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Therapeutic delivery of oxygen using artificial oxygen carriers demonstrates the possibility of treating a wide range of diseases

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Abstract

Artificial oxygen carriers have emerged as potential substitutes for red blood cells in situations of major blood loss, including accidents, surgical procedures, trauma, childbirth, stomach ulcers, hemorrhagic shock, and blood vessel ruptures which can lead to sudden reduction in blood volume. The therapeutic delivery of oxygen utilizing artificial oxygen carriers as red blood cell substitutes presents a promising avenue for treating a spectrum of disease models. Apart from that, the recent advancement of artificial oxygen carriers intended to supplant conventional blood transfusions draws significant attention due to the exigencies of warfare and the ongoing challenges posed by the COVID-19 pandemic. However, there is a pressing need to formulate stable, non-toxic, and immunologically inert oxygen carriers. Even though numerous challenges are encountered in the development of artificial oxygen carriers, their applicability extends to various medical treatments, encompassing elective and cardiovascular surgeries, hemorrhagic shock, decompression illness, acute stroke, myocardial infarction, sickle cell crisis, and proficient addressing conditions such as cerebral hypoxia. Therefore, this paper provides an overview of therapeutic oxygen delivery using assorted types of artificial oxygen carriers, including hemoglobin-based, perfluorocarbon-based, stem cell-derived, and oxygen micro/nanobubbles, in the treatment of diverse disease models. Additionally, it discusses the potential side effects and limitations associated with these interventions, while incorporating completed and ongoing research and recent clinical developments. Finally, the prospective solutions and general demands of the perfect artificial oxygen carriers were anticipated to be a reference for subsequent research endeavors.

Keywords Artificial oxygen carriers, Red blood cell substitutes, Therapeutic oxygen delivery, Hemoglobin, Perfluorocarbon, Stem cell, Disease models

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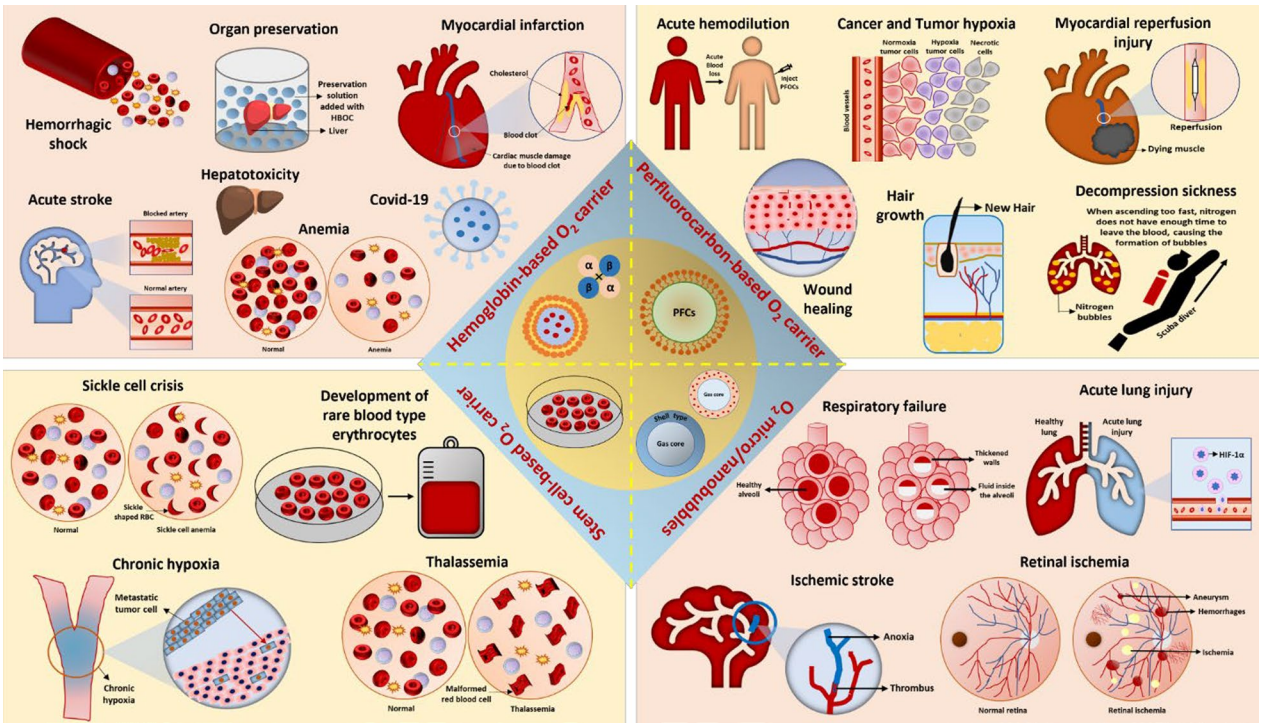
Graphical Abstract



Introduction

Blood, a vital bodily fluid within the circulatory system of humans and other vertebrates, is crucial in supplying essential substances like nutrients and oxygen to cells. Simultaneously, it facilitates the removal of metabolic waste products from these cells [1]. Blood comprises primarily red blood cells, commonly known as RBCs or erythrocytes, white blood cells, referred to as WBCs or leukocytes, and platelets, also known as thrombocytes in mammals. Hemoglobin, an iron-containing protein found in RBCs, plays a crucial role in transporting oxygen by reversibly binding to this respiratory gas, thereby enhancing its solubility in blood. Conversely, carbon dioxide is predominantly transported externally as bicarbonate ions in plasma [2]. Various factors can lead to blood loss, including accidents, surgical procedures, trauma, childbirth, stomach ulcers, hemorrhagic shock, and the rupture of blood vessels, all of which can result in a sudden reduction in blood volume. Furthermore, conditions such as sickle cell disease, thalassemia, cancer (chemotherapy), stem cell transplants, and leukemia frequently contribute to lower-than-normal levels of blood cells [3]. Besides these, trauma with massive blood loss is the leading cause of the battlefields, accounting for 50% of fatalities from exsanguinating hemorrhage [4]. According to the American Red Cross society, blood and platelets are needed by someone in the USA every 2 s. About 29,000 units of RBCs are needed daily in the United States, which adds up to almost 16 million blood components transfused yearly. Sickle cell disease impacts 90,000 to 100,000 individuals in the USA, and about 1000 newborns are born with the illness every year. Patients with sickle cell disease may necessitate blood transfusions throughout their lives [5].

To address this situation, the use of allogeneic RBC transfusions is implemented to enhance the oxygen-carrying capacity, representing the most prevalent method of resuscitation for hospitalized patients [6]. The onset of the modern era of blood transfusion aligned with World War II due to the significant need for widespread blood replacement [7]. The concerning situation is that blood donations are currently declining, exacerbated by the increasing number of canceled blood drives due to the pandemic. In the midst of the 2020 COVID-19 pandemic, shortages in blood supply were observed globally, these challenges also affected the USA [8]. In South Korea, the blood donor numbers declined cause of transfusion-transmitted human immunodeficiency virus (HIV) infections during 2004–2007, then in 2016

because of MERS-CoV infections, and finally in 2020 as a result of the COVID-19 pandemic [9]. Whenever various pandemic conditions arise, there is a significant and drastic drop in blood donations. Importantly, the SARS-CoV-2 pandemic has brought renewed focus on blood substitutes as a potential solution for treating hypoxemia associated with COVID-19. Additionally, it has gained significance in addressing the strain on blood banks [10]. Despite the life-saving nature of blood transfusions, significant side effects that impact patient outcomes have been documented, complications that can sometimes be fatal and originate from both infectious and noninfectious sources [7]. Immediate and delayed transfusion responses, transfusion-associated immunomodulation, transfusion-induced graft-versus-host disease (TA-GVHD), transfusion-associated acute lung injury (TRALI), reactions to hemolytic transfusions are just a few of the adverse effects that have spurred decades of research. Along these, transfusion-associated viral infections such as hepatitis B and C viruses, Zika virus, HIV, and parasitic contamination like babesiosis have been reported [7, 11]. Nevertheless, the primary challenge associated with allogeneic transfusion is the limited shelf life of blood, presenting potential issues in demanding environments [12]. Whole blood transfusion is naturally the initial preference for replenishing lost blood as it matches all of the natural components that are typically present in blood. Due to the evident concerns about the availability and safety matters, current efforts are directed toward developing artificial oxygen carriers (AOCs) that can serve as substitutes, restoring normal blood functions [13].

The advancement of oxygen carriers that are safe, efficient, and stable is of significant importance for replacing the recent condition of blood shortage. Furthermore, it can improve the synergistic therapeutic impact in hypoxic environments for various diseases. AOCs offer a range of amenities beyond allogeneic blood transfusions, potentially reducing morbidity and mortality for patients experiencing severe distress. It acts as an antiischemic agent in a range of pathogenic conditions that increase tissue oxygenation and deliver oxygen to organs and tissues [14]. Moreover, recent research indicates their potential utility in various areas, such as organ preservation for transplant surgery, sickle cell crisis, brain oxygenation during circulatory arrest, and so on [15]. Additionally, AOCs are suitable for Jehovah's Witnesses patients who abstain from blood transfusions. They reject both internal and external blood transfusions because they feel that receiving blood goes against God's will. Furthermore, blood management poses challenges for individuals with rare blood groups, like the Bombay type (Oh), and for highly immunocompromised patients [16]. As a result, AOCs serve as a safety net for patients experiencing severe blood loss and are a helpful system for life recovery. Numerous products have been used in several clinical emergencies in severely sick patients which increase tissue oxygenation and lower the risk of critical ischemia, however, research has not produced a commercial product that is equivalent to allogeneic erythrocytes without causing appreciable adverse effects [17]. We provided an overview of the details formulation, shelf-life, storage, overall perspectives, and pre-clinical (in vitro and in vivo) assessments of AOCs as RBC substitutes in our previous paper [15]. Therefore, this paper provides an overview of therapeutic oxygen delivery using assorted types of AOCs, including hemoglobin-based oxygen carriers (HBOCs), perfluorocarbon-based oxygen carriers (PFOCs), stem cell-derived, and oxygen micro/nanobubbles, in the treatment of diverse diseases. Additionally, it discusses the potential side effects and limitations associated with these interventions, while incorporating completed and ongoing research and recent clinical developments from 2017 to 2024. Additionally, this article reviewed prospective solutions and general demands of the perfect AOCs.

Artificial oxygen carriers as red blood cell substitutes

AOCs play a crucial role in addressing blood-related conditions in individuals with severe diseases. The primary advantage of these systems lies in their instant delivery of oxygen through the circulatory system, ensuring life-saving without any hindrance. AOC products can be used in trauma and austere environments where blood donation is not viable [15]. Moreover, they are also applicable in medical treatments, including elective and cardiovascular surgeries, decompression illness, acute stroke, sickle cell crisis, and effectively mitigating conditions like cerebral hypoxia [1]. Furthermore, oxygen carriers are frequently referred to as blood substitutes, even though these substances do not completely replace blood or all of its functions, including immune response, coagulation, and nutrition transport [18]. These are artificial solutions that can bind, transfer, and supply oxygen to any required tissue or organ of the body [19].

AOCs typically utilize hemoglobin (Hb), commonly derived from bovine or human sources. Alternatives, other non-hemoglobin oxygen carriers, such as perfluorocarbon (PFC) based oxygen carriers, and oxygen microbubbles/nanobubbles have been investigated for their O_2 delivery abilities. Furthermore, stem cell RBCs can also be used for patients with chronic blood transfusion.

The operational mechanisms of AOCs within the body differ fundamentally from one another. HBOCs and PFOCs are mainly administered through intravenous (IV) injection, enabling rapid systemic distribution essential for oxygen delivery in critical scenarios such as trauma and surgery. Alternative administration methods such intra-arterial delivery or inhalation for PFCs are being researched, but not yet accepted as standard procedure [13]. HBOCs imitate the oxygen transport mechanism of native hemoglobin by reversibly binding oxygen to heme groups, even though RBCs' protective cellular context is absent. This may make it easier for them to reach tissues, particularly in situations where blood flow is constrained. They readily move throughout the plasma, improving the supply of oxygen in areas where blood flow is impaired [20, 21]. In contrast, PFCs function by dissolving oxygen in accordance to the surrounding partial pressure of oxygen (pO_2). In alveolar capillaries, PFCs absorb oxygen and release it into tissues with lower pO_2 through passive diffusion [22].

AOCs must traverse several barriers, such as the cellular membranes, interstitial space, and vascular endothelium, to efficiently deliver oxygen to tissues [21]. HBOCs are eliminated by renal filtration, while they can lead to oxidative stress and methemoglobin buildup, they can also promote vasoconstriction and hypertension by scavenging nitric oxide [13, 23]. Conversely, PFOCs are mostly expelled through the lungs after dissolving gasses. Although PFOCs are resistant to enzymatic breakdown, there may be some uptake by the reticuloendothelial system (RES), particularly liver and spleen. Often, additional oxygen is needed to increase their efficacy [14]. The schematic illustration of oxygen delivery mechanism of AOCs within the body is shown in Fig. 1.

Overview of AOCs

Hemoglobin-based oxygen carriers (HBOCs)

HBOCs aim to mimic the oxygen and nutrient transport roles of RBCs which are applicable in various life-threatening conditions, including trauma, stroke, acute blood loss, hemorrhagic shock, and myocardial infarction [24]. Hemoglobin (Hb) is a tetrameric protein molecule, approximately 64 kDa in size, composed of two noncovalently bound αβ dimers with excellent ability to carry oxygen. However, free Hb has restrictions to clinical use due to several difficulties such as nephrotoxicity, easy oxidation, no longer circulation time in animals, and hypertension [25]. To address these challenges, diverse approaches for engineering Hb modifications have been suggested to create HBOCs where human, bovine, and recombinant Hb were used as raw ingredients. Hb is obtained through a process involving cell lysis, chromatography, sterile filtration, and low-heat sterilization which is carried out using outdated human or bovine RBCs [23, 26]. HBOCs is a semi-synthetic carrier system utilizing the natural oxygen carrier Hb which not only preserves the oxygen transport function of natural Hb but also mitigates the toxic effects associated with free Hb [27].

To date, various techniques have been devised to develop HBOCs through chemical modifications and

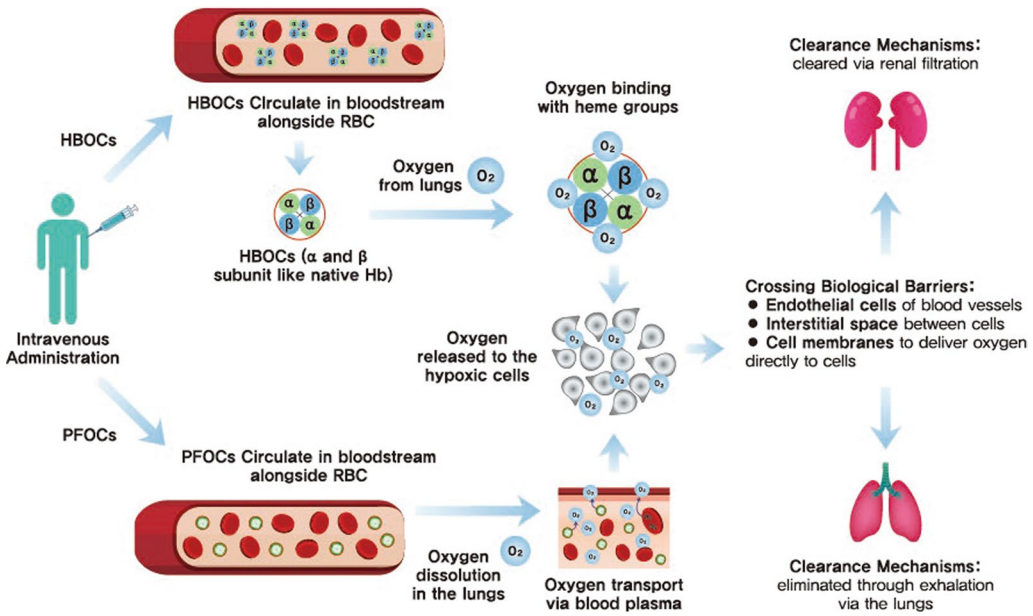


Fig. 1 Schematic illustration of oxygen delivery mechanism of AOCs

stabilization of the Hb molecule. The goal of these modifications is to enhance oxygen release, employing methods such as intramolecular cross-linking to maintain the tetramer. Additionally, the high affinity of oxygen has been diminished through the use of 2,3-DPG analogs or combinations of intramolecularly cross-linked structures and oxygen affinity modifiers like bis-(3,5-dibromosalicyl)-fumarate (DBBF) and 2-nor-2-formylpyridoxal phosphate (NFPLP) [28, 29]. HBOCs are categorized into two main types: chemically modified HBOCs and encapsulated HBOCs where Hb is enclosed within a protective shell [30], as shown in Fig. 2a.

