas delivery via microbubbles and liposomes. J Control Release 209:139–149. https://doi.org/10.1016/j.jconrel.2015.04.027

Fraker CA, Mendez AJ, Inverardi L et al (2012) Optimization of perfluoro nano-scale emulsions: the importance of particle size for enhanced oxygen transfer in biomedical applications. Colloids Surf B 98:26–35. https://doi.org/10.1016/j.colsurfb.2012.04.011

Fukui T, Kawaguchi AT, Takekoshi S et al (2017) Liposome-encapsulated hemoglobin accelerates skin wound healing in diabetic dB/dB mice. Artif Organs 41:319–326. https://doi.org/10.1111/aor.12864

Gerber F, Waton G, Krafft MP, Vandamme TF (2007) Long lived microbubbles for oxygen delivery. Artif Cells Blood Substit Immobil Biotechnol 35:119–124. https://doi.org/10.1080/10731190600974939

Gould SA, Moss GS (1996) Clinical development of human polymerized hemoglobin as a blood substitute. World J Surg 20:1200–1207. https://doi.org/10.1007/s002689900183

Graham K, Moon-Massat PF, Unger EC (2019) Dodecafluoropentane emulsion (DDFPE) as a resuscitation fluid for treatment of hemorrhagic shock and traumatic brain injury: a review. Shock 52:50–54. https://doi.org/10.1097/SHK.0000000000001060

Grapentin C, Barnert S, Schubert R (2015) Monitoring the stability of perfluorocarbon nanoemulsions by cryo-TEM image analysis and dynamic light scattering. PLoS ONE 10:e0130674. https://doi.org/10.1371/journal.pone.0130674

Haldar R, Gupta D, Chitranshi S et al (2019) Artificial blood: a futuristic dimension of modern day transfusion sciences. Cardiovasc Hematol Agents Med Chem 17:11–16. https://doi.org/10.2174/1871525717666190617120045

Haque A, Scultetus AH, Arnaud F et al (2016) The emulsified PFC Oxycyte® improved oxygen content and lung injury score in a swine model of Oleic Acid Lung Injury (OALI). Lung 194:945–957. https://doi.org/10.1007/s00408-016-9941-9

Harrington JP, Wollocko H (2011) Molecular design properties of OxyVita hemoglobin, a new generation therapeutic oxygen carrier: a review. J Funct Biomater 2:414–424. https://doi.org/10.3390/jfb2040414

Hegde MM, Prabhu S, Mutalik S et al (2022) Multifunctional lipidic nanocarriers for effective therapy of glioblastoma: recent advances in stimuli-responsive, receptor and subcellular targeted approaches. J Pharm Investig 52:49–74. https://doi.org/10.1007/s40005-021-00548-6

Hernot S, Klibanov AL (2008) Microbubbles in ultrasound-triggered drug and gene delivery. Adv Drug Deliv Rev 60:1153–1166. https://doi.org/10.1016/J.ADDR.2008.03.005

Hill SE (2019) Perfluorocarbons: knowledge gained from clinical trials. Shock Augusta Ga 52:60–64. https://doi.org/10.1097/SHK.0000000000001045

Jägers J, Wrobeln A, Ferenz KB (2021) Perfluorocarbon-based oxygen carriers: from physics to physiology. Pflugers Arch 473:139–150. https://doi.org/10.1007/s00424-020-02482-2

Jahr JS, Akha AS, Holtby RJ (2012) Crosslinked, polymerized, and PEG-conjugated hemoglobin-based oxygen carriers: clinical safety and efficacy of recent and current products. Curr Drug Discov Technol 9:158–165. https://doi.org/10.2174/157016312802650742

Jahr JS, Guinn NR, Lowery DR et al (2021) Blood substitutes and oxygen therapeutics: a review. Anesth Analg 132:119–129. https://doi.org/10.1213/ANE.0000000000003957

Jansman MMT, Hosta-Rigau L (2018) Recent and prominent examples of nano- and microarchitectures as hemoglobin-based oxygen carriers. Adv Colloid Interface Sci 260:65–84. https://doi.org/10.1016/j.cis.2018.08.006

