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 transport, 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 (O2) to tissues and organs via red blood cells (RBCs) and removes carbon dioxide (CO2) 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 short-age 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,
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 CO₂ 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 (Fe²⁺) redox state, which binds with oxygen. After oxidation (loss of an electron), Fe²⁺ (ferrous) becomes Fe³⁺ (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 (Mihaiescu and Russu 2001). After binding to oxygen, Hb can change its shape and prevail in a relaxed state (R-state) with high oxygen affinity (Mihaiescu and Russu 2001; Modery-Pawlowski et al. 2013). When oxygenated Hb reaches tissues containing low partial pressure of oxygen (pO₂), it is divided into Hb and oxygen, thereby providing increased local pO₂; if the oxygenated Hb reaches pulmonary circulation containing high pO₂, it results in less pO₂ 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 CO₂ levels: For cellular respiration, several biochemical reactions essential causing enhances metabolic activity in tissues results in the CO₂ production as a metabolic byproduct. CO₂ 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 (HCO₃⁻) and H⁺ (hydrogen) ions in the blood. When blood CO₂ level
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 O₂ in peripheral tissue and input O₂ in the lungs. But due to decrease pH, Hb acts as a buffering agent by discharging its O₂ and Hb decreased its 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 (Ohha 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
| Types                                      | Sub-types | Products name/compositions                                                                 | References                                                                 |
|--------------------------------------------|-----------|---------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Hemoglobin-based oxygen carriers (HBOCs)  | Chemically altered Hb-based HBOCs | Cell-Free Hb                                                                               | Modery-Pawłowski et al. (2013), Moradi et al. (2016), Sen Gupta (2017, 2019), Ferenz and Steinbicker (2019), Bialas et al. (2019), Jähr et al. (2021) |
|                                            |           | HemAssist                                                                                 |                                                                           |
|                                            |           | Optro                                                                                      |                                                                           |
|                                            |           | Hemolink                                                                                   |                                                                           |
|                                            |           | Polyheme                                                                                  |                                                                           |
|                                            |           | PHP (Pyridoxylated Hb) or Hemoximer                                                        |                                                                           |
|                                            |           | PolyHb-SOD-CAT-CA                                                                          |                                                                           |
|                                            |           | PolyHb-Fibrinogen                                                                          |                                                                           |
|                                            |           | Hemotech                                                                                   |                                                                           |
|                                            |           | Hemospan                                                                                  |                                                                           |
|                                            |           | Sanguinate                                                                                 |                                                                           |
|                                            |           | PEG-Hb                                                                                     |                                                                           |
|                                            |           | SanFlow (PNPH)                                                                            |                                                                           |
|                                            |           | HemO₂Life/ 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                                                                                   |                                                                           |
|                                            |           | Oxycele                                                                                    |                                                                           |
|                                            |           | 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-Pawłowski et al. (2013), Kitagishi et al. (2017), Sen Gupta (2017), Norvaiša et al. (2021) |
|                                            |           | LipidHeme porphyrin                                                                         |                                                                           |
|                                            |           | HSA-heme porphyrin                                                                          |                                                                           |
|                                            |           | HemoCD porphyrin                                                                            |                                                                           |
| O₂ microbubbles                           | Lipid shell types                           | 1,2-Distearyl-sn-glycero-3-phosphocholine (DSPC)                                           |                                                                           |
|                                            |           | 1,2-distearyl-sn-glycero-3-phosphethanolamine-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                                                                       |                                                                           |
|                                            | Chitosan                                     | Dextran with or without polyvinylpyrrolidone (PVP)                                        |                                                                           |
|                                            | Surfactants stabilized microbubbles         | Span 60, D-alpha tocopheryl polyethylene glycol 100 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 intranod inter-molecularly. The first chemically altered HBOC was HemAssist (Baxter, Illinois, USA), in which Hb was cross-linked with diaspinir 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-rafinnose 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 inter-molecularly with glutaraldehyde (Hemopure, Biopure; Cambridge, MA, USA and Polyheme, Northfield Labs; Evanston, IL, USA), polyoxyethylene pyridoxylated polymer (PHP (Pyridoxylated Hb)), or Hemoximer (Curacyte/Apx 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 PHoXylated hemoglobin (PHP), or hemoximer, which is sourced from human Hb and surface-modified with a pyrroxyethylene 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 CO2 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 polyxoroxylated polyethylene glycol conjugated (PEGylated) Hb, respectively (Bedőcs and Szébeni 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).