The chemically modified HBOCs include cross-linked Hb including both intra and inter-molecularly, polymerized Hb, polyethylene glycol conjugated Hb, natural extracellular biopolymer Hb, genetically engineered recombinant Hb [13–15]. Despite decades of devoted improvement, the majority of chemically modified HBOCs have fallen short of achieving sufficient circulatory half-lives like > 12–18 h, which significantly restricts their usefulness for short-term utilization in

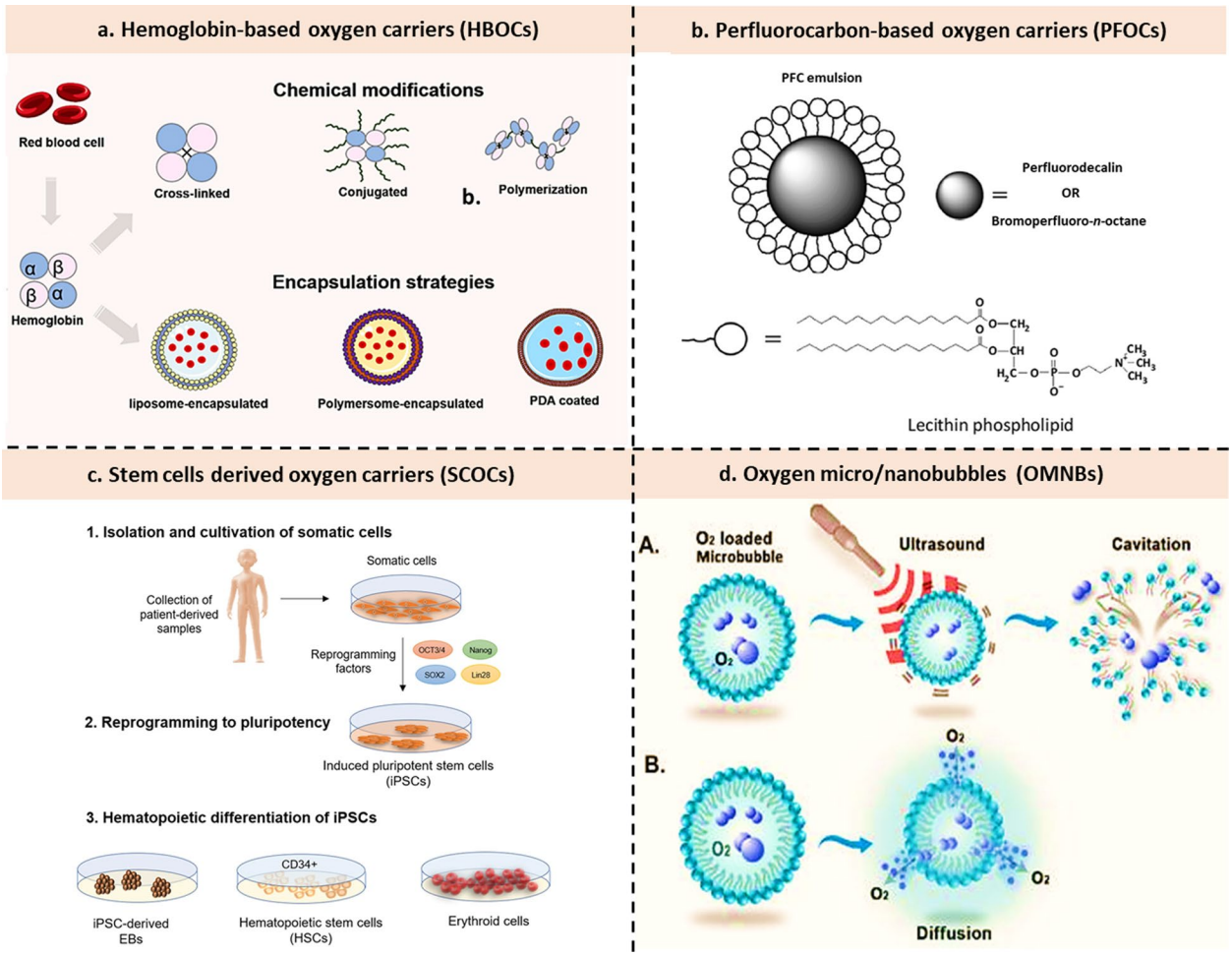


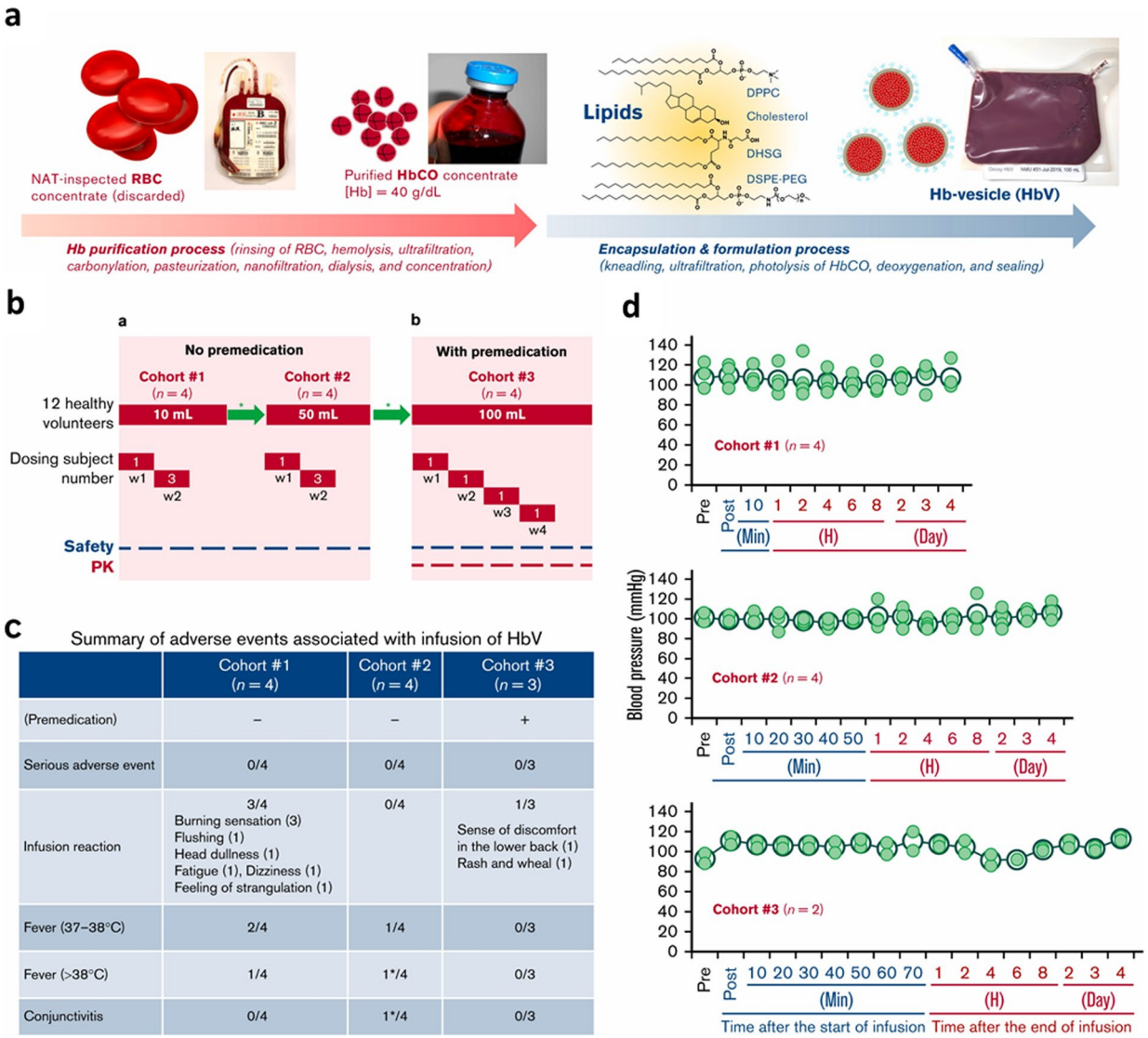
Fig. 2 Potential artificial oxygen carriers. **a** Hemoglobin-based oxygen carriers (HBOCs) with Hb modifications mechanisms; Chemical modification: where Hb α and β subunits cross-linked intramolecularly with glycine, glutaraldehyde, O-raffinose etc., conjugated on the surface by maleimide-activated Polyethylene glycol, and intramolecularly polymerized with glutaraldehyde, o-raffinose etc.; Encapsulation strategies: where Hb encapsulated with liposomes, sub-micron size polymeric vesicles (polymersomes) made from amphiphilic block copolymers, and polydopamine (PDA). Reprinted with permission from [30]; **b** Perfluorocarbon-based oxygen carriers (PFOCs), (Schematic illustration of structural compositions for PFC emulsions, where PFCs (perfluorodecalin/ Bromoperfluoro-n-octane) are surrounded by lecithin phospholipid emulsifier). Reprinted with permission from [56], **c** Stem cells derived oxygen carriers (SCOCs); Differentiation of human iPSCs into RBCs derived from primary fibroblasts using Oct4/Klf4/Sox2/LIN28 through three steps: generation of iPSCs, HSCs, and finally RBCs. Reprinted with permission from [73], **d** Oxygen delivery mechanism of Oxygen micro/nanobubbles (OMNBs), (A) OMNBs disruption using ultrasound and (B) diffusion of oxygen across the concentration gradient. Reprinted with permission from [86]

cases of acute blood loss [31, 32]. Furthermore, they generally exhibited undesirable toxicities in the clinical trials, such as, vigorous microvascular vasoconstriction leading to end-organ damage, hypertensive emergencies, cardiovascular dysfunction like myocardial infarction, renal toxicity, renal failure, gastrointestinal distress, and unfortunately enhanced mortality [33–36].

Despite the discontinuation of numerous HBOCs products, valuable insights can be gleaned from fundamental research analysis that has identified their limitations. For instance, the infiltration of modified Hb into blood vessel endothelium has been partially attributed to the vasoactivity of acellular HBOCs, leading to oxidative damage and nitric oxide (NO) consumption, which is a crucial paracrine signaling element essential for the underlying smooth muscle relaxation [37, 38]. Moreover, the delivery of oxygen carried by these permeable Hb initiates autoregulation of blood flow, accelerating the contraction of arteriolar vessels, which, in turn, elevates blood flow as well as hydrodynamic pressure [38, 39]. In order to mitigate the harmful impacts of blood substitutes arising from the inherent toxicity of hemoglobin, a new pharmacologic cross-linking blood substitute called HemoTech has been reported, which is both safe and efficacious. The cGMP manufacturing process of HemoTech incorporates an innovative and validated orthogonal technology platform designed for the efficient removal of endotoxin, prions, as well as both non-enveloped and enveloped viruses. The outcomes from preclinical and clinical investigations affirm that HemoTech demonstrates non-toxic characteristics which possesses vasodilatory properties, capable of mitigating vasoconstriction subsequent to hemorrhage [10, 40]. Significantly, certain polymerized Hb products like PolyHeme™, Hemopure™, Oxyglobin™ and Oxy-vita exhibit restricted NO sequestration. This is probably because their larger particle sizes hinder their ability to interact with the endothelium underneath [38, 41]. The surface alteration of Hb using inert polymers, such as polyethylene glycol (PEG) with molecular weight > 5 kDa seen in Hemospan, leads to larger particle sizes. This enhancement improves blood circulatory half-lives, oncotic properties, product viscosity, and, promotes heightened intravascular oxygen delivery with minimal vasoactivity [42]. The Phase III clinical trial of Hemospan was employed to prevent hypotension, but it was also terminated in 2013 [43]. Considering, like other chemically modified HBOCs, PEG-modified polymerized or conjugated Hb is unable to autonomously regulate the oxidative state of iron (Fe) in their heme groups. This deficiency leads to the irreversible transformation of Fe^{2+}-containing Hb into Fe^{3+}-containing methemoglobin (metHb) [44, 45]. In contrast to Hb, metHb has a reduced capacity to carry oxygen and exhibits a heightened affinity for oxygen. This characteristic impedes oxygen delivery at physiological oxygen tensions, consequently leading to the onset of adverse effects, including hypotension and bradycardia [46].

In the encapsulated HBOCs, Hb is encapsulated within a phospholipid bilayer capsule, which will resemble the RBC membrane. A phase 1 human trial of Hb vesicles was recently approved in Japan in 2022 [20]. The researcher groups created Hb vesicles (HbVs), which a cellular-structured HBOCs that encapsulate purified and concentrated Hb molecules within liposome (PEGylated phospholipid vesicles) with a mean particle diameter of 225–285 nm, utilize a lipid bilayer membrane to shield against the toxic effects of molecular hemoglobin, and mimicking erythrocytes in the process shown in Fig. 3a [20]. In phase 1 study, twelve healthy male volunteers were received single doses of HbVs across 3 cohorts 1, 2, and 3 (n = 4), respectively. Cohort 1, and 2 receiving 10, 50 mL dosages without premedication and cohort 3 receiving 100 mL dosage with premedication (Fig. 3b). To prevent volume overload in healthy volunteers, the trial's maximum dose was limited to 100 mL. In cohorts 2 and 3, HbVs was infused at 1 mL/min for the first 10 min, then increased to 2.5 mL/min and performed different laboratory test, pharmacokinetic analysis. Several adverse effects happened, regarded as liposome-induced infusion reactions which is shown in Fig. 3c. The appearance of adverse effects shortly after starting HbVs administration and their spontaneous resolution within minutes without medication support this inference. In cohort 3, prioritizing subject safety, premedication with dexamethasone (6.6 mg IV), famotidine (20 mg Orally), and acetaminophen (500 mg Orally) was administered 1 h before HbVs dosing, a common practice for liposomal drugs. The first two subjects completed the 100 mL infusion without adverse effects, but the third developed back discomfort and a chest rash after 10 mL, leading to an immediate stop. No clinically significant changes were observed in vital signs, except for body temperature (Fig. 3d). All deviated laboratory results resolved without related symptoms, and the half-life in the bloodstream was about 8 h [20].

A new biomimetic HBOC with high stability, high reactive oxygen species (ROS) scavenging ability is erythrocyte membrane-encapsulated Hb oxygen carrier which was produced by coating the PLGA (poly-lactide-co-glycolide) core of hemoglobin through the membrane of the erythrocyte which maintains its long circulation time, high biological safety, and low immunogenicity [47]. Furthermore, another HBOC is polydopamine (PDA)-encapsulated Hb in which PDA as a biocompatible coating on



	Cohort #1 ($n = 4$)	Cohort #2 ($n = 4$)	Cohort #3 ($n = 3$)
(Premedication)	–	–	+
Serious adverse event	0/4	0/4	0/3
Infusion reaction	3/4 Burning sensation (3) Flushing (1) Head dullness (1) Fatigue (1), Dizziness (1) Feeling of strangulation (1)	0/4	1/3 Sense of discomfort in the lower back (1) Rash and wheal (1)
Fever (37–38°C)	2/4	1/4	0/3
Fever (>38°C)	1/4	1*/4	0/3
Conjunctivitis	0/4	1*/4	0/3

Fig. 3 Phase 1 study of HbVs; **a** Schematic diagram of production of HbVs, **b** Study design of phase 1 with and without premedication, **c** Summary of adverse effects related with the administration of the HbVs, **d** Result of systemic systolic blood pressure. Reprinted with permission from [20]

the surface of Hb- nanoparticles imparts antioxidation properties, preventing the oxidation of Hb to metHb. Simultaneously, it diminishes Hb leakage and alleviates the toxicity linked to free Hb [48]. Moreover, a new candidate, ErythroMer is a bio-synthetic, first-in-class, and nano-cyte blood substitute and it contains deformable, hybrid peptidic-lipid nanoparticle that carries a high payload of hemoglobin (Hb) per particle. This structure enables (a) context-sensitive control of oxygen capture and release, while (b) reducing adverse interactions between hemoglobin and nitric oxide (NO). ErythroMer remains in the early testing phases. Recent preclinical studies have demonstrated that it can effectively deliver oxygen in mice with 70% of their blood volume replaced by ErythroMer. Similarly, in rabbits, when half of their blood volume was removed, infusing ErythroMer successfully resuscitated the animals, much like real blood [49, 50].

Furthermore, the production of genetically engineered hemoglobin involves the use of recombinant DNA technologies. The altered human Hb genes are introduced using plasmids into *Escherichia coli* or *Saccharomyces cerevisiae.* The engineered human hemoglobin genes are subsequently expressed within *E. coli* to generate human Hb molecules [51]. Rather than relying on them, current efforts aim to derive recombinant Hb from plants, such as *Nicotiana benthamiana* [52]. Natural Extracellular Biopolymer Hb HBOCs produced from *Lumbricus terrestris* (LtEc) which is under research, whereas

another HBOC Hemarina-M101 was approved for clinical use by the European Union for donor organ preservation. It could improve COVID-19 patients' survival [53, 54]. Additionally, researchers are increasingly directing their focus toward fetal Hb, known for its greater stability compared to adult Hb. Fetal Hb provides advantages attributed to its lower oxidative reactivity compared to adult Hb, although, both of them produced similar yields of purified functional protein [55]. The summary of different HBOCs is listed in Table 1.

Perfluorocarbon-based oxygen carriers (PFOCs)

Perfluorocarbons (PFCs) are hydrocarbons in which the hydrogen atoms are entirely replaced with fluorine atoms, occasionally with additional halogens. They are transparent, chemically inert, and seemingly non-toxic liquids characterized by low boiling points and they exhibit insolubility in water and alcohol. Clinical applications necessitate the solubilization of PFCs using an emulsifying agent, particularly advantageous for individuals who reject blood or proteins derived from humans or animals [21]. PFCs can dissolve substantial quantities of gases. The solubility of gases in PFC liquids follows the order $CO_2 >> O_2 > CO > N_2$, corresponding to the decreasing molecular volume of the solute. PFC emulsion can easily navigate through blood vessels obstructed in certain diseases due to their small sizes and help to enhance the oxygenation rate, where RBCs face challenges. [22]. It exhibits an impressive oxygen dissolution concentration, reaching around 40%-50%, which surpasses water's capacity by 20 times and exceeds plasma by 2 times [23]. Additionally, it can dissolve 130–160 mL of carbon dioxide, two to three times more than water's corresponding capacity [24]. Linear PFCs like perfluoro-octyl bromide exhibit greater effectiveness in dissolving O_2 compared to cyclic molecules such as perfluorodecalin. Overall, the solubility of O_2 in PFCs is inversely proportional to molecular weight and directly proportional to the number of fluorine atoms [22]. Along with PFOCs, PFC-based nanoparticles were engineered for multifunctional nanomedicines, such as bioimaging contrast agents and drug delivery vehicles for the diagnosis and treatment of various diseases [25]. In early efforts to create substitutes for RBCs, PFC emulsions emerged as the most advanced alternatives to donor RBC units, as shown in Fig. 2b [56].

To date, PFOCs can be categorized into five subclasses based on the primary PFC used in the emulsion product including perfluorodecalin-based PFOCs such as Fluosol-DA, Perftoran, Albumin-derived PFC-based AOC, PFOB (perfluorooctylbromide) -based PFOCs like Oxygent, perfluoro-dichloro octane- based PFOCs like Oxyfluor, tert-butyl perfluoro cyclohexane-based PFOCs such as Oxycyte, and Dodecafluoropentane (DDFPe)-based PFOCs [16]. The general properties of different PFOCs are listed in Table 2. Fluosol-DA demonstrated an oxygen-carrying capacity of only 7.2% (v/v), which is lower than that of human RBCs (17–20% v/v) and proved insufficient in critical situations like anemia with significant blood loss. Despite its tendency to accumulate in the liver and spleen, the substance could be cleared by the lungs for a specific duration [13]. Fluosol-DA's development was impeded due to low durability and stability in blood vessels, along with the complexity of its configuration and use [26]. Oxyfluor™ and Oxygent™ exhibited enhanced lipophilicity, facilitating increased encapsulation of higher amounts of oxygen-enriched PFCs in these formulations. Oxyfluor™ was discontinued following early clinical trials due to severe adverse events associated with its effective dosage [27, 28]. Likewise, Phase III clinical trials of Oxygent™ were halted due to an enhanced occurrence of strokes among coronary bypass patients [13]. While the Russian-developed Perftoran™ has received approval for clinical use in Russia and Mexico and rebranded as Vidaphor™ by Fluor02 Therapeutics, Inc., USA, in North America and Europe, which has been safely administered to over 30,000 patients with only minor side effects, with aspirations for future FDA approval [29, 30]. Oxycyte is made up of submicron particles of 60% perfluoro(t-butylcyclohexane), with an egg phospholipid emulsifier. Phase II trial of oxycyte was completed in 2008 with severe non-penetrating traumatic brain injury patients, and initiated a Phase III trial in the USA to evaluate the cardiac surgery patients, but terminated in September 2014 due to lack of patient assignation [57, 58]. In 2018, ABL-101, the new code name of oxycyte, was reinstated in the Phase II trial in the UK specifically for acute ischemic stroke, launched by Aurum Biosciences Ltd, Glasgow, UK, a collaborative work. This clinical trial is groundbreaking as it employs a PFC nanoemulsion as a theranostic platform [59]. Dodecafluoropentane, a unique oxygen carrier with high affinity and transport capacity, exhibits notable safety effects on oxygen-reliant organs like the brain and heart [60, 61]. As a result, it has been formulated for addressing brain damage and hemorrhagic shock in medical treatment [62]. Presently, PFC development focuses on creating smaller and more stable emulsions. This effort aims to achieve necessary enhancements in biodistribution, circulatory properties, and clearance, exemplified by the creation of kinetically stable PFC nanoemulsions. Hence, researchers must seek a surfactant characterized by excellent biocompatibility when formulating novel PFOCs.