Kakizaki T, Kobayashi K, Komatsu T et al (1994) Lipidheme-microsphere (LH-M). A new type of totally synthetic oxygen carrier and its oxygen carrying ability. Artif Cells Blood Substit Immobil Biotechnol 22:933–938. https://doi.org/10.3109/10731199409117932

Kaneda S, Ishizuka T, Goto H et al (2009) Liposome-encapsulated hemoglobin, TRM-645: current status of the development and important issues for clinical application. Artif Organs 33:146–152. https://doi.org/10.1111/j.1525-1594.2008.00699.x

Kaneda S, Ishizuka T, Sekiguchi A et al (2014) Efficacy of liposome-encapsulated hemoglobin in a rat model of cerebral ischemia. Artif Organs 38:650–655. https://doi.org/10.1111/aor.12358

Kano K, Kitagishi H (2009) HemoCD as an artificial oxygen carrier: oxygen binding and autoxidation. Artif Organs 33:177–182. https://doi.org/10.1111/j.1525-1594.2008.00704.x

Kano K, Kitagishi H, Mabuchi T et al (2006) A myoglobin functional model composed of a ferrous porphyrin and a cyclodextrin dimer with an imidazole linker. Chemistry 1:358–366. https://doi.org/10.1002/asia.200600070

Kano K, Ochi T, Okunaka S et al (2011) Preparation and function of poly(acrylic acid)s modified by supramolecular complex composed of porphinatoiron and a cyclodextrin dimer that bind diatomic molecules (O_2 and CO) in aqueous solution. Chemistry 6:2946–2955. https://doi.org/10.1002/asia.201100354

Kao I, Xiong Y, Steffen A et al (2018) Preclinical in vitro safety investigations of submicron sized hemoglobin based oxygen carrier HbMP-700. Artif Organs 42:549–559. https://doi.org/10.1111/aor.13071

Karasugi K, Kitagishi H, Kano K (2011) Gold nanoparticles carrying diatomic molecules (O_2 and CO) in aqueous solution. Chemistry 6:825–833. https://doi.org/10.1002/asia.201000756

Karasugi K, Kitagishi H, Kano K (2012) Modification of a dioxygen carrier, HemoCD, with PEGylated dendrons for extension of circulation time in the bloodstream. Bioconjug Chem 23:2365–2376. https://doi.org/10.1021/bc300303z

Kawaguchi AT, Yamano M, Haida M et al (2017) Effect of oxygen affinity of liposome-encapsulated hemoglobin on cerebral ischemia and reperfusion as detected by positron emission tomography in nonhuman primates. Artif Organs 41:336–345. https://doi.org/10.1111/aor.12905

Kawaguchi AT, Salybekov AA, Yamano M et al (2018) PEGylated carboxyhemoglobin bovine (SANGUINATE) ameliorates myocardial infarction in a rat model. Artif Organs 42:1174–1184. https://doi.org/10.1111/aor.13384

Keipert PE, MP4CO-SCD-105 Study Investigators (2016) Clinical evaluation of MP4CO: a phase 1b escalating-dose, safety and tolerability study in stable adult patients with sickle cell disease. Adv Exp Med Biol 923:23–29. https://doi.org/10.1007/978-3-319-38810-6_3

Khan MS, Hwang J, Lee K et al (2018a) Oxygen-carrying micro/nanobubbles: composition, synthesis techniques and potential prospects in photo-triggered theranostics. Mol Basel Switz 23:E2210. https://doi.org/10.3390/molecules23092210

Khan MS, Hwang J, Seo Y et al (2018b) Engineering oxygen nanobubbles for the effective reversal of hypoxia. Artif Cells Nanomed Biotechnol 46:S318–S327. https://doi.org/10.1080/21691401.2018.1492420

Khan F, Singh K, Friedman MT (2020) Artificial blood: the history and current perspectives of blood substitutes. Discov Craiova Rom 8:104. https://doi.org/10.15190/d.2020.1