HemO2Life/Hemarina-M101 (Hemarina) was created from lungworms, Arencia marina (invertebrate), which is not attached to Hb or other membranes (Ferenz and Steinbicker 2019; Bedőcs and Szébeni 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.
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). Furthermore, 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
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, HemO2-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). HemO2-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 vaso-activation 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-poly lactate 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, neoehemocytes, 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
| 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 | Cross-linked with glycine, O-raffinose             | Chen et al. (2009), Moradi et al. (2016), Bialas et al. (2019) |
|                          | Optro        | Somatogen     | Recombinant                   | Cross-linked with glycine   | Cross-linked with O-raffinose                      | Chen et al. (2009), Moradi et al. (2016), Bialas et al. (2019) |
|                          | Hemolink     | Hemosol       | Human                         | Cross-linked with glycine   | Cross-linked with O-raffinose                      | Chen et al. (2009), Moradi et al. (2016), Bialas et al. (2019) |
| Polymerized and/or tethered Hb | Hemopure (HBOC-201 and HBOC-301) | Biopure (afterward called OPKbiotech, now HbO₂ Therapeutics) | Bovine                       | Polymerization with glutaraldehyde                | Oxygen carrier, withdrawal of oxygen radical, CO₂ transportation | 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                         | Polyoxymethylene pyridoxylated polymer (Surface-modified) | Oxygen carrier and coagulation (platelet-like activity) |                                                     |
|                          | 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                                    |                                                     |
|                          | PolyHb-Fibrinogen | –             | Bovine                       | Cross-linking 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                                    |                                                     |
(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...
| Products name                  | Half-life                          | Indications                                                                 | Trials description                                                                 | Current status                                      | References                                                                 |
|-------------------------------|-----------------------------------|-----------------------------------------------------------------------------|------------------------------------------------------------------------------------|-----------------------------------------------------|---------------------------------------------------------------------------|
| HemAssist                     | Healthy volunteer: 2.5–3.3 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)               |
|                              | Hemodialysis patient: 2.1–4.3 h   |                                                                             |                                                                                   |                                                     |                                                                           |
|                              | Cardiac surgery patient: 24 h     |                                                                             |                                                                                   |                                                     |                                                                           |
| 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        | Severe anemia, hemorrhagic shock                                             | 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) |
|                              | Patient undergoing a liver section: 8.5 h |                                                                             |                                                                                   |                                                     |                                                                           |
| 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    | Critical limb ischemia, hemorrhagic Shock                                    | Phase III study was used to impede hypotension (NCT00421200)                      | Terminated in 2013                                  | Taguchi et al. (2017)                                                    |
|                              | Patient with orthopedic surgery: 14–23 h |                                                                             |                                                                                   |                                                     |                                                                           |
| 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)                                                |
| HemO₂Life                     | 2.5 days                          | For COVID-19 patients, sickle cell anemia, hemorrhagic Shock, organ preservation | Phase I is completed in 2018 (NCT04181710)                                         | Phase II and III in progress                        | Lupon et al. (2021), Varney et al. (2021)                                |
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

| Products name | Half-life | Indications | Current status | Trials description | References |
|---------------|-----------|-------------|----------------|--------------------|------------|
| OxyVita Hb    | 72 h      | Hemorrhagic shock, severe hemorrhage | In progress | Preclinical development in progress | Harrington and Wollrock (2011), Ferenz and Steinbicker (2019); In progress Harrington and Wollocko (2011), Ferenz and Steinbicker (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 Bri-
ceno 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 destabiliza-
tion of AOCs (Jägers et al. 2021). In coalescence, two nan-
odroplets 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 coa-
lescence, 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 desta-
bilization is dominated by molar volume, solubility, and dif-
fusion 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 ingre-
dients (Grapentin et al. 2015).