Human serum albumin (HSA) is a protein derived from human blood, possessing amphipathic properties, high biocompatibility, low toxicity, and minimal immunogenicity. Consequently, HSA is anticipated to offer a

Table 1 Summary of HBOC Products

HBOCs	Hb modifications mechanism(s)	Products Name	Sources	Notable side effects in human or animal trial	Current status	References
Cross-linked	α-α crosslinked with diaspirin	HemAssist (Baxter, IL, USA)	Human	Cerebrovascular emergency (stroke), Hypertension, Myocardial infarction, High mortality	Terminated in 1999	[16, 40]
	Cross-linked with O-raffinose	Hemolink (Hemosol, Toronto, Canada)	Human	Myocardial infarction, Transient ischemic attack, severe cardiotoxicity, Cerebrovascular emergency (stroke), Hypertension, High mortality	Terminated in 2004	[17]
	Cross-linked with ATP (Intra molecularly) and adenosine, glutathione (Inter molecularly)	Hemotech (Hemobiotech Inc, USA)	Bovine	-	Clinical 'proof-of-concept' was conducted in pediatric patients with sickle cell anemia	[10]
	Conjugated PHP (Pyridoxylated Hb) & Crosslinked human Hb	PHP or Hemoximer (Apex bioscience)	Human	Hypertension, High mortality, Myocardial infarction, Cerebrovascular emergency (stroke)	Phase II completed; Phase III terminated 2011 as AOC, but acts as a nitric oxide scavenger	[32]
Polymerized	Polymerization with glutaraldehyde	Hemopure (Biopure)	Bovine	Methemoglobinemia, Increased liver enzyme, oliguria, Hypertension	- Phase III completed & approved for human use in South Africa & Russia - Not approved by FDA in the USA yet, but FDA's Expanded Access Program (EAP) is ongoing, although the estimated completion date was December 2023, but no update till writing the manuscript	[24]
		Oxyglobin (Biopure); sister product of Hemopure		Circulatory overload causes tachypnea, dyspnea, harsh lung sounds, and pulmonary edema	Approved for veterinary use in USA, UK & Europe, although it is not commercially available now	[49]
	Polymerized Hb	Polyheme (Northfield Labs, Evanston, IL, USA)	Human	Acute renal failure, Transient ischemic attack, Hypertension, Myocardial infarction, Cerebrovascular emergency (stroke), High mortality	Phase III was completed in the USA, but not approved by FDA. Terminated in 2009	[17, 42]
	Polymerized Zero-linked Bovine Hb	OxyVita (OXYVITA Inc. Windsor, USA)	Bovine	-	Preclinical trial ongoing	[41]

Table 1 (continued)

HBOCs	Hb modifications mechanism(s)	Products Name	Sources	Notable side effects in human or animal trial	Current status	References
PEGylated modified Hb	Maleimide-PEGylated modified Hb	Hemospan (Sangart Inc. USA)	Human	Hypertension, Myocardial infarction, High mortality, Acute renal failure, Transient ischemic attack	Phase I and II trials completed with no serious adverse effects; Phase III trial was terminated in 2015	[42]
	PEGylated carboxy-Hb	PP-007 (previously known as Sanguinate) (South Plainfield, USA) HEMERA 1 (first clinical trial of PP-007 in acute ischemic stroke patients	Bovine	Musculoskeletal detrimental events, vertigo, lethargy	Phase I trials have been published, although several phase II trials have been completed 272 individuals received PP-007 treatment in 12 clinical trials conducted between 2013 and 2017	[45]
Encapsulated	Encapsulating a purified and concentrated Hb solution in PEGylated phospholipid vesicles	Hemoglobin vesicles (HbVs)	–	–	Phase I clinical trial in human was approved in Japan in November 2022	[20]
	Encapsulating Hb with a novel 2,3-DPG shuttle and incorporating a proprietary lipid known as KC1003	Erythromer	Human	–	Currently undergoing preclinical testing	[50]
Genetically Engineered Hb	Recombinant DNA technology	Optro by Somatogen		Significant nitric oxide scavenging	Terminated in 1999	[17]
Natural Extracellular Biopolymer Hb	Erythrocruorin	–	*Lumbricus terrestris* (LtEc)	–	Research ongoing	[53]
		HemO2Life/Hemarina-M101	*Arenicola marina* (AmEc)	–	Approved for clinical use by the European Union for donor organ preservation It could improve COVID-19 patients' survival	[53, 54]

Table 2 Summary of PFOC Products (Updated and modified from [15])

Products name	Formulations (%W/V PFC)	Current status	References
Fluosol-DA (Green Cross Corp.)	14% Perfluorodecalin and 6% Perfluorotripropylamine	Clinical trials finished in the 1980s; Approved in 1989 by FDA and discontinued in 1994 due to side effects	[12]
Perftoran (Perftoran, Russia)	14% Perfluorodecalin and 6% perfluoromethylcyclohexylpiperidin	Approved for clinical use in Russia, Kazakhstan, Kyrgyzstan, Ukraine, and Mexico from 2005 to 2010. Recently, re-branded as Vidaphor™ in North America	[12, 22]
Oxygent (Alliance Pharmaceutical Corp., USA)	58% Perfluorooctyl bromide and 2% perfluorodecyl bromide	Reached phase III trials, licensed and accepted in China for clinical studies in 2017	[12, 13]
Oxyfluor (HemaGen, St. Louis, USA)	78% Perfluoro-dichlorooctane	Phase III trials were suspended	[22]
Oxycyte (Oxygen Biotherapeutics Inc., North Carolina, USA)	60% tertbutylperfluorocyclohexane	Phase II was completed in 2008, but terminated in September 2014 due to lack of patient assignation	[60, 61]
ABL-101 (Aurum Biosciences Ltd, Glasgow, UK)	60% tertbutylperfluorocyclohexane	Although the estimated completion date was expired, but no update till writing the manuscript	[57, 58]
Dodecafluoropentane (DDFPe) (also known as perfluoropentane) (NuvOx Pharma, LLC, Tucson, Arizona)	2% DDFPe	Phase Ib/II completed in 2018 with acute ischemic stroke	[60, 61]
Albumin derived perfluorocarbon based artificial oxygen carrier (A-AOC)	17% Perfluorodecalin	Pre-clinical study	[63]
PFC@PLGA-RBC Membrane artificial red blood cells	–	Research ongoing (Biomimetic oxygen carrier)	[65]
Hybrid natural–synthetic biomimetic nanoemulsion	PFOB-RBC membrane nanoemulsions	Research ongoing (Biomimetic oxygen carrier)	[116]
Concave-shaped deformable PFC-based OCs (PFOB core and poly(lactide-co-caprolactone) shell)	–	Research ongoing (Young's modulus was close to that of human RBC (hRBC))	[66]

safer and more efficient emulsification process for developing perfluorocarbon (PFC) oxygen carriers. Wrobeln and colleagues employed HSA as an emulsifying agent to encase perfluorodecalin, creating a capsule containing perfluorocarbon [63]. The human erythrocyte membrane serves as a natural biological material containing several glycans and proteins on the surface, and retention time in the body is 120 days, which is employed as a natural carrier for long-circulating drugs. Moreover, it possesses excellent biocompatibility, nonimmunogenicity, and biodegradability [64]. Applying this functionalization method, some researchers prepared biomimetic PFC oxygen carriers.

A new novel oxygen self-enrichment biomimetic carbon–oxygen carrier was created which comprises two components: (1) an oxygen-carrying segment containing perfluorotributamine loaded with the near-infrared dye indocyanine green; (2) the external covering constituted the RBC vesicles extracted from the RBC membrane which helps to prevent uptake by macrophages, thereby extending the circulation time in vivo [64]. Gao and colleagues acquired nanoparticles by enclosing PFC within the biocompatible polymer poly (D, L-lactide-co-glycolide), PLGA. Subsequently, they coated the surface of these nanoparticles with the RBC membrane. This process resulted in nanoparticles characterized by a high oxygen-carrying capacity and an extended blood circulation time, referred to as PFC@PLGA-RBC membrane nanoparticles. For In vivo radiotherapy treatment of PFC@PLGA-RBC membrane nanoparticles, 4T1-tumor-bearing mice received a 200 μL IV injection of PFC@PLGA-RBCM. After 24 h, they were exposed to 8 Gy X-ray radiation. Mice provided with PFC@ PLGA-RBCM without X-ray exposure (PFC@PLGA-RBCM group), mice treated with X-ray radiation without nanoparticle (RT group), and mice administered with only PBS were used as control groups (Fig. 4a). PFC@PLGA-RBCM alone did not affect tumor growth, but merged with X-ray radiation, it significantly impeded tumor growth more effectively than radiation alone shown in Fig. 4b. 14 days post-treatment, mice were euthanized to evaluate tumors; average tumor weight was lowest in the group administered with both RT and PFC@PLGA-RBCM, significantly less than with RT alone (Fig. 4c). In the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling assay, significant damage

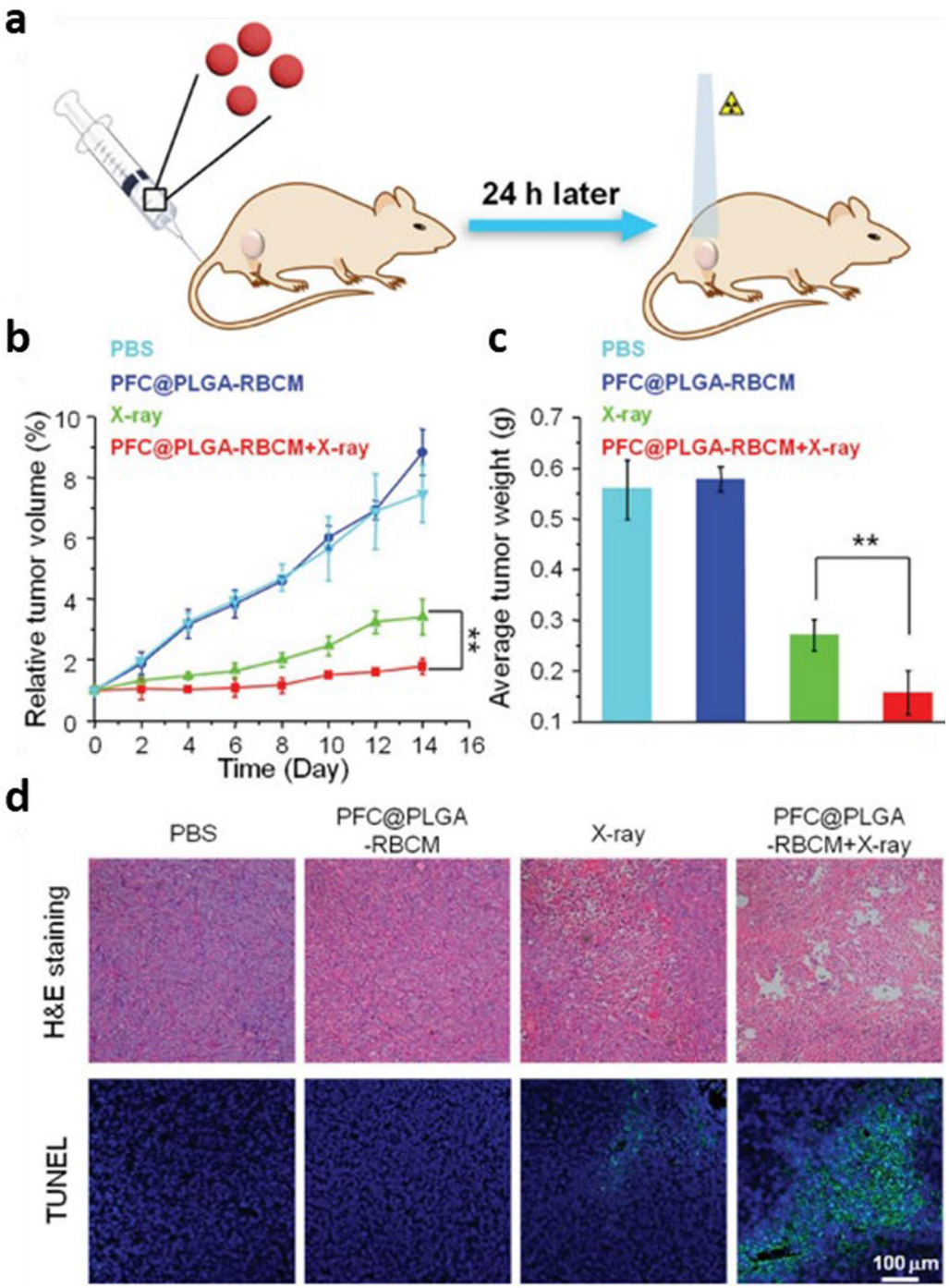


Fig. 4 In vivo radiotherapy treatment of PFC@PLGA-RBC membrane nanoparticles. **a** Experimental design of animal model; **b** Tumor growth curves of different groups; **c** Weight curves of tumor of different groups, **d** Stained tumor slices collected from 24 h post treatment of mice. Reprinted with permission from [65]

and high tumor cell apoptosis were found in the PFC@PLGA-RBCM + RT group, with minimal damage in control groups. Additionally, Hematoxylin & Eosin-stained images revealed no inflammation or organ damage in major organs 14 days after PFC@PLGA-RBCM + RT treatment, suggesting no in vivo toxicity (Fig. 4d) [65]. Furthermore, human erythrocytes, with their biconcave structure supported by ankyrin, possess a flexible

membrane that allows them to change shape, facilitating passage through narrow capillaries. Fu et al. featured a micron-sized, concave, and highly deformable core–shell PFC-based oxygen carrier following human RBC structure, called cDFCs whereas its core was PFOB, and poly(lactide-co-caprolactone) utilized as a thin and extremely deformable elastic shell [66].

Stem cell-based oxygen carriers (SCOCs)

Recent advancements in stem cell technologies have enabled the production of RBC-like cells from diverse cultured sources, mimicking the natural biogenesis process of human erythrocytes. In 2006, Takahashi and Yamanaka discovered a novel cell source capable of differentiating into cell types from endoderm, ectoderm, or mesoderm lineages [67]. These ex vivo-generated RBC oxygen carriers hold remarkable potential for advancing the creation of an unlimited source of RBC units for transfusion [68]. It is produced through induced hematopoietic stem cells of different origins which closely resemble natural RBC in terms of their physicochemical characteristics and biological functions. These carriers hold promise for fulfilling the objective of extended oxygen delivery to patients with chronic anemia, severe kidney or liver disease, acquired or congenital erythropoiesis defects, and chronic bleeding disorders. Additionally, they are well-suited for individuals with rare blood types or autoantibodies necessitating repeated transfusions [69].

Erythropoiesis is a developmental process whereby multipotent hematopoietic stem/progenitor cells (hSPCs) undergo specialization to produce circulating RBCs. The complex process of natural human erythropoiesis typically commences in the spleen and liver during early fetal development and later primarily takes place in the bone marrow [70]. In the case of ex vivo human RBC production, hSPCs undergo erythroid lineage differentiation through three sequential stages: commitment, expansion, and maturation. A combination of cytokines, often including stem cell factor (SCF) and erythropoietin (EPO), is incorporated into the culture medium to stimulate erythroid differentiation and maturation. Additionally, cells may be cocultured with feeder cells derived from murine or human sources. Broadly, RBC-like cells can be synthetically generated in vitro through the proliferation and differentiation of human embryonic stem cells (HESCs), natural hSPCs, or induced pluripotent stem cells (iPSCs). Moreover, another concept involves transdifferentiation, wherein human fibroblasts are directly reprogrammed into erythroblasts, circumventing the hSPCs stage [69].

For over a decade following the first bone marrow transplantation in the mid-50 s, bone marrow (BM) was regarded as the exclusive source of hSPCs. Subsequent animal-based studies demonstrated the existence of circulating hSPCs in the bloodstream of mice. However, Peripheral blood (PB) can supply hSPCs in smaller quantities in circulation, which can be expanded by administering recombinant growth factor, specifically granulocyte colony-stimulating factor (G-CSF). This process leads to a significant elevation in the number of circulating CD34 + progenitor cells in the bloodstream [71]. Apart from this, umbilical cord blood (UCB) is acknowledged as a valuable source of cells and is extensively utilized in hSPCs transplantations which is promptly accessible and can be gathered without endangering the mother or the infant. Nevertheless, the count of nucleated cells in the UCB unit is ten times less than that in BM and/or PB grafts [72].

The development of gene or cellular therapies for hematologic disorder can gain advantages from the generation of hSPCs or mature blood cells derived from iPSCs. hPSCs, encompassing both embryonic (hESCs) and induced (iPSCs) types, present an abundant source of blood cells for both experimental and therapeutic applications. Typically, iPSC differentiation into RBCs involves three stages: iPSC generation, HSC formation, and RBC maturation [73]. Human iPSCs are a promising therapeutic option for the in vitro or ex vivo production of RBCs. Reprogramming human somatic cells with transcription factors (OCT4, SOX2, KLF4, c-MYC, LIN28, and NANOG) offers new paths in disease modeling and regenerative medicine. Techniques now enable the production of enucleated RBCs from human iPSCs, presenting an alternative treatment for blood disorders which is shown in Fig. 2c [73]. Furthermore, iPSCs offer several advantages: (1) their cell source is readily accessible; (2) they can be generated at a relatively affordable cost; and (3) they serve as an essentially limitless source of both HSPCs and mature blood cells [74]. HEMOXCell, a mesenchymal stem cell-based RBC substitute is cultured in human platelet lysate–supplemented media. Based on their findings, there is a potential interest in utilizing HEMOXCell as a natural oxygen carrier in tissue engineering applications. Its role includes oxygenating hypoxic regions and ensuring the preservation of cell viability, functionality, and stemness [75]. Over the past decades, scientists have effectively produced SCOCs and demonstrated their therapeutic potential through animal models. Various approaches have been formulated for the ex vivo generation of RBCs: (1) mimicking the developmental hematopoiesis pathway towards erythrogenesis through pluripotent stem cells (PSCs), (2) redirecting the fate of either stem cells or somatic cells towards the hematopoietic lineage, and (3) fostering the expansion and maturation of circulating hematopoietic

Table 3 Summary of SCOC products

Cell source	Design method	Culture length (days)	Advantages	Disadvantages	References
Human embryonic stem cells (hESCs)	Differentiated into hematopoietic lineage to generate RBCs	46	- Entrenched quality control standards for Good Manufacturing Practice (GMP) grade - Exceptional expansion capacity	- Immature characteristics of produced RBCs - Inefficient induction of RBCs - Ethical and safety standards are required	[69]
Induced pluripotent stem cells (iPSCs)		46	- Appropriate for transfusions specific to a donor - Exceptional expansion capacity	- Immature characteristics of produced RBCs - Inefficient induction of RBCs - Safety standards are required	[76]
Umbilical cord blood (UCB)	CD34 + hSPCs are isolated from the blood	21	- Direct origin for hSPCs - Entrenched quality control standards for GMP grade - Has less sophisticated hSPCs than PB	- Restricted capacity for expansion - Immature characteristics of produced RBCs	[72]
Peripheral blood (PB)		30	- Direct origin for hSPCs - Exhibit more mature, adult-like characteristics	- Restricted capacity for expansion - The quality of hSPCs fluctuates based on individual variations	[71]
Immortalized RBC lines	hSPCs or erythroid progenitors are immortalized	–	- Suitable for subsequent gene editing - Potential for theoretically infinite expansion	- Safety standards are required	[76]
Human mesenchymal stromal cells (HMSCs)	Extracted from human bone marrow	Cultured until passage 3	- Promising candidates for tissue engineering - Entrenched quality control standards for GMP grade	- Requires an adequate quantity of expanded HMSCs within a confined timeframe of in vitro expansion	[75]

stem/progenitor cells (hSPCs) isolated from UCB and PB. The existing cell sources and strategies for ex vivo RBC generation are listed in Table 3 to acquire a satisfactory quantity of functional RBCs most conveniently and cost-effectively.