Kheir JN, Scharp LA, Borden MA et al (2012) Oxygen gas-filled microparticles provide intravenous oxygen delivery. Sci Transl Med 4:140ra88. https://doi.org/10.1126/scitranslmed.3003679

Kim HW, Greenburg AG (2004) Artificial oxygen carriers as red blood cell substitutes: a selected review and current status. Artif Organs 28:813–828. https://doi.org/10.1111/j.1525-1594.2004.07345.x

Kim D-H, Lee S-E, Pyo Y-C et al (2020) Solubility enhancement and application of cyclodextrins in local drug delivery. J Pharm Investig 50:17–27. https://doi.org/10.1007/s40005-019-00434-2

Kitagishi H, Kano K (2021) Synthetic heme protein models that function in aqueous solution. Chem Commun 57:148–173. https://doi.org/10.1039/d0cc07044k

Kitagishi H, Minegishi S (2017) Iron(II)porphyrin-cyclodextrin supramolecular complex as a carbon monoxide-depleting agent in living organisms. Chem Pharm Bull 65(4):336–340. https://doi.org/10.1248/cpb.c16-00767

Kitagishi H, Negi S, Kiriyama A et al (2010) A diatomic molecule receptor that removes CO in a living organism. Angew Chem Int Ed Engl 49:1312–1315. https://doi.org/10.1002/anie.200906149

Kitagishi H, Kawasaki H, Kano K (2015) Bioconjugation of serum albumin to a maleimide-appended porphyrin/cyclodextrin supramolecular complex as an artificial oxygen carrier in the bloodstream. Chemistry 10:1768–1775. https://doi.org/10.1002/asia.201500451

Kitagishi H, Minegishi S, Yumura A et al (2016) Feedback response to selective depletion of endogenous carbon monoxide in the blood. J Am Chem Soc 138:5417–5425. https://doi.org/10.1021/jacs.6b02211

Kitagishi H, Mao Q, Kitamura N, Kita T (2017) HemoCD as a totally synthetic artificial oxygen carrier: improvements in the synthesis and O_2/CO discrimination. Artif Organs 41:372–380. https://doi.org/10.1111/aor.12870

Kobayashi K, Watanabe M, Ishihara T et al (1991) Liposome-embedded-heme as a totally artificial oxygen carrier. ASAIO Trans 37:M443-444

Kobayashi K, Kakizaki T, Komatsu T et al (1994) Two types of totally artificial red blood cell substitutes liposome embedded heme(L/H) and lipidheme/microsphere(LHM). Artif Cells Blood Substit Immobil Biotechnol 22:895–900. https://doi.org/10.3109/10731199409117927

Komatsu T, Nishide H, Tsuchida E (1994) Structure and solution properties of lipidheme-microsphere. Artif Cells Blood Substit Immobil Biotechnol 22:855–860. https://doi.org/10.3109/10731199409117921

Komatsu T, Oguro Y, Teramura Y et al (2004) Physicochemical characterization of cross-linked human serum albumin dimer and its synthetic heme hybrid as an oxygen carrier. Biochim Biophys Acta Gen Subj 1675:21–31. https://doi.org/10.1016/j.bbagen.2004.08.010

Komatsu T, Ishihara S, Tsuchida E et al (2005a) Heat-resistant oxygen-carrying hemoproteins consist of recombinant xylanases and synthetic iron(II) porphyrin. Biomacromol 6:1489–1494. https://doi.org/10.1021/bm049255l

Komatsu T, Ohmichi N, Nakagawa A et al (2005b) O_2 and CO binding properties of artificial hemoproteins formed by complexing iron protoporphyrin IX with human serum albumin mutants. J Am Chem Soc 127:15933–15942. https://doi.org/10.1021/ja054819u

Krafft MP, Riess JG (2021) Therapeutic oxygen delivery by perfluorocarbon-based colloids. Adv Colloid Interface Sci. https://doi.org/10.1016/j.cis.2021.102407