Furthermore, Perftoran (14% PFD and 6% perfluorome-
ethyl-cyclohexylyphosphinid) was used to treat severe blood
loss and was approved for clinical use in Russia, Kazakh-
istan, 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). Sub-
sequently, new-generation products, such as Oxygent (58%
perfluoroocetyl bromide and 2% perfluorodecyl bromide)
(Alliance Pharmaceutical Corp., USA) and Oxyfluor (78%
perfluorodichlorooctane) (HemaGen, St. Louis, USA),
were used to resolve the side effects of Fluosol-DA. Ox-
ygent 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-butylperfluorocy-
lohexane) (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 con-

| Products name | Sources | Encapsulated components | Features/Characteristics | Functions | References |
|---------------|---------|-------------------------|-------------------------|-----------|------------|
| Hb corpuscles (artificial) | Hemoglobin | (i) Collodion (cellulose nitrate) | Increase circulation half-life, reduce antigenicity, expand the specific site targeting and generate water-soluble Hb | More oxygen transport performance, easily circulates due to less viscosity | Moradi et al. (2016), Sen Gupta (2019) |
| Liposome encapsulated hemoglobin (LEH) | Bovine hemoglobin | (i) PEG-polylactate polymer (ii) Cholesterol with phospholipid | Resemblance to human RBC, made in huge quantities, more Hb loading capability | Used as oxygen carrier, used as drug delivery for cancer treatment | Kaneda et al. (2009), Haldar et al. (2019) |
| Polymersome-encapsulated hemoglobin (PEH) | Human and bovine hemoglobin | Polymeric vesicles such as poly(lactic acid)/poly(e-caprolactone) and poly(lactic-coglycolic acid)/PEG copolymers | Resemblance to human RBC, made in huge quantities, more Hb loading capability | Used as oxygen carrier, used as drug delivery for cancer treatment | Rameez et al. (2008), Haldar et al. (2019) |
| 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                          | 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) |
| 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 ααHb (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                 | Abutarboush et al. (2014) |
### Table 5 (continued)

| Products | Purposes | Animal model/cell line | Administration (Route and dose) | Results | References |
|----------|----------|------------------------|---------------------------------|---------|------------|
| Liposome-encapsulated hemo-globin (LEH) with high O2 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 | Fukui et al. (2017) |
| Liposome-encapsulated hemo-globin (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 | 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 | Rao et al. (2015) |
| | To assess the high O2 affinity of LEH is better than the low O2 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 O2 affinity of LEH reduces histological damage in the cerebral cortex and protected the cerebral metabolic rate of O2 | Kawaguchi et al. (2017) |
| LEH arisen on the use of polyethylene glycol connected with non-phospholipid hexadecylcarbamoylmethylhexadecanoate | 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 | Yadav et al. (2014) |
| | 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 | Baek et al. (2012) |
| | 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) |
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 O₂ (Fig. 4c) (Spiess 2009; Wrobeln et al. 2017b; Jägers et al. 2021).
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 CO₂, which was better for improved oxygenation. It can

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**Fig. 4** a Fluosol-DA (perfluorodecalin), Perftoran (perfluorodecalin+perfluoromethyl-cyclohexylpiperidine), Oxygent (perfluorooctyl bromide), Oxyfluor (perfluorodichloro octane), Oxy- cyt (tertbutylperfluorocylohexane) (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 O₂ uptake into the PFC droplet occurs rapidly which decrease the diffusion distance between RBCs and endothelium and act as stepping-stones for O₂ (Reprinted with permission from (Jägers et al. 2021) Copyright © 2020, The Author(s), Springer Nature)
| 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 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 corp. And Alpha therapeutinc) | 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) |
| Oxygen (Alliance Pharmaceutical Corp., USA) | 58% Perfluoroctyl 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 (Hema-Gen, St. Louis, USA) | 78% Perfluorodichloro octane | 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) |
| 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 pre-clinical 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 enhanced 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, CO₂, 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 (Cabralaes 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” Fe²⁺ 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(α-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
| 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 CO₂. Finally, this capsule impeded hypoxic tissue damage | Wrobeln et al. (2020) |
| Perfortran | 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) |
| | To evaluate the properties (potential vasoactive) of perfortran by measurement of pial arteriolar diameters in a healthy rat brain | Rat (Sprague–Dawley, Male, 300–450 g) | Perfortran 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 Perfortran (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 Perfortran (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 perfortran infusion, no platelet effect was found | Zhu et al. (2021) |
| 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 oxycyte™ 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, oxycyte™ 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)         |
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) (O2), Fe(TpivPP)(1-MeIm) (O2), and Fe(TpivPP)(2-MeHIm) (O2), 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) (FeII)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 FeII)TPPS, which is necessary for oxygen binding and has a longer half-life (Kano and Kitagishi 2009). In addition, another new 1:1 complex, FeIIPImCD (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) |

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**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) Lipid-Heme porphyrin vesicles as an artificial O₂ carrier [Reprinted with permission from (Tsuchida et al. 2009). Copyright © 2009, American Chemical Society].  
(c) HSA-heme porphyrin as an artificial O₂ 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.