Oxygen micro/nano bubbles (OMNBs)

The transport of oxygen via micro/nanobubbles represents an alternative method for oxygen delivery in hypoxic conditions. Oxygen micro/nanobubbles (OMNBs), also referred to as ultra-fine bubbles or submicron bubbles, are minute gas-filled spherical entities encapsulated by an interface (gas–liquid or gas–solid). The OMNBs ranging in size from 0.1 to 10 μm, possess the capability to enter both major and minor blood vessels. Their stability is governed by factors such as Laplace pressure (the difference between inside and outside shell pressure), coalescence, and Ostwald ripening [77]. Additionally, micro/nanobubbles not only consist of oxygen but also contain other gases like nitrogen, which is enclosed in a liquid, typically water. These OMNBs are stabilized by a thin layer of molecules at the gas–liquid interface [78]. Structurally, they are spherical vesicles, comprising a shell-like phospholipid, proteins, polymers, and a core-holding gas. Various shells serve multiple essential functions, including enhancing bubbles' mechanical stability and protection. OMNBs have garnered considerable interest across diverse medical fields, including gas delivery, molecular imaging, gene therapy, and drug delivery [79].

OMNBs function as echogenic particles, responding to applied ultrasonic fields. Echogenicity is an inherent characteristic of OMNBs, resulting from the encapsulated gas within the shell. This creates a difference in the acoustic impendence of the shell and gas. The effective medical gas solubility of OMNBs inside the shell is their most promising characteristic [80]. OMNBs undergo oscillation in response to applied acoustic waves, driven by the density difference between the gas and the surrounding aqueous solution. Under low acoustic pressures, OMNBs exhibit persistent oscillation, a phenomenon referred to as stable cavitation. This phenomenon enhances the diffusion of the core gas out of the bubble [81]. OMNBs demonstrate shelf lives ranging from a few weeks to several months, contingent upon the type of shell materials used. In case of protein- shelled

microbubbles, experienced a loss of nearly half of the oxygen gas during a 12-day storage period [82], while lipid-shelled oxygen bubbles retained 80% of the gas over a two-week observation period. Meanwhile, polymer shells have been reported to exhibit a shelf life of approximately six months. The in vivo half-life of OMNBs varies from a few seconds to several hours, once again contingent upon the composition of the shell [83].

OMNBs can be injected into the circulatory system, creating supersaturated suspensions. Consequently, they have been employed to enhance oxygen content and alleviate hypoxia. Researchers have explored the use of OMNBs to improve the effectiveness of photodynamic therapy, chemotherapy, and radiotherapy in overcoming the effects of hypoxia [84]. Under hypoxic conditions, there is an overexpression and stabilization of the hypoxia-inducible factor-1α (HIF-1α) protein, identified as a major contributor to increased resistance in therapeutic interventions which contributes to elevated tumor resistance and a poorer prognosis [85]. Numerous researchers have focused on inhibiting or silencing HIF-1α as a strategy to enhance tumor treatment.

Figure 2d illustrates two primary methods for delivering gas through OMNBs. In one approach, OMNBs are intravenously injected, and subsequently, they are disrupted by applying high-intensity ultrasound. High-intensity ultrasound generates zones of high and low pressure along its propagating wave, causing the bubbles to resonate and rupture, releasing the core gas. A second method involves enabling the diffusion of gas along the concentration gradient. The majority of OMNB shells are permeable to gases, particularly soluble gases such as oxygen, which can readily diffuse out [86].

Applications of AOCs

AOCs comprise a wide array of synthetic compounds and substances designed to mimic the oxygen-carrying capabilities of RBCs or facilitate the transportation of oxygen within the bloodstream. The use of AOCs is justified by their capacity to enhance tissue oxygenation in circumstances when there is a shortage of oxygen or poor oxygen delivery. Hypoxia, characterized by insufficient oxygen supply to tissues, can manifest in a multitude of disease processes, spanning from wound healing and anemia to tumors, myocardial infarction, cerebral apoplexy, and hemorrhagic shock. If adequate oxygen is supplied in a hypoxic environment, it can slow down or halt the progression of the disease. For example, restoring the oxygen supply to ischemic tissues is essential in ischemic disorders like myocardial infarction and stroke to stop irreparable damage and encourage tissue healing. AOCs can also act as resuscitative agents in hemorrhagic shock, which is a condition in which blood loss results in systemic hypoxia and organ failure. In this case, oxygen delivery is maintained until normal blood circulation is restored. While AOCs hold promises for therapeutic use, their clinical translation is hindered by various challenges, including concerns regarding safety, efficacy, and regulatory approval. The subsequent sections will immerse into the utilization of oxygen carriers in diverse diseases, including acute blood loss, hemorrhagic shock, myocardial infarction, ischemia, tumors, and so on. We classified the application of AOCs into three categories: application of AOCs in diverse diseases, in different medical conditions, and in medical procedures.

Application of AOCs on diverse diseases

Anemia/ sickle cell anemia

Anemia is characterized by a reduced level of Hb or a lower-than-normal number of RBCs, leading to impaired oxygen transport, causes recurrent painful vaso-occlusive crises and damage to multiple organs, leading to decreased life expectancy. The purpose of using AOCs to treat anemia is to enhance oxygen delivery and reduce hypoxia, minimizing the risk of vaso-occlusive crises. Specially HBOCs is currently being given via "compassionate use" programs to Jehovah's Witnesses who refuse natural blood transfusions or who suffer from severe autoimmune hemolytic anemia.

HBOCs Three severely ill sickle cell disease patients with multiple organ failure showed successful outcomes following HBOC-201 treatment, which can provide an bridge of oxygen, until Hb levels in RBCs recover sufficiently to meet metabolic demands [87]. The Vel-negative blood type found in approximately 1 in 4000 individuals which is a rare autosomal recessive trait. The Vel-negative patients with leukemia pose significant challenges who may require frequent transfusions. Ayyoubi et al. successfully managed severe acute anemia in a patient diagnosed with refractory acute myeloid leukemia (AML) and anti-Vel antibodies, using the HBOC-201, when compatible blood was not available [88]. Gomez and colleagues noted that a Jehovah's Witness patient faced significant anemia, who diagnosed with acute lymphoblastic leukemia with a hemoglobin level dropping as low as 3.1 g/dl during chemotherapy. Nonetheless, the patient maintained hemoglobin levels between 3.6 and 5.3 g/dl during the HBOC-201 infusion, and there were no documented instances of ischemia or impaired organ function [89]. Zumberg et al. reported effective treatment outcomes after administration of at least ten units of HBOC-201 in 10 patients with life-threatening anemia and dismal prognosis. All patients survived and was released without any lasting effects. These results provide evidence for the safety and feasibility of long-term, high-dose HBOC-

201 administration as a preventative measure for treating severe anemia [90].

PFOCs An oxygenated perflubron-based fluorocarbon emulsion (PFE) was evaluated for its effectiveness in mitigating vaso-occlusive events in the ex vivo rat mesocecum vasculature. The effectiveness of PFE is attributed to its superior oxygen dissolution capacity, which is ten times higher than plasma. This enables the dislodgment of trapped Sickle cell RBCs and increases wall shear rates, potentially reversing partial obstructions. Oxygenated PFE shows promise in reducing Sickle cell RBC-induced vaso-occlusion, suggesting the need for further development in this approach [91].

SCOCs Allogeneic hematopoietic stem cell transplantation stands as the sole potentially curative option for these conditions. Hladun et al. transplant hematopoietic stem cells of three sickle cell disease patients from HLA-genotypically identical siblings. The results suggested that allogeneic hematopoietic stem cell transplantation from siblings with identical HLA genotypes offers a good chance of survival without complications. In addition, there were no new vaso-occlusive crises observed, and stabilization of pulmonary and neurological function was evident [92].

Anemia is a foremost application framework for AOCs. Common adverse effects of acellular Hb substitutes include increase in concentration of methemoglobin and vasopressor effects leading to elevated blood pressure, fluid overload, and myocardial infarction, but HBOC-201 has shown tolerability in critically anemic patients, offering a potential bridge when RBC transfusions are not feasible [88]. For vaso-occlusive crises, oxygenated PFE shows potential in reducing sickle cell RBC-induced vaso-occlusion, underscoring the need for further development of this approach [91].

Cancer and tumor hypoxia

Hypoxia is common in tumors due to their rapid, disordered growth, which depletes oxygen and creates a hypoxic tumor microenvironment (TME). The decreased oxygen levels within tumors can diminish the effectiveness of treatment modalities such as chemotherapy, radiation, photodynamic, and sonodynamic therapies. AOCS improve oxygen delivery, alleviate tumor hypoxia, and enhance treatment efficacy. Studies show that oxygen concentrations within highly hypoxic tumors are less than 5 mmHg, while normal tissue normally has oxygen concentrations between 30 and 50 mmHg. Therefore, optimizing the hypoxic microenvironment inside tumors is essential to treating hypoxic cancers successfully [93].

HBOCs Radiotherapy is an invaluable method in cancer therapy; however, its therapeutic efficacy is constrained by both its side effects and the development of tumor radiation resistance. Xu and colleagues administered fractionated radiotherapy combined with HBOC to treat Miapaca-2 cell and HeLa cell xenografts in nude mice and noted that HBOC ameliorated the hypoxic environment and suppressed the expression of hypoxia-inducible factor-1α (HIF-1α) in both regular (100 mm^3) and large (360/400 mm^3) tumors [94] which is shown in Fig. 5a.

Furthermore, A novel engineered erythrocyte biomimetic nanovesicle, based on hemoglobin (SPN-Hb@RBCM), has been developed as an oxygen supply agent. It aims to alleviate tumor hypoxia, thereby enhancing

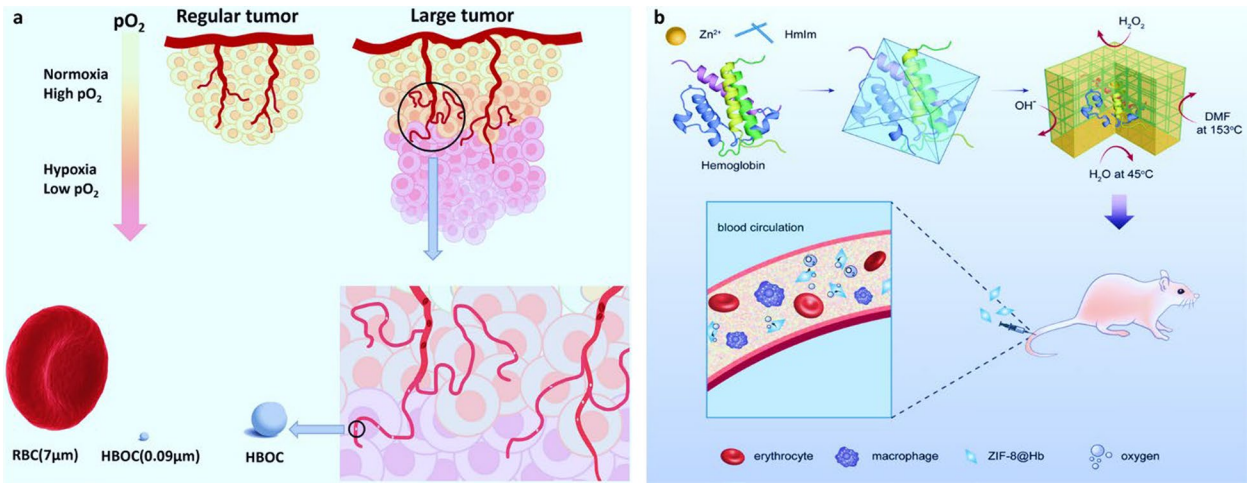


Fig. 5 Application of HBOCs. **a** Diagram of tumor oxygenation after HBOC administration with fractionated RT in the large tumor model. Reprinted with permission from [94], **b** Application of a new biomimetic oxygen carrier (ZIF-8@Hb), created by encapsulating Hb with nanoporous-coated MOFs in mice model of hemorrhagic shock. Reprinted with permission from [115]

tumor oxygenation and augmenting the antitumor effects of near-infrared light II (NIR-II)-guided synergistic chemodynamic therapy and photodynamic therapy [95]. Hemoglobin stands as the most abundant natural metalloporphyrin protein carrier, encompassing four hemes (iron-based porphyrins). Hb can serve to enhance the therapeutic efficacy of tumor sonodynamics which facilitates the release of oxygen and acts as a sound sensitizer within tumor cells. This process generates ROS, inducing mitochondrial damage in tumor cells and effectively suppressing tumor cell proliferation [96]. In China, researchers have developed a liposome incorporating both Hgb and doxorubicin, to improve tumor oxygen delivery while concurrently enhancing the delivery of an anti-cancer drug [97]. There are currently no completed or ongoing human trials involving the use of HBOCs in conjunction with adjuvant therapies such as chemotherapy, radiation therapy, or immunotherapy. The foundational knowledge and history of low oxygen levels in cancers make this an effective therapeutic field for research.

PFOCs Nanoscale PFC oxygen carriers circulate in the bloodstream, accumulate in tumors, and aid in oxygen delivery for antitumor therapies like radiotherapy, chemotherapy, and photodynamic therapy. Cheng et al. devised a method for oxygen self-enhanced photodynamic therapy by incorporating photosensitizers into PFC nanodroplets which are co-encapsulated by lipids called Oxy-PDT agent

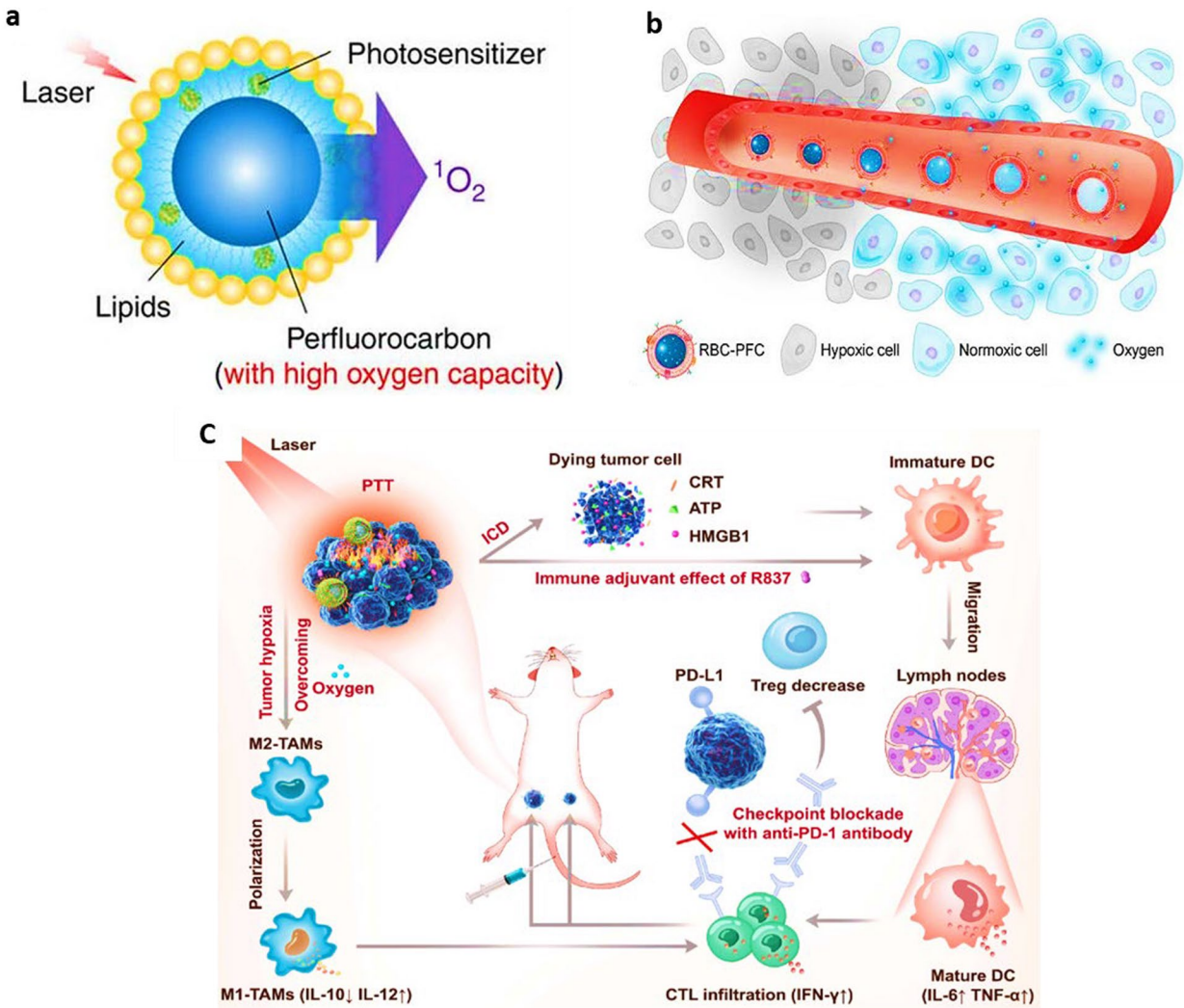


Fig. 6 Application of PFOCs. **a** Structure of the Oxy-PDT agent, whereas photosensitizer and perfluorocarbon are co-encapsulated by lipids. Reprinted with permission from [98], **b** Delivery of oxygen and release to hypoxic tissues by RBC-PFC nanoformulation. Reprinted with permission from [117], **c** Mechanism of oxygen-carrying LIP/PFOB nanoformulation mediated photothermal immunotherapy, Reprinted with permission from [100]

which is shown in Fig. 6a. The photosensitizer is spread evenly in the lipid layer, and the PFC is in the nanoparticle core. When the photosensitizer is exposed to laser light, it transfers energy to the oxygen in the perfluorohexane (PFH) and produces O_2, resulting in an impressive tumor inhibition rate of nearly 80%. In vivo tests show that Oxy-PDT-treated animals exhibit tumor growth suppression following direct injection into tumors. Moreover, a single-dose intravenous injection of Oxy-PDT in tumor-bearing mice markedly impedes tumor growth, whereas traditional photodynamic therapy shows no effect [98].