Kwan JJ, Kaya M, Borden MA, Dayton PA (2012) Theranostic oxygen delivery using ultrasound and microbubbles. Theranostics 2:1174–1184. https://doi.org/10.7150/thno.4410

Lambert E, Janjic JM (2021) Quality by design approach identifies critical parameters driving oxygen delivery performance in vitro for perfluorocarbon based artificial oxygen carriers. Sci Rep 11:5569. https://doi.org/10.1038/s41598-021-84076-1

Lambert E, Gorantla VS, Janjic JM (2019) Pharmaceutical design and development of perfluorocarbon nanocolloids for oxygen delivery in regenerative medicine. Nanomed 14:2697–2712. https://doi.org/10.2217/nnm-2019-0260

Le Daré B, Ferron PJ, Bellamri N et al (2021) A therapeutic oxygen carrier isolated from *Arenicola marina* decreases amanitin-induced hepatotoxicity. Toxicon 200:87–91. https://doi.org/10.1016/j.toxicon.2021.07.004

Lee M, Lee EY, Lee D, Park BJ (2015) Stabilization and fabrication of microbubbles: applications for medical purposes and functional materials. Soft Matter 11:2067–2079. https://doi.org/10.1039/C5SM00113G

Legband ND, Feshitan JA, Borden MA, Terry BS (2015) Evaluation of peritoneal microbubble oxygenation therapy in a rabbit model of hypoxemia. IEEE Trans Biomed Eng 62:1376–1382. https://doi.org/10.1109/TBME.2015.2388611

Li J, Noll BC, Oliver AG et al (2013) Correlated ligand dynamics in oxyiron picket fence porphyrins: structural and Mössbauer investigations. J Am Chem Soc 135:15627–15641. https://doi.org/10.1021/ja408431z

Li X, Dao M, Lykotrafitis G, Karniadakis GE (2017) Biomechanics and biorheology of red blood cells in sickle cell anemia. J Biomech 50:34–41. https://doi.org/10.1016/j.jbiomech.2016.11.022

Lowe KC (2001) Fluorinated blood substitutes and oxygen carriers. J Fluor Chem 109:59–65. https://doi.org/10.1016/S0022-1139(01)00374-8

Lupon E, Lellouch AG, Zal F et al (2021) Combating hypoxemia in COVID-19 patients with a natural oxygen carrier, HEMO2Life® (M101). Med Hypotheses 146:110421. https://doi.org/10.1016/j.mehy.2020.110421

Mahon RT, Auker CR, Bradley SG et al (2013a) The emulsified perfluorocarbon Oxycyte improves spinal cord injury in a swine model of decompression sickness. Spinal Cord 51:188–192. https://doi.org/10.1038/sc.2012.135

Mahon RT, Hall A, Bodo M, Auker C (2013b) The intravenous perfluorocarbon emulsion Oxycyte does not increase hyperbaric oxygen-related seizures in a non-sedated swine model. Eur J Appl Physiol 113:2795–2802. https://doi.org/10.1007/s00421-013-2720-x

Mallet V, Dutheil D, Polard V et al (2014) Dose-ranging study of the performance of the natural oxygen transporter HEMO2 Life in organ preservation. Artif Organs 38:691–701. https://doi.org/10.1111/aor.12307

Malte H, Lykkeboe G (2018) The Bohr/Haldane effect: a model-based uncovering of the full extent of its impact on O_2 delivery to and CO_2 removal from tissues. J Appl Physiol Bethesda Md 125:916–922. https://doi.org/10.1152/japplphysiol.00140.2018

Mao Q, Kawaguchi AT, Mizobata S et al (2021) Sensitive quantification of carbon monoxide in vivo reveals a protective role of circulating hemoglobin in CO intoxication. Commun Biol 4:1–15. https://doi.org/10.1038/s42003-021-01880-1

Matsuki N, Ishikawa T, Ichiba S et al (2014) Oxygen supersaturated fluid using fine micro/nanobubbles. Int J Nanomed 9:4495–4505. https://doi.org/10.2147/IJN.S68840