**O₂ 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 amphipilic 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 were 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 O₂ 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, SE61O₂, 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)(O2), Fe(TpivPP)(1-MeIm)(O2), Fe(TpivPP)(2-MeHIm)(O2)) | (i) Fe(TpivPP)(1-EtIm)(O2) (ii) Fe(TpivPP)(1-MeIm)(O2) (iii) Fe(TpivPP)(2-MeHIm)(O2) | The results indicate the bond of Fe-oxygen was temperature-dependent, not orientational preference | Li et al. (2013)             |
|                               |                                                                          | (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(Me2Im)) | The results indicate the O2 binding process was similar for Hb and that model                | Collman et al. (1978)       |
|                               |                                                                          | (i) 2-[8-(2-Methylimidazolyl)-octanoyloxymethyl]-5,10,15,20-tetrakis [R, R, R-o-pivalamido] phenyl| The heat resistant recombinant enzymes were incorporated with synthetic iron (II) porphyrin which can make O2 complexes up to 90 °C temperature. It was the first synthetic heat-resistant O2-carrying enzyme | Komatsu et al. (2005a)      |
|                               |                                                                          | porphinatoiron(III) bromide [FeP Br-] (ii) Thermostoga maritima xylanase B (iii) Dictyoglomus thermophilum xylanase B |                                                                                  |                             |
|                               | Picket-fence cobalt porphyrin                                           | (i) meso-R, R, R-R-tetakis(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-vinylimidazoleco-o-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 Clm-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-tetakis (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) (ii)Liposome-embedded-heme/ triglyceride microsphere (LH-M)      | In the case of L/H, 12.7–24.4% oxygen consumed of the total volume                                                                                   | Kobayashi et al. 1994 |
|                                           |                                                                         |                                                                                                                                                             | In the case of (LH-M), 13.1–16.4% of oxygen consumed of the total volume                                                                               |                     |
| 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)histidinidobutanoyloxy]methyl]-5,10,15,20-tetrakis[ R,R,R,R-o-(1'-methylcyclohexanamido)phenyl]porphinatoiron; (iii) 2-[4-methoxycarbonyl(3-methyl)histidinidobutanoyloxy]methyl]-5,10,15,20-tetrakis[ R,R,R,R-o-(1'-methylcyclohexanamido)phenyl]porphinatoiron | 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, incorporated 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-[[N-(2-Methylimidazolyl)]octanoyloxy)methyl]-5,10,15,20-tetrakis(o-pivalamido)phenylporphinatoiron(II) (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[ (+, +, +, 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[ (+, +, +, 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) |
| 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)  |
|                  |                                                                          | (ii) Per-O-methylated b-cyclodextrin dimer                                       | Fe(II)TPPS encapsulated by two cyclodextrins is necessary for oxygen binding                     | Kano and Kitagishi (2009)               |
|                  | To know the benefits of hemoCD synthesis and its O₂/CO selectivity       | (i) 5,10,15,20-tetrakis(4-sulfonatophenyl) porphinatoiron(II) (Fe(II)TPPS)       | It acts as a good artificial oxygen carrier                                                     | Kitagishi et al. (2017)                 |
|                  |                                                                          | (ii) a cyclodextrin (CD) dimer having a pyridine linker                          | The synthesis of CD dimer was successful to gain its in gram quantities                         |                                          |
|                  |                                                                          |                                                                                 | HemoCD was the appropriate compound for oxygen carrier due to its adequate affinity of O₂ 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 PAA (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)                 |
|                  | 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)          |
| 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)                 |
| Fe₃PImCD         | To examine activity (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 (Fe₃PImCD) | (i) 1:1 inclusion complex (Fe₃PImCD) of 5,10,15,20-tetrakis(4-sulfonatophenyl) porphinatoiron(II) (Fe(II)TPPS)  |
|                  |                                                                          | (ii) O-methylated b-cyclodextrin dimer with an imidazole linker (ImCD)           | Fe₃PImCD 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)                      |
| Name                  | Purposes                                      | Used components                                                                 | Results                                                                                                     | References                        |
|-----------------------|-----------------------------------------------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|-----------------------------------|
| HemoCD -AuNPs         | To evaluate the circulation time in the blood | (i) Tris (4-sulfonatophenyl)-porphinoiron (III) (FeIIIp2)                       | HemoCD-AuNPs used as oxygen and carbon monoxide carrier of diatomic molecules                                | Karasugi et al. (2011)            |