Hou and colleagues presented a technique for fabricating a pH-sensitive nano-oxygen carrier to counter tumor hypoxia and boost PDT efficacy and PFC oxygen carrier exploits the tumor's acidic environment, achieving an 84.2% inhibition rate [99]. In addition, enhancing the tumor microenvironment with intratumorally oxygen delivery boosts tumor photothermal immunotherapy. This process facilitates in situ exposure of tumor-associated antigens and transition of tumor-associated macrophages from M2 to M1 phenotype, suppressing tumor growth via eliciting antitumor immune responses and promoting synergistic photothermal therapy and immunotherapy which is shown in Fig. 6C [100]. Zhang and colleagues reported a "oxygen bomb" in which PFC oxygen carriers can augment photothermal therapy for tumors. Under NIR-II light irradiation, oxygen is explosively released, facilitating the simultaneous release of chemotherapy drug doxorubicin. This enables a synergistic antitumor effect combining chemotherapy and PDT [101]. Song et al. used a PFOB emulsion for tumor oxygenation to overcome the hypoxia-induced chemoresistance to cisplatin. The study revealed that preoxygenated PFOB nanoemulsion, coupled with carbogen breathing, enhanced cisplatin-induced apoptosis in cancer cells and suppressed tumor growth at a low dose of cisplatin (1 mg/kg) treatment. Importantly, this treatment did not induce nephrotoxicity [102].

OMNBs Hypoxia-inducible factor-1α (HIF-1α) is a key regulator of cellular oxygen balance and adaptation to hypoxia. It is well-documented that cancer cells exposed to hypoxic conditions develop resistance to radiation therapy and various types of chemotherapy, largely due to HIF-1α accumulation. Iijima et al. creating single nanometer-sized ultrafine oxygen bubbles aims to counter hypoxia-induced resistance to radiation therapy by suppressing hypoxia-inducible factor-1α in cancer model and the oxygen nanobubble medium markedly reduced hypoxia-induced resistance to radiation compared to the use of standard medium [103]. Owen and colleagues seek to assess the potential of reducing tumor hypoxia by administering orally delivered suspension of surfactant-stabilized oxygen nanobubble in a mouse xenograft tumor model of human pancreatic cancers. Analysis of HIF1α and VEGF expression, however, revealed that oxygen nanobubbles could induce a statistically significant reduction to a level linked with improved treatment outcomes, although there is a pressing need for enhanced methods to alleviate tumor hypoxia, aiming to enhance the effectiveness of existing cancer therapies [104]. Luo et al. successfully synthesized multifunctional folate-targeted and oxygen/paclitaxel loaded microbubbles (TOPLMBs) for ultrasound (US) mediated delivery in a combination therapy approach, demonstrated in an intraperitoneal ovarian cancer xenograft model and the results suggests that the intraperitoneal injection of dual-targeting TOPLMBs, coupled with ultrasound mediation, shows promise for treating ovarian cancer and its microenvironment [105].

Hypoxia in the tumor microenvironment promotes growth and reduces treatment effectiveness. AOCs like HBOCs, PFOCs and OMNBs can be loaded with cancer drugs that target tumor cells and can improve synergistic therapy by providing a stable oxygen supply. Studies show AOCs produce significant therapeutic effects in vitro and in vivo for tumor treatment. Combination of AOCs with cancer drugs could lead to breakthroughs in clinical tumor treatment.

COVID-19/ corona patients

COVID-19 is characterized by respiratory distress, often leading to hypoxia due to lung damage and impaired oxygen exchange. HBOCs are used to improve oxygen delivery, stabilize oxygen levels, and support respiratory function in severe cases of COVID-19, particularly when the body struggles to maintain adequate oxygenation.

HBOCs Lupon and colleagues hypothesized that intravenous administration of $HEMO_2Life^®$ is safe and could alleviate symptoms in COVID-19 patients. It could increase arterial oxygen content despite lung failure and improve tissue oxygenation control. Additionally, its antioxidative effect may prevent cytokine storms induced by SARS-CoV-2. $HEMO_2Life^®$, possesses an oxygen capacity 40 times greater than vertebrate hemoglobin. With a size 250 times smaller than a human RBC, it diffuses throughout the microcirculation without exiting the vascular sector. Additionally, it exhibits antioxidative properties due to superoxide dismutase activity. In preclinical in vivo models involving mice, rats, and dogs, $HEMO_2Life^®$ has demonstrated improved tissue oxygenation, particularly in the brain [106].

HBOCs such as $HEMO_2Life^®$ can provide critical support in COVID-19 treatment during the pandemic by improving patient survival, reducing the need for tracheal intubation, shortening oxygen supplementation

time, and helping treat more patients amid respirator shortages, however, it provides only temporary oxygen support and do not address the underlying viral infection or long-term lung damage. Given these factors, AOCs may be beneficial in emergency settings, they should be used in conjunction with other therapies, and further research is needed to optimize their role in COVID-19 management.

β-Thalassemia

β-Thalassemia is an autosomal recessive blood disorder that results in insufficient production of Hb, causing anemia and reduced oxygen delivery. In β-thalassemia, there is a deficit in β-globin chain production alongside an abundance of free α-globin chains. The surplus α-globin chains precipitate within RBCs, causing increased destruction (hemolysis) and ineffective erythropoiesis [107]. SCOCs are used to enhance oxygen supply, potentially reducing the need for blood transfusions, and supporting patients by improving oxygenation in the absence of normal Hb production.

SCOCs Hladun and colleagues assess the efficacy and safety of hematopoietic stem cell transplantation in hemoglobinopathies with transfusion-dependent β-thalassemia. Nine donors were HLA (Human leukocyte antigen) -genotypically identical siblings, two were partially matched related donors (with one HLA allele mismatch), and three were unrelated donors. The graft was successful in all patients. However, secondary graft rejection occurred in two thalassemia patients, necessitating their reliance on blood transfusions. Finally, they concluded that for thalassemia major, allogeneic hematopoietic stem cell transplantation from HLA-genotypically identical siblings presents a favorable likelihood of complication-free survival. Allogeneic hematopoietic stem cell transplantation from siblings with identical HLA genotypes offers a good chance of survival without complications [92].

Allogenic hematopoietic stem cell transplantation from identical siblings provides a high chance of survival without complications. Despite promising results, transplantation from unrelated donors carries morbidity and mortality risks, unlike non-curative treatments that offer long-term survival.

Application of AOCs on different medical conditions

Trauma and hemorrhagic shock (HS)

Hemorrhagic shock involves severe blood loss, reduced blood volume, and tissue hypoxia, risking organ failure. Artificial oxygen carriers help by restoring oxygen delivery to tissues, stabilizing patients and mitigating the effects of hypoxia when immediate blood replacement isn't available [108].

HBOCs Numerous HBOC agents have undergone efficacy testing in the treatment of HS. Gould and colleagues compared the therapeutic effect of allogeneic RBCs versus PolyHeme (human polymerized hemoglobin) in treating trauma patients with acute blood loss. Despite the decrease in RBC hemoglobin, PolyHeme preserved total hemoglobin concentration and decreased the need for allogeneic blood. Later, the same researchers looked examined PolyHeme in patients undergoing emergency surgery and with severe bleeding trauma who did not receive RBCs [109, 110]. The first generation HBOC was diaspirin cross-linked hemoglobin (DCLHb), which administering initial hospital resuscitation could potentially decrease 28-day mortality in cases of traumatic hemorrhage [111]. In an animal model that combined traumatic brain injury (TBI) with HS, SanFlow/ PNPH significantly decreased intracranial edema, acidosis, and hyperkalemia while increasing mean arterial pressure (MAP) and heart rate [112]. MP4 was shown to enhance tissue oxygenation in animal models of uncontrolled hemorrhage and improve functional capillary density [46, 113]. Furthermore, another group of researcher synthesized liposome-encapsulated hemoglobin (LEH), a nanoparticulate oxygen carrier with a size of 216 ± 2 nm and a hemoglobin content of 7.2 g/dl and investigated HS (45% blood loss) in a rat model leading to improved oxygenation and perfusion, reduced inflammation, stress and protected the function of organ [114]. A new biomimetic mineralized oxygen carrier (ZIF-8@Hb), created by encapsulating hemoglobin with nanoporous-coated Metal–Organic Frameworks (MOFs) known as ZIF-8, demonstrates excellent biocompatibility, reduced protein adsorption, extended blood circulation time and significantly enhances the survival time of mice experiencing hemorrhagic shock [115] which is shown in Fig. 5b.

PFOCs To assess the effectiveness of a perfluorochemical emulsion like oxyfluor, designed for oxygen transport, as a prehospital therapy, Goodin et al. utilized a canine HS model, focusing on compromised tissue oxygenation. The results suggests that the emulsion enhanced oxygen transport and reinstated global tissue oxygenation following severe hemorrhage [116]. In addition, Dodecafluoropentane emulsion (DDFPe) efficiently dissolves oxygen due to its low boiling point and linear chain structure. It acts as a cutting-edge nanoformulation to provide oxygen in HS. In a preclinical porcine model of HS, 100% of DDFPe-treated animals survived to 6 h, compared to only 30% of control animals [60]. To enhance the long-term stability of PFC oxygen carriers, Zhuang et al. utilized

RBC membranes to encapsulate PFC and create a biomimetic PFC nano formulation designed as an oxygen delivery vehicle which is shown in Fig. 6b, thereby enhancing bioavailability and reducing immunogenicity. In a mouse model of HS, this approach achieved efficacy equivalent to resuscitation with whole blood transfusion [117]. Paxian and colleagues investigate the comparison of oxygent and stored blood in terms of hepatocellular ATP content recovery, hepatocellular injury, and the expression pattern of glutamine synthetase 1 (GluS-1) depending on the hepatic partial pressure of oxygen after HS; it enhanced hepatocellular oxygen availability led to the normalization of oxygen-dependent gene expression but had no impact on early hepatocellular injury [118].

HS is a key application for AOCs, but treatment often requires high doses and repeated infusions, demanding strict safety standards. They offer quick oxygenation but may cause oxidative stress and their effects are often temporary, requiring repeated doses. Considering these aspects, AOCs needs further research to enhance their safety and efficacy for broader use.

Stroke and myocardial infarction (MI)

Stroke and MI involve reduced blood flow, leading to tissue hypoxia and damage. AOCs are used to enhance oxygen delivery to affected tissues, helping to minimize damage and support recovery by compensating for impaired circulation.

HBOCs Due to its smaller diameter of HBOC compared to RBCs, it can efficiently navigate through plasma to inaccessible areas where RBCs cannot penetrate, thereby facilitating oxygen transport and release and consequently renders it more advantageous than whole blood in emergency situations like stroke and MI. When HBOC-201 was infused half an hour before myocardial ischemia, the area at risk (AAR) and the extent of the infarct were significantly reduced [119]. Similar research by George et al. found that infusing HBOC-201 15 min after ischemia can likewise drastically lower Inf/AAR and increase myocardial survival [120]. Kawaguchi and colleagues studied the effect of PEGylated carboxyhemoglobin bovine (Sanguinate) on MI. They ligated 20 rats underwent of the left anterior descending artery which induced MI and treated with Sanguinate. The findings indicate that short-term repeated doses of SANGUINATE may expedite weight recovery while preserving myocardial integrity, mitral competence, and cardiac function following MI [121]. Saxena and colleagues evaluated the safety and tolerability of DCLHb initiated within 18 h of symptom onset in patients experiencing acute ischemic stroke. In the multivariate logistic regression analysis, DCLHb treatment emerged as an independent predictor of a poorer outcome, with an odds ratio of 4.0 and a confidence interval of 1.4–12.0 [122]. Sanguinate was also used to acute stroke treatment and improve the outcomes. It enhanced collateral flow following a 30-min blockage that persisted for 1.5 h of ischemia [123]. Gao et al. reported neurointerventional infusion of HBOC to prevents brain damage in acute cerebral infarction. They used interventional microcatheter technology to perfuse HBOC for treatment of cerebral infarctions areas and observed preserving brain structure via inflammation and apoptosis reduction post-induced infarction [124].

PFOCs To evaluate the efficacy of DDFPe emulsion, Culp and colleagues used an insoluble emboli rabbit stroke model whereas they used 2% w/v DDFPe emulsion at a rate of 0.6 mL/kg intravenously. The results demonstrated intravenous DDFPe reduces infarct volumes and protects brain tissue from ischemia in animal models [62]. A Phase Ib/II randomized and controlled trial of dose-escalation in DDFPe emulsion in acute ischemic stroke model trial was designed to identify the maximum tolerated dose and the study trial was approved by the FDA (NCT02963376). Intravenous DDFPe was safe in animal studies, transporting 3–7 times more oxygen than other perfluorocarbons and 9–15 times more than blood. Its efficacy is observed at levels below those expected to cause adverse events [61]. In addition, Oxycyte® was used as a therapeutic potential of Glasgow Oxygen Level Dependent (GOLD) Technology for acute stroke models in rats. The results showed that oxycyte combined with 50% O_2 hyperoxia, integrated into GOLD penumbral imaging, holds promise for enhancing stroke prognosis and serving as a supplement to reperfusion therapy [125].

AOCs offer potential in ischemic stroke treatment by targeting drug delivery, crossing the damaged blood–brain barrier, and alleviating brain tissue hypoxia. Their unique advantages include co-loading drugs for thrombolysis, anti-inflammation, and neuroprotection, promoting rehabilitation and offering a comprehensive treatment approach.

Acute normovolemic hemodilution (ANH) / acute blood loss

Acute blood loss results in a rapid decrease in blood volume, leading to reduced oxygen delivery and tissue hypoxia. AOCs are used to quickly restore oxygen supply to tissues, compensating for the loss of red blood cells and stabilizing the patient until blood volume is restored.

HBOCs Cross-linked and polymerized hemoglobin have demonstrated effectiveness in numerous studies, both in cardiac and noncardiac surgery settings, with blood conservation during the perioperative period as the primary efficacy endpoint [126]. Hemopure is derived from

bovine hemoglobin and undergoes polymerization with glutaraldehyde which is utilized in acute normovolemic hemodilution during cardiac surgery [3]. Hemolink was employed to examine its impact on ANH, and research indicated that when utilized as a volume replacement alongside ANH, adverse hemodynamic effects stemming from vasoconstriction could be mitigated. This effect is likely attributed to the reduction in blood viscosity, while concurrently sustaining blood flow [127]. A new generation of HBOC called OxyVita Hb was created using a zero-linked polymerization method with the goal of improving oxygen delivery in circumstances which used in hemodilution [128].

PFOCs PFC emulsions have been developed to deliver oxygen in the body due to their high oxygen-carrying capacity. Despite advancements in developing various PFC emulsions for acute blood loss, progress in clinical use has been limited due to instability, storage challenges, and poor immune response [12]. Lundgren et al. investigated as a therapy for maintaining homeostasis during blood loss and providing a survival advantage in male Wistar rats were bled from a carotid artery and after reducing the rats' hemoglobin levels by approximately half, they were injected with DDFPe (0.014 mL/kg). The results found all control rats had died, but there was 100% survival in the DDFPe-treated rats in less than 2 h [129]. Wrobeln and colleagues used albumin-derived PFOCs to investigate the hypoxic tissue damage in massive hemodilution in a rat model. Following hemodilution, treated animals exhibited elevated arterial blood pressure and maintained stable body temperature. Furthermore, they demonstrated stable pH levels, increased oxygen partial pressure (pO_2), and decreased carbon dioxide partial pressure (pCO_2) [63].

AOCs help treat acute blood loss by rapidly restoring oxygen delivery to tissues, compensating for blood volume loss. Their advantage is quick oxygenation without the need for blood transfusions, which is crucial in emergencies. However, AOCs may cause side effects like inflammation or oxidative stress and provide only temporary support. AOCs should be used alongside other treatments, with further research recommended to improve safety and long-term effectiveness.

Ischemia/ reperfusion (I/R) injury

Ischemia/Reperfusion (I/R) injury is a clinical condition caused by the disruption of blood flow to the brain due to various cerebrovascular diseases, leading to localized brain ischemia, hypoxic necrosis, and neurological deficits. It is commonly associated with cerebral infarcts and has become a leading cause of mortality in recent years due to its rising incidence and severity. AOCs are used to improve oxygen delivery during reperfusion, helping to reduce tissue damage and support recovery by mitigating hypoxia and oxidative stress.

HBOCs Subsequently developed, stroma-free hemoglobin nanoparticles (SFHbNP), functioning as a HBOCs, are characterized by a spherical polymerized stroma-free hemoglobin core encased within a HSA shell. These nanoparticles have been under investigation for the treatment of cerebral ischemia in a rat transient middle cerebral artery occlusion (tMCAO) model. During the early phase of reperfusion, within 6 h, microvascular HBOC perfusion and cerebral blood flow remained notably high in the SFHbNP groups, and this observation suggests that the superior oxygen transport capacity of SFHbNP may contribute to enhanced neuroprotective effect [130]. Apart from this, ischemia/reperfusion injury represents an inevitable complication in liver surgery and transplantation. Zapletal and colleagues investigated the combined advantages of hemodilution along with improved oxygen delivery to reperfused liver tissue through the utilization of HBOC-201 (Hemopure®) and demonstrated a reduction in ischemia/reperfusion (I/R) injury in the liver while simultaneously enhancing oxygenation of postreperfusion liver tissue [131]. Wei et al. investigated the cardioprotective effect of a HBOCs like PolyPHb on cold I/R Injury. The findings revealed that HBOC offered protection against cold I/R injury in isolated rat hearts and this protective effect was linked to the suppression of Toll-like receptor 2 (TLR2) and Toll-like receptor 4 (TLR4)/NF-κB signaling pathway expression, which potentially mitigates the inflammatory response [132].