Matton APM, Burlage LC, van Rijn R et al (2018) Normothermic machine perfusion of donor livers without the need for human blood products. Liver Transplant 24:528–538. https://doi.org/10.1002/lt.25005

Mayer D, Ferenz KB (2019) Perfluorocarbons for the treatment of decompression illness: how to bridge the gap between theory and practice. Eur J Appl Physiol 119:2421–2433. https://doi.org/10.1007/s00421-019-04252-0

Mayer D, Guerrero F, Goanvec C et al (2020) Prevention of decompression sickness by novel artificial oxygen carriers. Med Sci Sports Exerc 52:2127–2135. https://doi.org/10.1249/MSS.0000000000002354

McEwan C, Owen J, Stride E et al (2015) Oxygen carrying microbubbles for enhanced sonodynamic therapy of hypoxic tumours. J Control Release 203:51–56. https://doi.org/10.1016/j.jconrel.2015.02.004

Mihailescu M-R, Russu IM (2001) A signature of the T → R transition in human hemoglobin. Proc Natl Acad Sci USA 98:3773–3777. https://doi.org/10.1073/pnas.071493598

Modery-Pawlowski CL, Tian LL, Pan V, Sen Gupta A (2013) Synthetic approaches to RBC mimicry and oxygen carrier systems. Biomacromol 14:939–948. https://doi.org/10.1021/bm400074t

Moon-Massat PF, Abutarboush R, Pappas G et al (2014) Effects of perfluorocarbon dodecafluoropentane (NVX-108) on cerebral microvasculature in the healthy rat. Curr Drug Discov Technol 11:220–226. https://doi.org/10.2174/1570163811666140709110301

Moradi S, Jahanian-Najafabadi A, Roudkenar MH (2016) Artificial blood substitutes: first steps on the long route to clinical utility. Clin Med Insights Blood Disord 9:33–41. https://doi.org/10.4137/CMBD.S38461

Moritz ED, Winton CS, Tonnetti L et al (2016) Screening for *Babesia microti* in the U.S. Blood Supply N Engl J Med 375:2236–2245. https://doi.org/10.1056/NEJMoa1600897

Nakagawa A, Komatsu T, Huang Y et al (2007) O2-binding albumin thin films: solid membranes of poly(ethylene glycol)-conjugated human serum albumin incorporating iron porphyrin. Bioconjug Chem 18:1673–1677. https://doi.org/10.1021/bc070086m

Nakagawa A, Komatsu T, Iizuka M, Tsuchida E (2008) O2 binding to human serum albumin incorporating iron porphyrin with a covalently linked methyl-L-histidine isomer. Bioconjug Chem 19:581–584. https://doi.org/10.1021/bc700400n

Noh G, Keum T, Bashyal S et al (2022) Recent progress in hydrophobic ion-pairing and lipid-based drug delivery systems for enhanced oral delivery of biopharmaceuticals. J Pharm Investig 52:75–93. https://doi.org/10.1007/s40005-021-00549-5

Norvaiša K, Yeow K, Twamley B et al (2021) Strategic synthesis of "Picket Fence" porphyrins based on nonplanar macrocycles. Eur J Org Chem 2021:1871–1882. https://doi.org/10.1002/ejoc.202100154

Nosé Y (2004) Is there a role for blood substitutes in civilian medicine: a drug for emergency shock cases? Artif Organs 28:807–812. https://doi.org/10.1111/j.1525-1594.2004.07393.x

Ohta H, Kaga M, Li H et al (2017) Potential new non-invasive therapy using artificial oxygen carriers for pre-eclampsia. J Funct Biomater 8:E32. https://doi.org/10.3390/jfb8030032

Ohyanagi H, Nakaya S, Okumura S, Saitoh Y (1984) Surgical use of fluosol-DA in Jehovah's Witness patients. Artif Organs 8:10–18. https://doi.org/10.1111/j.1525-1594.1984.tb04238.x