| (Gold nanoparticles) | stream of PEGylated AuNPs (20 nm in diameter) | (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) porphinoiron (II) and |                                                                                                              |                                   |
|                       |                                               | Py3CD                                                                            |                                                                                                              |                                   |
| In vivo study         |                                               |                                                                                  |                                                                                                              |                                   |
| HemoCD1               | To develop a new technique to detect and quantify CO using hemoCD1               | (i) 5,10,15,20-Tetrakis (4 sulfonatophenyl) porphinoiron (III) (FeIIITPPS)        | HemoCD1 acts as significant adjuvant to oxygenation for elimination of excess CO from organs, even the brain | Mao et al. (2021)                 |
|                       |                                               | (ii) Py3CD                                                                       |                                                                                                              |                                   |
|                       |                                               | Animal preparation:                                                              |                                                                                                              |                                   |
|                       |                                               | Lewis and Sprague–Dawley rats (5 weeks)                                          |                                                                                                              |                                   |
| Oxy-HemoCD            | To study the effect of oxy-hemoCD as a CO   | (i) 1:1 inclusion complex of meso-tetrakis (4-sulfonatophenyl) porphinoiron (II)  | The oxy-hemoCD able to expel CO from cell-free CO-Hb                                                        | Kitagishi et al. (2016)            |
|                       | depleting agent                               | (ii) per-O-methylated β-cyclodextrin dimers                                       |                                                                                                              |                                   |
|                       |                                               | Animal preparation:                                                              |                                                                                                              |                                   |
|                       |                                               | Male C57BL/6 N mice (20–22 g)                                                    |                                                                                                              |                                   |
|                       |                                               | Administration: HemoCD (0.15 mL); Intraperitoneally                               |                                                                                                              |                                   |
| HemoCD                | To study the effect of HemoCD as a CO removal | (i) 5,10,15,20-tetrakis (4-sulfonatophenyl) porphyrinato iron (II) (FeIIITPPS)  | The CO was successfully detected and expelled by hemoCD                                                      | Kitagishi et al. (2010)            |
|                       | agent from living body                        | (ii) per-O-methylated β-cyclodextrin dimer with a pyridine linker (Py3CD)          |                                                                                                              |                                   |
|                       |                                               | Animal preparation:                                                              |                                                                                                              |                                   |
|                       |                                               | Wistar male rat (270–350 g); Intravenously                                       |                                                                                                              |                                   |
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).

**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]

**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
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 13 Properties of different O2 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) | − 4 μm | 95% oxygen, 5% perfluorobutane | Sonication | Kwan et al. (2012), McEwan et al. (2015), Fix et al. (2015), Khan et al. (2018a) |
| | (ii) 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N- [amino(polyethyleneglycol)] (DSPE-PEG) | 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-methoxy(polyethylene glycol 5000)-1,2-dipalmitoylcephalin sodium (DPPE-MPEG5000) | 1033 ± 72 nm (1 day) 1069 ± 53 nm (3 days) 1055 ± 89 nm (7 days) | Increase sixfold of pO2 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; Perfluorobutane | 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; Perfluorobutane | 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) |
| 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-O₂ mL⁻¹) and rapid diffusion capability (kLa = 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) |
| SE61O₂ (mixture of Span 60 and vitamin E (water-soluble)) | To determine the capability of SE61O₂ 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 | SE61O₂ microbubbles more workable for oxygen transport to hypoxic cells in solid tumors | Eisenbrey et al. (2015) |
| DSPC, (DSPE-PEG-2000)Amine), (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-1α 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.

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