PFOCs Ischemia or hypoxia can lead to heart failure, neurological complications, and organ damage in numerous patients. Swyer and colleagues developed an oxygen carrier emulsion containing nanoparticle dodecafluoropentane which is advantageous because their smaller size (250 nm) allows them to carry oxygen past blockages which obstruct RBCs (6–8 μm). In the murine model of Ischemia/ Reperfusion (I/R) injury, the utilization of dodecafluoropentane resulted in a 60% reduction in injury/ infarct size [133]. In a rat Langendorff-heart perfusion model, albumin-derived perfluorocarbon-based nanoparticles is used to evaluate the functionality of this oxygen carrier. The results suggest that the oxygen capsules prevented myocardial dysfunction in the presence of ischemia, which would otherwise occur without them [134]. Furthermore, non-hemodiluting doses of PFC emulsions were investigated in the hamster window chamber model to assess their

impact on ischemia–reperfusion injury. PFC-treated animals showed significantly reduced leukocyte rolling and adhesion in postcapillary venules, along with restored functional capillary density and blood flow after ischemia [135].

OMNBs Ho et al. created a murine ischemic stroke-reperfusion (S/R) model following tPA-induced thrombolysis to explore the mechanisms and outcomes of oxygen-loaded microbubbles (OMBs) treatment. In vivo monitoring of blood flow and tissue oxygen levels provided real-time insights into the targeted vessel's OMB treatment conditions. In the murine model, microscopic images showed clot formation at 60 min post-stroke induction and thrombolysis after 60 min of reperfusion (Fig. 7a). After 60 min of a stroke, 20 min of reperfusion, and 10 min of OMB treatment, the blood flow percentages were 45 ± 3%, 70 ± 3%, and 86 ± 2%, respectively, indicating sonoperfusion, and the corresponding pO_2 levels were 60 ± 1%, 76 ± 2%, and 79 ± 4%, demonstrating reoxygenation (Fig. 7b). 2% 2,3,5-triphenyltetrazolium chloride staining showed reduced infarct size post-reperfusion, with infarct areas of 11.9 ± 1.1%, 7.5 ± 1.7%, and 5.2 ± 0.6% in the stroke, 50 + 50, and 10 + 90 groups, respectively (Fig. 7c). The research showed that OMB treatment synergizes the benefits of sonoperfusion and local oxygen therapy, resulting in reduced brain infarction and activation of neuroprotection to prevent ischemic stroke-reperfusion injury [136].

Ischemic stroke-reperfusion (S/R) injury is a critical concern in preserving brain function following thrombolysis. AOCs should be considered as a complementary therapy to enhance oxygenation and neuroprotection in ischemic stroke-reperfusion injury, with ongoing research to optimize their safety, efficacy, and long-term benefits.

Skin wound healing/ regeneration

Oxygen is essential for wound healing, as it promotes cell proliferation, accelerates angiogenesis, reduces infection, and enhances collagen synthesis. Oxygen levels in the wound microenvironment are crucial for healing;

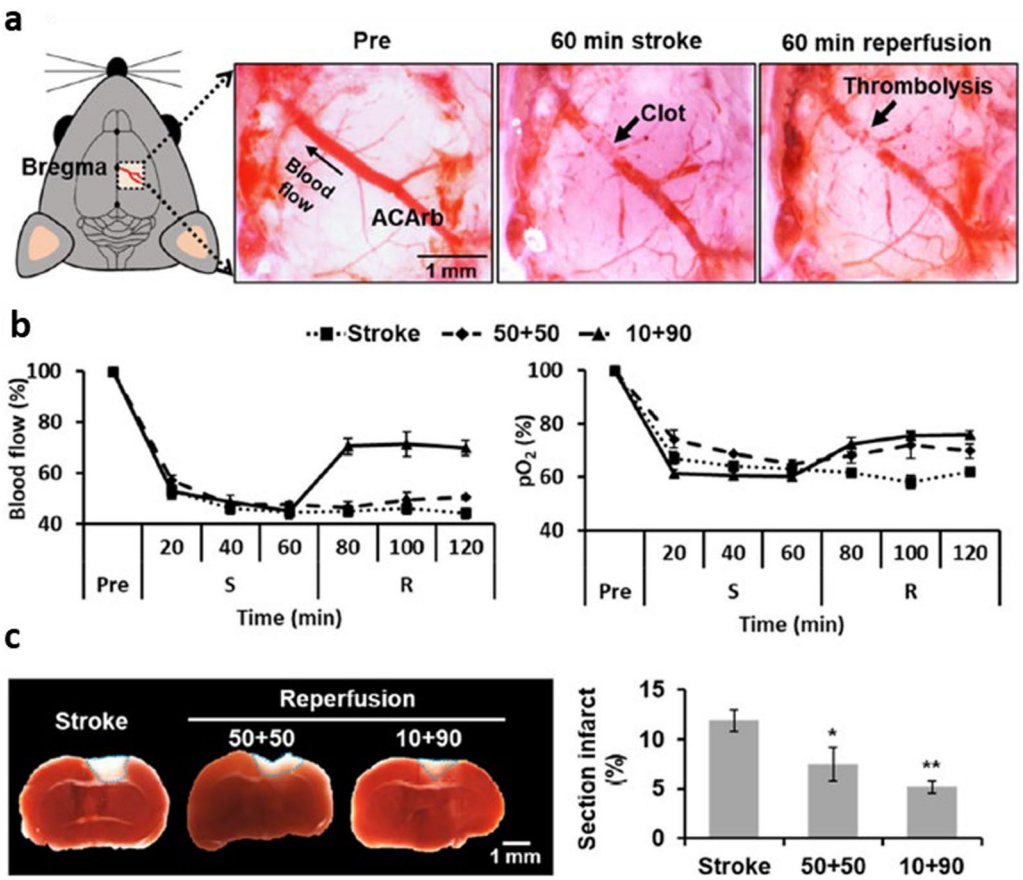


Fig. 7 Application of OMNBs. **a** Microscopic view from dissecting show normal vessel condition (pre), clot formation (60 min stroke), and thrombolysis (60 min reperfusion), **b** Percentage of blood flow and pO_2 during pre, stroke (S), and reperfusion (R). **c** Staining with 2% 2,3,5-triphenyltetrazolium chloride shows white areas of brain infarction. Reprinted with permission from [136]

prolonged hypoxia hinders neovascularization and limits wound repair, while controlled hypoxia can promote the healing process. AOCs are used to boost oxygen supply are employed to relieve wound hypoxia, stimulate angiogenesis, and improve collagen remodeling, ultimately accelerating healing.

HBOCs Fukui and colleagues investigated the impact of LEH as an artificial oxygen carrier on skin wound healing in mice and found LEH treatment accelerated granulation, increased epithelial thickness, and suppressed early granulocyte infiltration, leading to reduced ulcer size. However, it did not affect surface blood flow or CD31 expression [137].

PFOCs PFC oxygen carriers promote wound healing by stimulating the growth of epithelial cells, vascular endothelial cells, and fibroblasts. Additionally, they enhance the efficacy of photodynamic anti-bacterial by supplying oxygen and combating bacterial infection at the wound site. Lee and colleagues use Chitosan hydrogel containing perfluorocarbon nanoemulsion, antimicrobials, and growth factor-loaded nanoparticles to act as a multifunctional dressing for wound healing in a diabetic foot ulcer model. The results demonstrated that hydrogel enhanced the efficacy of wound healing through decreased inflammatory response, re-epithelization, faster and advanced collagen deposition and maturation which is promising for clinical diabetic wound treatment [138]. A perfluoropentane core, chitosan-shell carrier Oxygen-loaded nanobubbles efficiently delivers oxygen to hypoxic tissues and combats infection by methicillin-resistant *Staphylococcus aureus* and *Candida albicans* which is a novel, promising, non-toxic, and cost-effective antimicrobial devices that promote repair processes are poised for use in wound healing treatments [139]. Patil et al. created a perfluorocarbon chain-modified methacrylamide chitosan hydrogel (MACF) dressing for the treatment of dermal wounds. The results confirm that elevated oxygen delivery facilitated by MACF + O_2 hydrogels boosts the process of wound healing, emphasizing that metabolomics analyses offer a potent means to evaluate the physiological aspects of wound recovery [140].

OMNBs Oxygen delivery, a critical factor in wound healing, can be enhanced using micro/nanobubbles (MNBs) to augment the dissolved oxygen in solution and facilitate increased oxygen delivery to a wound. A pilot study started involving an oxygen micro/nanobubbles (NCT05169814). This study aims to evaluate the safety and effectiveness of MNBs in promoting the healing of both acute and chronic wounds which will be completed in 2025 [141].

Research on the use of HBOCs for wound healing is more limited compared to PFOCs. Studies indicate that PFOCs can significantly enhance wound healing by reducing inflammation, promoting re-epithelialization, and accelerating collagen deposition and maturation. PFOCs have also shown promising results in diabetic wound healing, offering potential benefits for diabetic patients with chronic wounds. Expanding research into HBOCs could provide valuable insights and broaden their application in wound care, potentially leading to new, effective therapeutic options.

Acute lung injury

Acute lung injury is characterized by inflammation and damage to the alveolar-capillary barrier, leading to impaired gas exchange and hypoxia. PFOCs are used to improve oxygen delivery to tissues, helping to stabilize oxygen levels and support respiratory function during acute lung injury when the lungs cannot efficiently oxygenate the blood.

PFOCs In a swine model, intravenous administration of oleic acid was utilized to induce acute lung injury, aiming to investigate the hypothesis that intravenous administration of a perfluorocarbon (PFC) emulsion would enhance plasma oxygen levels and alleviate lung injury. This investigation explored whether the PFC emulsion would be effective either when used prophylactically before oleic acid-induced injury (OALI) or when administered as treatment for OALI. The results concluded that when administered intravenously as a treatment subsequent to OALI, the PFC Oxycyte significantly enhances blood oxygen content and improves lung histology. However, when Oxycyte is administered before OALI, it is linked with increased mortality rates [142].

OMNBs Nanobubbles are tiny gas-filled spheres enclosed by an interface (gas–liquid or gas–solid) with diameters typically ranging from tens to hundreds of nanometers. Kheir and colleagues have developed microparticles designed for intravenous injection to deliver oxygen systemically to vital organs. The lipidic oxygen-containing microparticles have a 4 μm diameter and consist of a lipid shell surrounding an oxygen gas core. They are designed to mix with venous blood and deliver oxygen to oxygen-deprived hemoglobin, vital for tissue oxygenation. Upon intravenous injection of the microparticles into hypoxemic rabbits, arterial saturations rapidly increased to near-normal levels within seconds. The administration of oxygen microparticles notably alleviated hypoxemia in these rabbits, leading to a decrease in the incidence of cardiac arrest and organ injury compared to control groups [83]. Legband and colleagues evaluated the peritoneal

microbubble oxygenation (PMO), involving the delivery of phospholipid-coated oxygen microbubbles (OMBs) into the peritoneal cavity in the rabbit model. The results demonstrate that PMO therapy can effectively double the survival time of rabbits with full tracheal occlusion, doubling it from 6.6 ± 0.6 min for saline controls to 12.2 ± 3.0 min for the bolus PMO-treated group. In summary, these findings affirm the viability of PMO technology in offering extrapulmonary ventilation for the recovery of serious hypoxic patients [143].

Alveolar injury causing diffuse damage may benefit from PFCs, which carry 50 times more oxygen than plasma and help preserve tissue in low-oxygen conditions. However, research has focused on the oleic acid lung injury model, and further studies across different models are needed to fully explore PFCs' potential. No data currently exists on the use of HBOCs; further research is needed to optimize their effectiveness on lung injury.

Amanitin-induced hepatotoxicity

Amanitin-induced hepatotoxicity is caused by the toxin α-amanitin, which inhibits RNA polymerase and leads to liver cell damage and dysfunction. HBOCs are used to improve oxygen delivery to the liver, supporting cell function and reducing tissue hypoxia, which can help mitigate liver damage and enhance recovery.

HBOCs Amanitins, including α- and β-amanitin found in certain mushrooms, are bicyclic octapeptides responsible for potentially lethal hepatotoxicity when ingested. Le Dare et al. evaluated the effect of M101 for reducing amanitin-induced toxicity in an in vitro hepatic cell model. In differentiated HepaRG cells, α- and β-amanitin cause cell apoptosis and the generation of reactive oxygen species in the mitochondria. M101 effectively lowers the generation of reactive oxygen species in the mitochondria and the death of cells generated by amanitin. The findings suggest its potential effectiveness in reducing amanitin-induced hepatotoxicity, indicating therapeutic development potential [144].

Although limited studies exist on HBOCs in hepatotoxicity, current research suggests they can be effective for minimizing amanitin-induced cell death and mitochondrial ROS production in vitro. The advantage is their potential to sustain cellular function when liver oxygenation is compromised, potentially mitigating liver damage. HBOCs should be considered experimentally in severe cases, with a strong recommendation for further research to establish their safety and therapeutic value in hepatotoxicity management.

Decompression sickness (DCS)

Decompression sickness (DCS) occurs when nitrogen bubbles form in the bloodstream after rapid decompression, causing joint pain, tissue damage, and organ dysfunction. It usually occurs during ascent from a dive, exit from a caisson or hyperbaric chamber, or ascent to altitude [145]. PFOCs are used to enhance oxygen delivery, promote nitrogen elimination, and reduce hypoxia, aiding in the treatment of DCS by improving tissue oxygenation and helping to resolve gas embolisms.

PFOCs To treat the severe DCS, Dromsky et al. used perfluorocarbon emulsion like Oxygent™. After diving, animals treated with Oxygent™ intravenously, which combines oxygen and corticosteroids, showed lessened and delayed onset of cardiac decompression sickness and prevented neurological symptoms [146]. On the other hand, perfluorotertbutylcyclohexan application was observed to increase oxygen delivery to and utilization in ischemic tissues in sheep experiments, offering a potential therapeutic avenue for DCS therapy [147]. Cronin et al. investigated the effect of oxycyte emulsion on platelet count and function of DCS in a swine model. They declared that there is no effect on platelet counts, whole blood coagulation by thromboelastometry, or clinical bleeding in DCS treated with PFC oxycyte emulsion [148]. In a 2010 study, Mahon et al. affirmed these findings, showing that Oxygent™ effectively reduced mortality in swine with DCS, even when administered with a delay (not immediately post-dive) after symptom onset [149]. However, not all PFC preparations demonstrate efficacy. A recent study by Sheppard et al. found that DDFPe was linked to high mortality and did not show any beneficial effects in a rat model of DCS [150].

DCS is important because it can lead to serious health complications even in death, when divers, aviators, or individuals in high-pressure environments transition too quickly to lower-pressure conditions. PFOCs may offer benefits due to their low viscosity can help improve blood flow, potentially aiding faster recovery. PFOCs can also have limited duration in the bloodstream and may require repeated doses which is a challenge that requires further research. The current DDFPe emulsion formulation, specifically, has not demonstrated significant therapeutic benefits and needs more study to improve its efficacy and functionality in DCS applications.

Hair growth

Hair growth is a process driven by follicular cell regeneration, nutrient supply, and oxygenation. PFOCs are used to enhance oxygen delivery to hair follicles, promoting cell metabolism, stimulating growth, and improving follicle health, which can help support and accelerate hair regrowth.

PFOCs Hair dermal papilla cells (hDPCs) are pivotal in hair growth and regeneration, and their performance is impacted by the availability of nutrients and oxygen. An environment characterized by markedly low oxygen levels termed anoxic conditions (< 0.2%) due to oxygen deficiency, impedes the promotion of hDPCs and slows down hair regrowth. Park and colleagues evaluated the effect of the supply of O_2 on human hair growth using PFOB-based nanoemulsion (PFOB-NE). Demonstrating its efficacy, the PFOB-NE exhibits a continuous release of oxygen for 36 h, effectively elevating and sustaining the oxygen concentration in the anoxic microenvironment to levels of up to 0.8%, with the sustained oxygen supply notably enhancing human hair organ elongation approximately fourfold in comparison to the control group under anoxic conditions [151].

PFOCs show promise for boosting hair growth by enhancing oxygen delivery to hair follicles. However, current research has only been conducted in ex vivo conditions. Validation through animal studies or direct application to human scalps is needed to overcome current limitations and support clinical use.

Severe arterial gas embolism

Arterial gas embolism poses a clinical challenge, manifesting directly in cardiopulmonary bypass machines during open-heart surgeries or indirectly through cardiac or pulmonary right-to-left shunts in dive accidents. It can lead to severe morbidity and mortality. It occurs when gas bubbles enter the bloodstream, blocking blood flow and causing tissue damage. PFOCs help by improving oxygen delivery, supporting tissue oxygenation, and promoting the elimination of gas bubbles, aiding recovery and reducing hypoxia-related damage.

PFOCs Torres and colleagues employed PFC emulsions for treating arterial gas embolism in animal models. They hypothesized that PFC emulsions improve microvascular blood flow, accelerate bubble resolution, and enhance oxygenation in arterial gas embolism model. The outcomes were arteriolar and tissue oxygen levels, as well as oxygen delivery, were higher in the PFC groups compared to the control. Additionally, there was an increase in arteriolar blood flow, a decrease in diffusional resistance of oxygen in the plasma, and improved tissue oxygenation [152].

Although studies on PFOCs in arterial gas embolism are limited, current research suggests PFOCs may be an effective therapeutic option by enabling arteriolar blood flow, reduction in diffusional resistance of O_2 in the plasma, and improved tissue oxygenation.

Chronic hypoxia

Chronic hypoxia is a condition where tissues experience prolonged oxygen deficiency, leading to impaired cellular function and potential organ damage. SCOCs are used for chronic hypoxia because they can enhance oxygen delivery, promote tissue regeneration, and support long-term healing by improving cell survival and repair in oxygen-deprived areas.

SCOCs Cell-based oxygen carriers, cultured through induced hematopoietic differentiation, closely resemble natural RBCs in their physiochemical features and biological functions. In 2008, Lu et al. introduced a method to generate functional erythroid cells from cultured hESCs, starting with the formation of hemangioblasts on cell plates. Following in vitro differentiation into erythroid cells, the researchers noted physiological oxygen loading and releasing patterns, along with the regulation of cellular oxygen binding capacity in response to variations in pH or concentrations of 2,3-DPG [153]. In 2011, French researchers conducted a phase I clinical trial involving artificial erythroid cells. These functional cell-based oxygen carriers were derived from peripheral CD34 + HSPCs and exhibited properties comparable to natural human RBCs. These properties included mechanical stability, protein content, oxygen affinity, presence of blood group antigens, and circulatory half-life, which was approximately 26 days compared to 28 ± 2 days for human RBCs [68]. Japanese researchers achieved large-scale production of RBC-like cells by overexpressing c-MYC and BCL-XL in multipotent hSPCs, enabling sustained self-replication and differentiation into erythrocytes [154].

SCOCs can enhance oxygen delivery and promote tissue regeneration in chronic hypoxia, improving healing and function. However, further research is needed to address challenges such as inconsistent cell engraftment, immune rejection risks, and ethical concerns.

Retinal ischemia

Retinal ischemia occurs when the blood supply to the retina is impaired, leading to oxygen deprivation and potential vision loss. OMNBs are used to enhance oxygen delivery to the retina, reducing hypoxia, promoting tissue repair, and preserving vision by supporting retinal cell function.