Padsalgikar AD (2017) Cardiovascular system: structure, assessment, and diseases. In: Padsalgikar AD (ed) Plastics in medical devices for cardiovascular applications. William Andrew Publishing, Norwich, pp 103–132

Pittman RN (2011) Regulation of Tissue Oxygenation. Morgan & Claypool Life Sciences, San Rafael (CA)

Płoszczyca K, Czuba M, Chalimoniuk M et al (2021) Red blood cell 2,3-diphosphoglycerate decreases in response to a 30 km time trial under hypoxia in cyclists. Front Physiol. https://doi.org/10.3389/fphys.2021.670977

Police AM, Waxman K, Tominaga G (1985) Pulmonary complications after Fluosol administration to patients with life-threatening blood loss. Crit Care Med 13:96–98. https://doi.org/10.1097/00003246-198502000-00008

Prolong Pharmaceuticals (2018) A Phase 2 Study of the Safety and Effectiveness of SANGUINATE™ in the Treatment of Vaso-Occlusive Crises in the Ambulatory Setting: A Placebo-Controlled, Single-Dose, Single-Blind Study in Adults With Sickle Cell Disease. clinicaltrials.gov

Rameez S, Alosta H, Palmer AF (2008) Biocompatible and biodegradable polymersome encapsulated hemoglobin: a potential oxygen carrier. Bioconjug Chem 19:1025–1032. https://doi.org/10.1021/bc700465v

Rao G, Hedrick AF, Yadav VR et al (2015) The brain metabolic activity after resuscitation with liposome-encapsulated hemoglobin in a rat model of hypovolemic shock. J Cereb Blood Flow Metab 35:1528–1536. https://doi.org/10.1038/jcbfm.2015.82

Riess JG (2001) Oxygen carriers ("blood substitutes")–raison d'etre, chemistry, and some physiology. Chem Rev 101:2797–2920. https://doi.org/10.1021/cr970143c

Rogers DM, Crookston KP (2006) The approach to the patient who refuses blood transfusion. Transfusion (paris) 46:1471–1477. https://doi.org/10.1111/j.1537-2995.2006.00947.x

Ryzhkov IA, Zarzhetsky YV, Novoderzhkina IS (2016) Effect of perfluorane on the regulation of skin blood flow in acute blood loss: experimental study. Gen Reanimatol 11:19–27. https://doi.org/10.15360/1813-9779-2015-6-19-27

Sakai H, Sou K, Horinouchi H et al (2009) Review of hemoglobin-vesicles as artificial oxygen carriers. Artif Organs 33:139–145. https://doi.org/10.1111/j.1525-1594.2008.00698.x

Sarkar S (2008) Artificial blood. Indian J Crit Care Med Peer-Rev off Publ Indian Soc Crit Care Med 12:140–144. https://doi.org/10.4103/0972-5229.43685

Sen Gupta A (2017) Bio-inspired nanomedicine strategies for artificial blood components. Wiley Interdiscip Rev Nanomed Nanobiotechnol. https://doi.org/10.1002/wnan.1464

Sen Gupta A (2019) Hemoglobin-based oxygen carriers: current state-of-the-art and novel molecules. Shock Augusta Ga 52:70–83. https://doi.org/10.1097/SHK.0000000000001009

Seno S, Wang J, Cao S et al (2020) Resuscitation with macromolecular superoxide dismutase/catalase mimetic polynitroxylated PEGylated hemoglobin offers neuroprotection in guinea pigs after traumatic brain injury combined with hemorrhage shock. BMC Neurosci 21:22. https://doi.org/10.1186/s12868-020-00571-7

Shellington DK, Du L, Wu X et al (2011) Polynitroxylated pegylated hemoglobin: a novel neuroprotective hemoglobin for acute volume-limited fluid resuscitation after combined traumatic brain injury and hemorrhagic hypotension in mice. Crit Care Med 39:494–505. https://doi.org/10.1097/CCM.0b013e318206b1fa

Shentu B, Nishide H (2003) Facilitated oxygen transport membranes of picket-fence cobaltporphyrin complexed with various polymer matrixes. Ind Eng Chem Res 42:5954–5958. https://doi.org/10.1021/ie020770e