OMNBs Messerschmidt and colleagues create an oxygen delivery system comprising oxygen nanobubbles (ONBs) (220 nm in diameter) capable of alleviating retinal ischemia during severe hypoxic events like central retinal artery occlusion. According to studies on oxygen release, after 12 h at 37 °C, 74.06 µg of O_2 are released from the ONBs. Despite their ability to preserve mitochondrial

function and viability, histological sections from rabbit eyes indicated no toxicity associated with ONBs [155].

Conditions such as retinal vein occlusion, central retinal artery occlusion, or diabetic retinopathy resemble stroke-like conditions, causing functional blindness in the affected eye. AOCs can help bypass compromised blood vessels, making them beneficial in such conditions focusing on increasing their stability, duration in circulation, and minimizing side effects.

Application of AOCs on medical procedures

Organ preservation and transplantation

Organ preservation and transplantation involve maintaining organ viability outside the body to ensure successful transplantation. AOCs are used to improve oxygen delivery to preserved organs, reducing ischemic damage, supporting cellular metabolism, and enhancing the success of organ transplants.

HBOCs The most widely employed extracorporeal organ preservation method in clinical practice is static cold storage. However, tissues and organs do not entirely cease metabolism even in a low-temperature environment. Consequently, the absence of metabolic substrates for aerobic respiration can still lead to organ damage. In addition, the earliest clinical series utilized hypothermic machine perfusion without oxygenation. However, recent studies have demonstrated that providing oxygen dissolved in the perfusate can help mitigate solid organ damage. HBOC-201 is a polymerized bovine hemoglobin with low immunogenicity and a comparable oxygen-carrying capacity to human hemoglobin at normothermic temperatures. In comparison to cold static preservation or blood perfusion alone, the sub normothermic machine perfusion combined with HBOC-201 system significantly enhanced graft function and effectively oxygenated the tissue [122]. Laing et al. reported the initial experience utilizing HBOC-201 in discarded high-risk human livers undergoing normothermic machine perfusion (NMP). The vascular blood flow parameters and lactate clearance rate of livers perfused with HBOC-201 were comparable to those perfused with RBCs, while the HBOC group demonstrated a greater capacity for oxygen extraction [156]. In addition, the most recent research has identified natural extracellular hemoglobin, known as $HemO_2$life or Hemarina-M101, isolated from the polychaete *Arenicola marina*, as a promising new hemoglobin oxygen carrier. The M101 has been evaluated in both resuscitation and organ preservation scenarios. A multicenter study assessing the safety and efficacy of M101 in preserving 60 deceased donor kidneys revealed improved renal function following kidney transplantation with an organ preservation solution containing HBOC agents. The study also observed a significant reduction in the incidence of delayed graft function recovery and a shortened time to recovery of renal function. Phase I clinical trials for kidney transplantation have further demonstrated the safety of M101 for both patients and grafts [157]. Recently, a safety study involving 60 renal grafts using $HEMO_2$life® as an additive to organ preservation solution, known as the Oxyop study (NCT02652520), was completed. The study confirmed that the use of $HEMO_2$life® is safe for both patients and grafts [158].

PFOCs The utilization of PFC in organ preservation is constrained by its inadequate solubility in water. The utilization of a PFC oxygen carrier for preserving the donor's liver resulted in notable benefits. Throughout the 30-min ischemic period in orthotopic liver transplantation in rats, there were significant reductions in levels of aminotransferase, malondialdehyde, serum aspartate transferase, alanine, and inflammatory factors. Furthermore, there was a marked improvement in the surgical survival rate [159]. Albumin, serving as an excellent biocompatible protein, can envelop PFC to create albumin-based AOCs. These carriers can be utilized as the normothermic mechanism perfusion (EVNP) fluid for transplanted organs, ensuring adequate oxygen supply and shielding the kidney from harm during EVNP of rat kidneys [160]. The standard approach for lung graft preservation involves perfusion via the pulmonary artery (antegrade perfusion) or pulmonary vein (retrograde perfusion) using a cold low-potassium dextran (LPD) solution. In a cold ischemia rat model, lungs preserved with vaporized PFC + LPD displayed elevated concentrations of superoxide dismutase compared to those preserved with LPD alone. This suggests that the utilization of vaporized PFC decreases the production of free radicals, subsequently safeguarding lung grafts from oxidative stress-induced damage [161].

AOCs can enhance organ preservation and transplantation by improving oxygen supply to donor organs, reducing ischemic damage, and extending preservation time. This improves transplant success and organ viability. However, potential disadvantages include risks of immune response, limited long-term efficacy, and high costs. Further research is needed to optimize AOC formulations and minimize complications for broader clinical use in organ preservation.

Stem cells for the development of rare blood type erythrocytes

The development of rare blood type erythrocytes involves producing specific blood types that are challenging to find for transfusions, especially in emergency situations. Stem cells are used because they can be differentiated

into erythrocytes with the required rare antigen profiles, providing a sustainable and customizable source of rare blood types.

SCOCs Research has focused on the in vitro production of mature human RBCs from induced pluripotent stem cells (iPSCs) to address the high demand for blood transfusions. Park et al. developed a comprehensive protocol for generating iPSC lines derived from the peripheral blood of donors with O D-positive blood and rare blood types (D-negative and Jr(a-negative)), followed by erythroid differentiation. Cells from all donors were successfully utilized to generate iPSC lines, which were then differentiated into erythroid precursors without any detectable chromosomal mutations. This differentiation protocol yielded a moderate amount of erythrocytes per iPSC [162].

Stem cell-based oxygen carriers can generate customized blood supplies for rare blood types, reducing donor dependence and improving oxygen delivery. However, high costs, complex processing, and immune rejection risks limit their scalability and accessibility.

The application of different AOCs such as HBOCs, PFOCs, SCOCs, and OMNBs are summarized in Tables 4, 5, 6, 7 respectively to help readers better understand the outcomes of each type.

Overview of oxygen-generating and producing materials for regenerative medicine

Oxygen is crucial for the survival, retention, and proliferation of cells. Significant progress has been made in the field of regenerative medicine and tissue engineering towards tissue regeneration. Despite advancements, tissue regeneration falls short of solid organ implantations due to challenges like limited cell survival and retention caused by oxidative stress and hypoxia in deeper tissues [163]. Before neovascularization, hypoxia is a significant constraint as oxygen delivery becomes vital for cell survival across the tissue-engineered construct. Oxygen-releasing biomaterials improve therapy and minimize tissue damage caused by hypoxia. Apart from PFCs and hemoglobin, various oxygen-releasing biomaterials have been assessed to counteract hypoxia-induced cell loss and enhance tissue perfusion under in vivo conditions [164]. These encompass solid peroxides (e.g., calcium peroxide, CaO_2,) [165, 166], urea peroxide, polymer-based oxygen carriers, hydrogen peroxide (H_2O_2) [167], microalgae [168], manganese peroxide (MnO_2) [169], substitutes for hemoglobin and myoglobin [170], fluorinated compounds, specifically perfluoro methyl-cyclohexyl piperidine, and also PFCs [171], perfluorodecalin-encapsulated albumin NPs can generate or deliver oxygen within engineered structures, aiming to aid tissue regeneration or repair processes [164]. Various types of oxygen generating materials, their structures, mechanisms, benefits, and limitations are summarized in Table 8. Among the solid peroxides commonly utilized are calcium peroxide (CaO_2) and magnesium peroxide (MgO_2) [163, 164, 172]. Research has demonstrated that these oxygen-producing biomaterials enable adequate oxygen diffusion, promoting enhanced cell survival both in vitro and in vivo. Various biomaterial systems have been employed for oxygen release to harness therapeutic advantages, achieve therapeutic benefits, including hydrogels, emulsions, NPs, nanofibers, solvent casting and evaporation method, nano/ microstructures, electro spun scaffolds, gelation method, 3D-printed scaffolds, and more. However, a significant drawback of both peroxides, percarbonates, and H_2O_2 may involve an excess production of reactive oxygen species [163, 172, 173].

Clinical developments of different oxygen carriers

Oxygen carriers represent a vital component in clinical advancements, particularly in scenarios where oxygen delivery to tissues is compromised. Despite nearly a century of development in oxygen carriers, encompassing a range of methods and carriers, and despite a significant increase in preclinical studies, progress toward clinical application often halts due to concerns regarding safety matters, optimizing efficacy, and navigating regulatory approval for broader utilization. There have been instances where drugs that were already on the market have been recalled by the Drug Enforcement Administration. At present, the most recent clinical applications continue to concentrate on organ preservation, ischemic conditions such as stroke, and hypoxic diseases like sickle cell disease. HBOC-201, a polymerized bovine hemoglobin developed by Biopure Corporation, is presently accessible in the USA and has been previously employed for the treatment of severe sickle cell anemia as well as in cases of multi-organ failure [122]. Erythromer, currently in development, aims to address key limitations in AOCs. It targets inadequate physiological interaction and mitigates NO scavenging by regulating oxygen release and incorporating novel 2,3-DPG with Hb molecules [50]. These endeavors aim to mitigate the dangers associated with inadequate oxygen supply to tissues and organs. The recent clinical developments of different AOCs such as HEMO2life® [158], HBOC-201 (Hemopure) [174], PP-007 [175], Sanguinate™ [176], Hemoglobin Vesicles (HbVs) [20], ABL-101 [177], DDFPe [178], NanO2™ [179], NVX-108 [180], Oxygen Micro/Nanobubbles (MNB's) [181] from 2017 to 2024 are shown in Table 9. The developments suggest a growing interest in the field of oxygen carriers and their potential applications in addressing

Table 4 Summary of the Application of HBOCs

Categories	Diseases/ Medical conditions/ Medical procedures	HBOCs formulation	Clinical indication	Treated dose and administration route	Results	References
Diseases	Anemia/ Sickle cell anemia	HBOC-201	Sickle cell disease patients (Jehovah's Witnesses)	More than 20 units; IV	Survived all critically ill patients and can provide an oxygen bridge, until corpuscular Hb levels recover to meet metabolic demand	[87]
			Acute anemia in a leukemic patient with anti-vel antibodies	32-g units; IV	Bridging treatment for treating critically anemic individuals, such as hematologic malignancies	[88]
		Hemopure	Acute anemia in a Jehovah's Witness patient	–	Patient's total hemoglobin level maintained between 3.6 and 5.3 g/dL over 12 days, with no observed evidence of ischemia or organ dysfunction	[89]
		HBOC-201	Severe anemia	Hb concentration of 3.3 g/dl; IV	Feasible and safe option as an oxygen bridge for severely anemic patients	[90]
	Cancer and Tumor hypoxia	HBOC	Alleviated tumor hypoxia during radiotherapy	–	Relieved hypoxic environment and down-regulate expression of hypoxia-inducible factor-1α (Hif-1α)	[94]
		SPN-Hb@RBCM	Synergetic Chemo-Photodynamic Therapy against Hypoxic Tumors	–	Enhanced tumor oxygenation and facilitate NIR-II fluorescence-guided synergistic CDT/PDT in hypoxic tumors	[95]
		Hb@ZIF-8	Sonodynamic Cancer Therapy	–	Demonstrated potent tumor suppression under ultrasound irradiation	[96]
		DOX-Hb-lipo (DHL)	Hypoxia-induced chemoresistance	–	Significantly enhances chemotherapy efficacy against hypoxic tumors	[97]
	Covid-19/ Corona Patients	$HEMO_2Life^{®}$	Alleviate symptoms in COVID-19 patients		Increased arterial oxygen content despite lung failure and improve tissue oxygenation; particularly in the brain	[106]

Table 4 (continued)

Categories	Diseases/ Medical conditions/ Medical procedures	HBOCs formulation	Clinical indication	Treated dose and administration route	Results	References
Medical condition	Trauma and Hemorrhagic Shock (HS)	PolyHeme (human polymerized hemoglobin)	Acute trauma and emergent surgery	4.4 ± 2.0 U' Intravenous (IV)	Safe in acute blood loss, maintains hemoglobin levels without red blood cells, and reduces the need for allogeneic blood transfusions, while efficiently supplying and withdrawing oxygen	[109]
		DDFPe emulsion	Severe bleeding leading to HS	1 to 20 units (1000 g, 10 L); IV	Increasing survival at life-threatening levels of RBC hemoglobin concentration	[110]
		Diaspirin cross-linked hemoglobin (DCLHb)	Severe traumatic HS	500–1000 mL; IV	Mortality rates were elevated among patients treated with DCLHb, does not seem to be effective as a resuscitation fluid	[111]
		SanFlow/ PNPH	Hemorrhagic hypotension and traumatic brain injury in mice	MAP ≥ 50 mmHg for 30 min; and then MAP ≥ 60 mmHg was re-infused; IV	PNPH is a distinctive non-neurotoxic therapeutic HBOC with novel neuroprotective properties demonstrated in both in vitro and in vivo traumatic brain injury (TBI) models	[112]
		MalPEG-hemoglobin (MP4)	Hemodynamics, and uncontrolled hemorrhage in swine	8 mL/min, delivering 250 mL 31 min; IV	Demonstrated that restoring oxygen delivery with a small volume of MP4 leads to significant recovery from hemorrhage without inducing systemic vasoconstriction	[113]
		Liposome-encapsulated hemoglobin (LEH)	Multi-organ injuries with HS in a rat model	1 mL/min; IV	Prevented the accumulation of markers indicating injury to critical organs and pro-inflammatory cytokines induced by hemorrhagic shock	[114]
	Acute stroke and Myocardial Infarction (MI)	HBOC-201	To test the impact of HBOC-201 on infarct size after acute myocardial ischemia	1 g/ Kg; IV	Infarct size/area at risk was significantly reduced after HBOC-201 therapy	[120]
		Sanguinate	MI in a rat model	10 mL/kg; IV	Preserving myocardial integrity, mitral competence, and cardiac function	[121]

Table 4 (continued)

Categories	Diseases/ Medical conditions/ Medical procedures	HBOCs formulation	Clinical indication	Treated dose and administration route	Results	References
			Acute stroke in a rat model	8 mL/kg; IV	Increased collateral flow suggests that useful as an adjunct to endovascular therapy and extends the time window for treatment	[123]
		HBOC	Acute stroke leading to brain damage	Ischemia + low perfusion:1.2 mL/h Ischemia + high perfusion:4 mL/h	Controlled perfusion protects ischemic brain tissue in occluded cerebral vascular regions	[124]
	Hemodilution/ Acute blood loss	Hemopure	Anemia after cardiac surgery	Initial transfusion- 60 g in 500 mL Two subsequent transfusions- 30 g in 250 mL	Oxygen extraction was greater in the HBOC group	[126]
		Diaspirin cross-linked hemoglobin (DCLHb)		250 mL infusions; IV	DCLHb allowed 19% of cardiac surgery patients to avoid allogeneic blood exposure	[3]
		Hemolink™	ANH	40 mL/kg; IV	Well-suited for volume replacement with acute normovolemic hemodilution	[127]
		OxyVita Hb	Hemodilution	2–3 mL/kg	Impaired maximum clot strength compared with whole blood at 2 higher dilutions	[128]
	Ischemia/ Reperfusion (I/R) injury	SFHbNP	Neuroprotective effects on I/R injury in tMCAO model	IV	Neurological function improved, alongside reductions in cerebral infarction, edema, oxidative stress, leukocyte recruitment, and BBB disruption	[130]
		HBOC-201	Partial warm liver ischemia model of the rat	–	Equivalent to Dextran in reducing I/R injury in the liver, but enhanced oxygenation of post-reperfusion liver tissue	[131]
		PolyPHb	Cold I/R Injury in a rat model	–	Profound cardioprotective effect and downregulation of TLR 2 and TLR 4/NF-κB signaling pathway expression	[132]
		HBOC-201	Myocardial reperfusion injury	0.2 mL/kg-min^{-1}; IV	Treatment with HBOC-201 before myocardial ischemia–reperfusion reduces myocardial inflammation and injury	[119]

Table 4 (continued)

Categories	Diseases/ Medical conditions/ Medical procedures	HBOCs formulation	Clinical indication	Treated dose and administration route	Results	References
	Wound healing	Liposome-encapsulated hemoglobin (LEH)	Accelerates skin wound healing in mice model	2 mL/kg; IV	Accelerate skin wound healing by reducing inflammation and increasing metabolism, rather than by improving hemodynamics or endothelial regeneration	[137]
	Amanitin-induced hepatotoxicity	M101	Reducing amanitin-induced hepatotoxicity	–	Effectively lowers the generation of reactive oxygen species in the mitochondria and the death of cells generated by amanitin	[144]
Medical procedures	Organ transplantation	Hemopure	Normothermic machine perfusion of the liver	–	Showed no histological damage and can substitute packed red cells in NMP-L perfusion fluid	[156]
		M101	Organ preservation	–	M101 is safe as preservation solution	[157]
		$HEMO_2$life (OxyOp2)	Organ Preservation	–	Improving graft survival by limiting ischemic lesions and safe as preservation solution	[158]

Table 5 Summary of the application of PFOCs

Categories	Diseases/ Medical conditions/ Medical procedures	PFOCs formulation	Clinical indication	Treated dose and administration route	Results	References
Diseases	Anemia/ Sickle cell anemia	Perflubron emulsion	Sickle red blood cell–induced obstruction	A bolus of fully oxygenated and undiluted PFE (0.3 mL)	Oxygenated PFE shows promise in reducing SS RBC-induced vaso-occlusion	[91]
	Cancer and Tumor hypoxia	Perfluorocarbon nanoparticles	Photodynamic therapy	–	Oxy-PDT injections inhibit tumor growth both locally and systemically, outperforming traditional PDT	[98]
		PFC nano oxygen carrier		24.5 mg L^{-1}	PFOCs exploit the tumor's acidic environment, achieving an 84.2% inhibition rate	[99]
		Oxygen-carrying nanoplatforms (IR@LIP/PFOB)	Synergistic PTT and immunotherapy	–	Suppressed tumor growth via eliciting antitumor immune responses	[100]
		"Oxygen bomb" containing PFC core	PDT and chemotherapy	–	Enables a synergistic antitumor effect combining chemotherapy and photodynamic therapy	[101]
		PFOB nanoemulsion	Hypoxia-induced chemoresistance	–	Enhanced cisplatin-induced apoptosis in cancer cells and suppressed tumor growth at a low dose of cisplatin treatment	[102]
Medical condition	Trauma and Hemorrhagic Shock (HS)	Oxyfluor	HS in canine model	15 mL/kg; IV	Enhanced oxygen transport and reinstated global tissue oxygenation	[116]
		DDFPe emulsion	Combination of hemorrhage (50% blood loss) and trauma (fractured bone) in porcine	0.6 mL/kg; IV	100% of DDFPe-treated animals survived to 6 h	[60]
		RBC-PFC formulation	HS	–	Demonstrated a high capacity for oxygen delivery, the mice were resuscitated with an efficacy comparable to that of whole blood infusion	[117]
		Oxygent (Perflubron)	Hepatocellular injury after HS	2.7 or 5.4 g/kg; IV	Enhanced hepatocellular oxygen availability normalized gene expression but had no impact on early hepatocellular injury	[118]