Simoni J, Simoni G, Wesson DE, Feola M (2012) ATP-adenosine-glutathione cross-linked hemoglobin as clinically useful oxygen carrier. Curr Drug Discov Technol 9:173–187. https://doi.org/10.2174/157016312802650797

Sirsi S, Borden M (2009) Microbubble compositions, properties and biomedical applications. Bubble Sci Eng Technol 1:3–17. https://doi.org/10.1179/175889709X446507

Spahn DR (2018) Artificial oxygen carriers: a new future? Crit Care Lond Engl 22:46. https://doi.org/10.1186/s13054-018-1949-5

Spahn DR, Waschke KF, Standl T et al (2002) Use of perflubron emulsion to decrease allogeneic blood transfusion in high-blood-loss non-cardiac surgery results of a European phase 3 study. Anesthesiology 97(6):1338–1349. https://doi.org/10.1097/00000542-200212000-00004

Spiess BD (2009) Perfluorocarbon emulsions as a promising technology: a review of tissue and vascular gas dynamics. J Appl Physiol Bethesda Md 1985 106:1444–1452. https://doi.org/10.1152/japplphysiol.90995.2008

Swanson EJ, Borden MA (2010) Injectable oxygen delivery based on protein-shelled microbubbles. Nano LIFE 01:215–218. https://doi.org/10.1142/S1793984410000195

Swanson EJ, Mohan V, Kheir J, Borden MA (2010) Phospholipid-stabilized microbubble foam for injectable oxygen delivery. Langmuir ACS J Surf Colloids 26:15726–15729. https://doi.org/10.1021/la1029432

Taguchi K, Yamasaki K, Maruyama T, Otagiri M (2017) Comparison of the pharmacokinetic properties of hemoglobin-based oxygen

carriers. J Funct Biomater 8:E11. https://doi.org/10.3390/jfb8010011

Tao Z, Ghoroghchian PP (2014) Microparticle, nanoparticle, and stem cell-based oxygen carriers as advanced blood substitutes. Trends Biotechnol 32:466–473. https://doi.org/10.1016/j.tibtech.2014.05.001

Themes UFO (2017) Porphyrins and Hemoglobin. In: Basicmedical Key. https://basicmedicalkey.com/porphyrins-and-hemoglobin/. Accessed 29 Dec 2021

Tsuchida E, Nishide H, Komatsu T et al (1992) Lipid microsphere containing lipophilic heme: preparation and oxygen transportation under physiological conditions. Biochim Biophys Acta 1108:253–256. https://doi.org/10.1016/0005-2736(92)90033-I

Tsuchida E, Komatsu T, Matsukawa Y et al (1999) Human serum albumin incorporating tetrakis(o-pivalamido) phenylporphinatoiron(II) derivative as a totally synthetic O_2-carrying hemoprotein. Bioconjug Chem 10:797–802. https://doi.org/10.1021/bc990019v

Tsuchida E, Sou K, Nakagawa A et al (2009) Artificial oxygen carriers, hemoglobin vesicles and albumin-hemes, based on bioconjugate chemistry. Bioconjug Chem 20:1419–1440. https://doi.org/10.1021/bc800431d

Vandegriff KD, Malavalli A, Lohman J et al (2014) Impact of acellular hemoglobin-based oxygen carriers on brain apoptosis in rats. Transfusion (paris) 54:2045–2054. https://doi.org/10.1111/trf.12643

Varney J, Rivera A, Dong V et al (2021) Mini-review on the properties and possible applications of therapeutic oxygen carrier Hemarina-M101. Transfus Apher Sci off J World Apher Assoc off J Eur Soc Haemapheresis 60:103016. https://doi.org/10.1016/j.transci.2020.103016

Wang R-M, Komatsu T, Nakagawa A, Tsuchida E (2005) Human serum albumin bearing covalently attached iron(II) porphyrins as O_2-coordination sites. Bioconjug Chem 16:23–26. https://doi.org/10.1021/bc049859m

Watanabe K, Ishikawa N, Komatsu T (2012) Human serum albumin-based peroxidase having an iron protoporphyrin IX in artificial heme pocket. Chemistry 7:2534–2537. https://doi.org/10.1002/asia.201200373

Weiskopf RB, Beliaev AM, Shander A et al (2017) Addressing the unmet need of life-threatening anemia with hemoglobin-based oxygen carriers. Transfusion (paris) 57:207–214. https://doi.org/10.1111/trf.13923

Wong NSW, Chang TMS (2007) Polyhemoglobin-fibrinogen: a novel oxygen carrier with platelet-like properties in a hemodiluted setting. Artif Cells Blood Substit Immobil Biotechnol 35:481–489. https://doi.org/10.1080/10731190701586210

Wrobeln A, Laudien J, Groß-Heitfeld C et al (2017a) Albumin-derived perfluorocarbon-based artificial oxygen carriers: a physico-chemical characterization and first in vivo evaluation of biocompatibility. Eur J Pharm Biopharm off J Arbeitsgemeinschaft Pharm Verfahrenstechnik EV 115:52–64. https://doi.org/10.1016/j.ejpb.2017.02.015

Wrobeln A, Schlüter KD, Linders J et al (2017b) Functionality of albumin-derived perfluorocarbon-based artificial oxygen carriers in the Langendorff-heart†. Artif Cells Nanomed Biotechnol 45:723–730. https://doi.org/10.1080/21691401.2017.1284858

Wrobeln A, Jägers J, Quinting T et al (2020) Albumin-derived perfluorocarbon-based artificial oxygen carriers can avoid hypoxic tissue damage in massive hemodilution. Sci Rep. https://doi.org/10.1038/s41598-020-68701-z

Xiong Y, Liu ZZ, Georgieva R et al (2013) Nonvasoconstrictive hemoglobin particles as oxygen carriers. ACS Nano 7:7454–7461. https://doi.org/10.1021/NN402073N

Yabuki A, Yamaji K, Ohki H, Iwashita Y (1990) Characterization of a pyridoxalated hemoglobin-polyoxyethylene conjugate as a physiologic oxygen carrier. Transfusion (paris) 30:516–520. https://doi.org/10.1046/j.1537-2995.1990.30690333482.x

Yadav VR, Nag O, Awasthi V (2014) Biological evaluation of liposome-encapsulated hemoglobin surface-modified with a novel pegylated nonphospholipid amphiphile. Artif Organs 38:625–633. https://doi.org/10.1111/aor.12304

Yadav VR, Rao G, Houson H et al (2016) Nanovesicular liposome-encapsulated hemoglobin (LEH) prevents multi-organ injuries in a rat model of hemorrhagic shock. Eur J Pharm Sci 93:97–106. https://doi.org/10.1016/j.ejps.2016.08.010

Yang C, Xiao H, Sun Y et al (2018) Lipid microbubbles as ultrasound-stimulated oxygen carriers for controllable oxygen release for tumor reoxygenation. Ultrasound Med Biol 44:416–425. https://doi.org/10.1016/j.ultrasmedbio.2017.08.1883

Yao Y, Zhang M, Liu T et al (2015) Perfluorocarbon-encapsulated PLGA-PEG emulsions as enhancement agents for highly efficient reoxygenation to cell and organism. ACS Appl Mater Interfaces 7:18369–18378. https://doi.org/10.1021/acsami.5b04226

Zhou Z, Sun D, Levasseur JE et al (2008) Perfluorocarbon emulsions improve cognitive recovery after lateral fluid percussion brain injury in rats. Neurosurgery 63:799–806. https://doi.org/10.1227/01.NEU.0000325493.51900.53

Zhu J, Parsons JT, Yang Y et al (2021) Platelet and white cell reactivity to top-load intravenous perfluorocarbon infusion in healthy sheep. J Surg Res 267:342–349. https://doi.org/10.1016/j.jss.2021.05.044