Table 5 (continued)

Categories	Diseases/ Medical conditions/ Medical procedures	PFOCs formulation	Clinical indication	Treated dose and administration route	Results	References
	Acute stroke and Myocardial Infarction (MI)	DDFPe emulsion	Ischemic Stroke Model in Rabbit	0.6 mL/kg; IV	Reduces infarct volumes and protects brain tissue from ischemia	[62]
			Acute Ischemic Stroke (Phase Ib/II trial)	–	Demonstrated safety of DDFPe with standard stroke therapies with no sign of dose-limiting toxicity	[61]
		Oxycyte®	Acute Stroke	3 mL/kg + 50% O_2	Oxycyte combined with 50% O_2 hyperoxia enhancing stroke prognosis	[125]
	Hemodilution / Acute blood loss	Dodecafluoropentane (DDFPe) emulsion	Fatal anemia in rats	0.014 mL/kg of DDFP in a 2% emulsion; IV	In less than 2 h, all control rats had died, but there was 100% survival in the DDFPe-treated rats	[129]
		Albumin-derived PFOCs	Hypoxic tissue damage massive hemodilution	5% HSA solution with 10 mM glucose containing 12 vol% treatment capsules	Elevated arterial blood pressure, stable body temperature, demonstrated stable pH, increased pO_2, and decreased pCO_2	[63]
	Ischemia/ Reperfusion (I/R) injury	Dodecafluoropentane emulsion	Ischemic heart in murine model	0.6 mL/kg; IV	60% reduction in injury/ infarct size	[133]
		Albumin-derived PFOCs	Langendorff-heart perfusion model in a rat	2, 4, and 6 vol% capsules	The 4 vol% capsules delivered sufficient oxygen to prevent myocardial dysfunction	[134]
		PFC emulsion	Ischemia reperfusion in hamster model	–	Reduced leukocyte adhesion, restored capillary density, and improved blood flow post-ischemia	[135]
	Wound healing	Hydrogel Containing PFC Nanoemulsions	Diabetic Wound Healing	Hydrogel sample with a size of 4.5 × 1.7 × 0.2 cm	Enhanced efficacy of wound healing	[138]
		Oxygen-loaded nanobubbles (OLNBs)	Wound healing	–	OLNB efficiently delivers oxygen to hypoxic tissue and promotes wound healing	[139]
		MACF		–	Boosts the process of wound healing and promotes a regenerative metabolic process	[140]
	Decompression sickness (DCS)	Oxygent™	DCS	6 mL/kg; IV	PFC emulsion-treated animals sustained less DCS ($p < 0.01$)	[146]

Table 5 (continued)

Categories	Diseases/ Medical conditions/ Medical procedures	PFOCs formulation	Clinical indication	Treated dose and administration route	Results	References
		PFC emulsions	DCS in sheep model	6 mmol per L of PFC emulsion; IV	Enhanced arterial oxygen content, mixed venous oxygen content, oxygen delivery, and tissue oxygen consumption	[147]
		Oxycyte	Effect of platelet count and function of DCS in swine model	5 cc/kg; IV	No significant additive or synergistic effect of PFC and DCS on platelets or platelet function	[148]
		Oxycyte	DCS in swine model	–	Effectively reduced mortality in swine with DCS	[149]
		DDFPe emulsion	DCS in rat model	0.07 mL/kg; 0.5 mL/kg, 1 mL/kg	Showed no beneficial effects in a rat model	[150]
	Human hair growth	PFOB based nanoemulsion	Hair growth	–	Enhanced human hair organ elongation approximately fourfold in comparison to the control group	[151]
	Lung injury	Oxycyte®	Oleic Acid Lung Injury (OALI)	Pre-Injury/ Post-injury: 5 mL/kg; IV	IV administration of PFC Oxycyte post-OALI significantly boosts blood oxygen levels and improves lung histology	[142]
	Gas embolism	PFC emulsion (Oxycyte and PHER-O_2)	Severe arterial gas embolism	Oxycyte: 12 mL/kg BW PHER-O_2: 4 mL/kg BW	Increased blood flow and decreased oxygen diffusion resistance, facilitating the delivery of oxygenated RBCs to the tissue	[152]
Medical procedures	Organ transplantation	PFC oxygen carrier	Liver transplantation in a rat model	–	In the PFC group, there was a notable enhancement in the postoperative survival rate	[159]
		Albumin based PFOCs	Ex-vivo normothermic kidney perfusion in a rat model	Different volume fractions (0%, 2%, 4%, or 8%)	The 4% concentration of A-AOCs in EVNP proved to be the most effective in preserving physiological renal function	[160]
		Vaporized perfluorocarbon	Lung graft preservation	7 mL/kg	Vaporized PFC decreased the free radical's production and mitigated the occurrence of pulmonary structural changes	[161]

Table 6 Summary of the application of SCOCs

Categories	Disease/ Medical conditions/ Medical procedures	Cell source for in vitro production	Results	References
Diseases	Anemia/ Sickle cell anemia	hSPCs transplantation	Allogeneic hSPCs transplantation from siblings with identical HLA genotypes offers a good chance of survival without complications	[92]
	β-Thalassemia	hSPCs transplantation in hemoglobinopathies	Allogeneic hSPCs transplantation from HLA-genotypically identical siblings offers a favorable chance of complication-free survival	[92]
Medical conditions	Chronic Hypoxia	Human embryonic stem cells (hESCs)	The differentiation and maturation of hESCs into functional oxygen-carrying erythrocytes on a large scale is indeed feasible	[153]
		CD34 + hSPCs	The artificial erythroid cells displayed properties similar to natural human RBCs, including mechanical stability, protein content, oxygen affinity, blood group antigen presence, and circulatory half-life	[68]
		Multipotent hematopoietic stem/progenitor cells	Induced immortalized erythrocyte progenitor cells with critical factors offers a potential model for stable, donor-independent erythrocyte transfusion	[154]
Medical procedures	Development of Rare Blood Type Erythrocytes	Induced pluripotent stem cells (iPSCs)	All donor cells were effectively utilized to create iPSC lines, with no observable chromosomal mutations	[162]

Table 7 Summary of the application of OMNBs

Categories	Disease/ Medical conditions/ Medical procedures	Formulation type	Clinical indication	Treated dose and Administration Route	Results	References
Disease	Cancer	Oxygen nanobubble water by ΣPM-5	Lung cancer	–	Oxygen nanobubble medium markedly reduced hypoxia-induced resistance to radiation compared to the use of standard medium	[103]
		Surfactant-stabilised oxygen nanobubbles	Pancreatic cancer	Orally administration	Oxygen nanobubbles did not lead to a statistically significant alteration in HIF1α expression at the transcriptional level, there is a pressing need for enhanced methods to alleviate tumor hypoxia	[104]
		TOPLMBs	Ovarian cancer	–	Intraperitoneal injection of dual-targeting TOPLMBs, coupled with ultrasound mediation, shows promise for treating ovarian cancer and its microenvironment	[105]
Medical conditions	Acute lung injury and respiratory failure	Lipidic oxygen–containing microparticles	Hypoxemia model in rabbits	Contain between 50 and 90 mL of oxygen gas per deciliter of suspension; IV	Alleviated hypoxemia in rabbits, leading to a decrease in the incidence of cardiac arrest and organ injury compared to control groups	[83]
		Phospholipid-coated oxygen microbubbles (OMBs)	Peritoneal microbubble oxygenation therapy in a Rabbit model of hypoxemia	80 mL/min for 4 min, and then at 12.6 mL/min/kg; perfused through the peritoneal cavity with a peristaltic pump	Demonstrate that PMO therapy can effectively double the survival time of rabbits with full tracheal occlusion	[143]
	Ischemic Stroke Re-perfusion	Lipid shell containing oxygen-loaded microbubbles	Neuroprotection after ischemic stroke reperfusion	Two rounds of OMB administration (1×10^7 MBs/mouse/injection) and US sonication (1-MHz, 5000-cycle, PRF 1 Hz, 300 kPa, 10 min)	Synergizes the benefits of sonoperfusion and local oxygen therapy, resulting in reduced brain infarction and activation of neuroprotection to prevent ischemic stroke-reperfusion injury	[136]
	Retinal Ischemia	Polymer shell (TPGS, Palmitic acid, dextran) containing oxygen nanobubbles (ONBs)	Retinal ischemia during central retinal artery occlusion	25 µL of sterile ONBs; IV	Oxygen release studies revealed that 74.06 µg of O_2 is released from the ONBs after 12 h at 37 °C	[155]
	Wounds	Oxygen Micro/nanobubbles (MNB's)	Acute and chronic wounds	–	Ongoing study (Estimated completion date- June 2025) (NCT05169814)	[141]

Table 8 Summary of oxygen generating materials

Materials	Fabrication methods	Mechanisms	Benefits	Limitations	References
PFCs	Hydrogel, Emulsification, Grafting, Microparticles	Intermolecular forces	Dissolve large amounts of oxygen, facilitating the effective transport of oxygen to hypoxic tissues	Accumulate in tissues, posing clearance challenges, vasoconstriction by binding to nitric oxide	[171]
Hemoglobin	Hydrogel, Microparticles, Electrostatic spraying, Grafting	Binding with ferrous ions	Efficiently mimic RBCs for rapid oxygen transport in hypoxic conditions	Scavenge nitric oxide and produce oxidative byproducts, which may narrow blood vessels and raise blood pressure, short half life	[170]
CaO_2	Emulsion, Electrospray, Hydrogel, Microparticles, Grafting	Hydrolysis	Sustainably releases oxygen, promoting cell survival and tissue healing in hypoxic conditions	Raise local pH, risk of cytotoxicity, requiring controlled release to prevent tissue irritation	[165, 166]
MgO_2	Emulsion, Microparticles, Hydrogel, Electrospray, Grafting	Hydrolysis	Extended oxygen release that promotes tissue regeneration and cell survival in hypoxic conditions	Increase pH and cause oxidative stress, hence their use needs to be carefully regulated	[163, 164, 172]
H_2O_2	Microparticles, Hydrogel, In situ generation	Disproportionate decomposition	Efficient source of oxygen, enhancing applications in medical fields due to their ease of handling and controlled release	Restricted shelf life and possible safety risks, such as the possibility of exothermic reactions and material corrosive effects	[167]
Microalgae	Hydrogel, Microparticles, 3D printing scaffold	Photosynthesis	Provide a sustainable, reliable supply of oxygen under suitable conditions	Regulations of light, temperature, and nutrients are necessary for the creation of oxygen	[168]
MnO_2	Emulsion, Hydrogel, Powder Blending, Porous Scaffold Incorporation	Catalytic decomposition	Offer rapid, controlled oxygen release	Require activation and generate heat during decomposition	[169]

Table 9 Clinical Developments of Different AOCs from 2017 to 2024

Name	Manufacturer	Oxygen carriers	Clinical indication	Intervention / treatment	Study starts	Clinical phase	Status	Clinical ID	References
HEMO2life®	Hemarina, Sponsor: University Hospital, Brest	HBOCs	End Stage Renal Diseases	Kidney transplant	July 2020	Phase I	Completed September, 2023	NCT04181710	[158]
HBOC-201 (Hemopure)	HbO2 Therapeutics LLC		Life-Threatening Anemia	Blood alternative	October 2013	Not Applicable	Completed December, 2023	NCT01881503	[174]
PP-007	Prolong Pharmaceuticals		Acute Ischemic Stroke	Standard of care	October 2021	Phase I	Completed March, 2023	NCT04677777	[175]
Sanguinate™			Anemia, Sickle Cell disease	Vaso-Occlusive Crisis	November 2016	Phase II	Completed December, 2017	NCT02411708	[176]
Hemoglobin Vesicles (HbVs)	Hokkaido University, Japan (Academia-initiated)		Life-threatening massive hemorrhage	Transfusion alternative	October 2020	Phase I	Completed August, 2021	jRCT2011200004	[20]
ABL-101	Aurum Biosciences Ltd	PFOCs	Acute Ischaemic Stroke	Oxygenation	September 2018	Phase II	Completed March, 2020	NCT03463551	[177]
DDFPe	NuvOx LLC		Ischemic Stroke		February 2017	Phase I	Completed July, 2018	NCT02963376	[178]
NanO2™			Ischemic Stroke	Cerebroprotectant	April 2023	Phase II	Ongoing	U44NS130589 (award tracking no)	[179]
NVX-108			Glioblastoma	Radiation Sensitizer	May 2014	Phase I	Completed April, 2018	NCT02189109	[180]
Oxygen Micro/ Nanobubbles (MNB's)	University of California, Irvine	Micro/ Nanobubbles	Acute and Chronic Wounds	-	October 2021	Phase I	Ongoing (Estimated completion date- June 2025)	NCT05169814	[181]

oxygen deficiency-related disorders. Oxygen carriers remain underutilized in tumor hypoxia and dynamic therapies for tumors, but their potential is promising for advancement in the next decade.

Challenges in AOCs development

HBOCs and PFOCs development

Assorted clinical trials show that unmodified acellular Hb, even when highly purified, is unsafe. Processing is necessary for Hb sourced from bovine or humans to remove toxicity and replicate RBC functions. Ideal HBOCs should possess stable tetramers, low oxygen affinity, non-vasoconstrictive, non-nephrotoxic, non-immunogenic properties, and should not interfere with physiological functions. Exposure of stromal-free Hb tetramers to internal conditions prompts rapid elimination via glomerular filtration, causing a short half-life and renal toxicity [23]. To counter this, methods such as chemical or genetic crosslinking, polymerization, crosslinking to polymers, and encapsulation in liposomes have been explored. These approaches aim to stabilize the tetramer, minimize endothelial interactions, and decrease renal excretion. To prevent vasoconstriction, methods like PEGylation and Hb encapsulation in liposomes are utilized. Mutant Hb with a preference for oxygen over NO could offer a promising approach, given their similar binding sites in heme [182]. Altering oxygen affinity is crucial. Acellular Hb lacks 2,3-DPG, leading to increased oxygen affinity and reduced tissue release. Additionally, the higher plasma pH boosts Hb's oxygen affinity. Point mutations and recombinant Hb expression, combined with 2,3-DPG supplementation, are advanced techniques for reducing oxygen affinity [52].

The body's immune response to modified Hb is a key concern, leading to accelerated clearance upon repeated dosing, macrophage accumulation, and altered T cell and lymphocyte ratios. Both genetically and chemically modified Hb may induce immune reactions, requiring careful consideration in HBOC development [183, 184]. Sterility and endotoxin elimination are top priorities for the

final product, but conventional heat sterilization methods are unsuitable for preserving Hb. Despite the stability of modified Hb variants like cross-linked and PEGylated forms after pasteurization, achieving complete elimination of endotoxins from bacterial-expressed recombinant Hb, such as that from *E. coli*, remains a persistent scientific challenge [185].

The primary issues identified with existing PFOCs include their large particle size, extended persistence in the body, low storage stability, high tissue distribution characteristics, and high content of PFCs in some PFOCs [186]. First-generation PFOCs like Fluosol-DA, Oxypherol, and Perftoran couldn't be improved by increasing content due to synthetic technical problems, resulting in low purity and unfavorable physical properties of PFCs. In addition, their lower oxygen delivery efficiency was attributed to the low PFC content, which was below 25%. As mentioned earlier, emulsion stability and intravascular half-life depend significantly on droplet size, ideally remaining below 0.5 µm. Larger droplets reduce oxygen transport efficiency and may cause micro embolism, leading to oxygen toxicity and lung embolism. Thus, older formulations like Fluosol-DA and Perftoran were particularly linked to pneumonia [22, 187]. Furthermore, in emulsion preparation, the formulation incorporates a synthetic polymer-based surfactant like poloxamer, and toxicity issues arose from the use of synthetic polymer-based surfactants, which led to discontinuation after clinical trials.

Second-generation of PFOCs like Oxygent, Oxyfluor, and Oxycyte have improved purity and physical properties compared to their predecessors, due to advancements in synthesis technology and processes. They are utilized in artificial RBC preparations and have enhanced biocompatibility with the use of synthetic polymer-based and phospholipid-based surfactants, mimicking biomembrane materials. The fusion of PFCs wrapped in phospholipids with natural lipid vesicles in the bloodstream can aid particle clearance. This fusion rate is proportional to the vapor pressure of the PFC used [22]. Due to these technical advancements, several products entering the clinical stage have been reported compared to first-generation products.

Apart from this, another challenge is the introduction of NO poses a complex interplay with potential implications for patient safety. When AOCs are used, the production of NO can lead to several potential side effects. These may include alterations in vascular tone, which could result in hypertension or hypotension, as well as disruptions in platelet function, increasing the risk of thrombosis or bleeding [188]. Additionally, NO production could impact immune, respiratory, and neurological functions, potentially leading to immune dysregulation, respiratory distress, or neurological manifestations [189]. Understanding and managing these side effects are crucial for the safe and effective use of AOCs.

SCOCs development

SCOCs face critical developmental obstacles, particularly in order to achieve full functional maturity, immunological compatibility, and scalable production. It is difficult to differentiate stem cells into completely enucleated, adult hemoglobin-rich cells, which may reduce their ability to carry oxygen [69]. The risk of immune rejection and potential tumorigenicity from any remaining undifferentiated cells pose significant challenges to the safe clinical application. Additionally, large-scale production of these cells is very expensive, and they have serious storage stability problems, which are essential for wider clinical application. Regulatory and ethical challenges also hinder the progression of SCOCs from research stages to clinical application [70, 71].

Challenges of producing reactive oxygen species (ROS) during AOCs using (a relationship between ROS, HIF-1α, and AOCs)

Oxygen is crucial for the survival and metabolic activities of cells. Research indicates that intracellular signals can fluctuate depending on oxygen levels. Specifically, the signals from ROS and HIF-1α are highly significant [190]. ROS, generated as a by-product resulting from the biological process of oxygen metabolism, especially under stress conditions like hypoxia. HIF-1α is a transcription factor that aids cells in adapting to low oxygen levels through the activation of genes involved in erythropoiesis, angiogenesis, and metabolic shifts. Under normal oxygen concentrations, the cellular abundance of HIF-1 is notably low, whereas in hypoxic conditions, cells increase ROS production, which stabilizes HIF-1α to promote survival by activating adaptive pathways. During hyperoxia, ROS levels are elevated due to prolyl hydroxylase inhibition, preventing HIF stabilization. However, HIF stabilization can be triggered by ROS, while in turn, HIF stabilization may lead to either a decrease or increase in ROS formation [191, 192]. The correlation between ROS formation and HIF activation shifts with changing oxygen levels is shown in Fig. 8.

The interaction between ROS, HIF-1α, and AOCs poses significant challenges for AOCs development and application. AOCs, such as HBOCs and PFOCs, are intended to ameliorate hypoxia by enhancing oxygen delivery, potentially lowering ROS and HIF-1α activity [193]. However, AOCs, particularly in high doses, may cause to spikes in oxygen availability that stimulate excessive ROS production, exacerbating oxidative damage instead of reducing it. This oxidative damage can

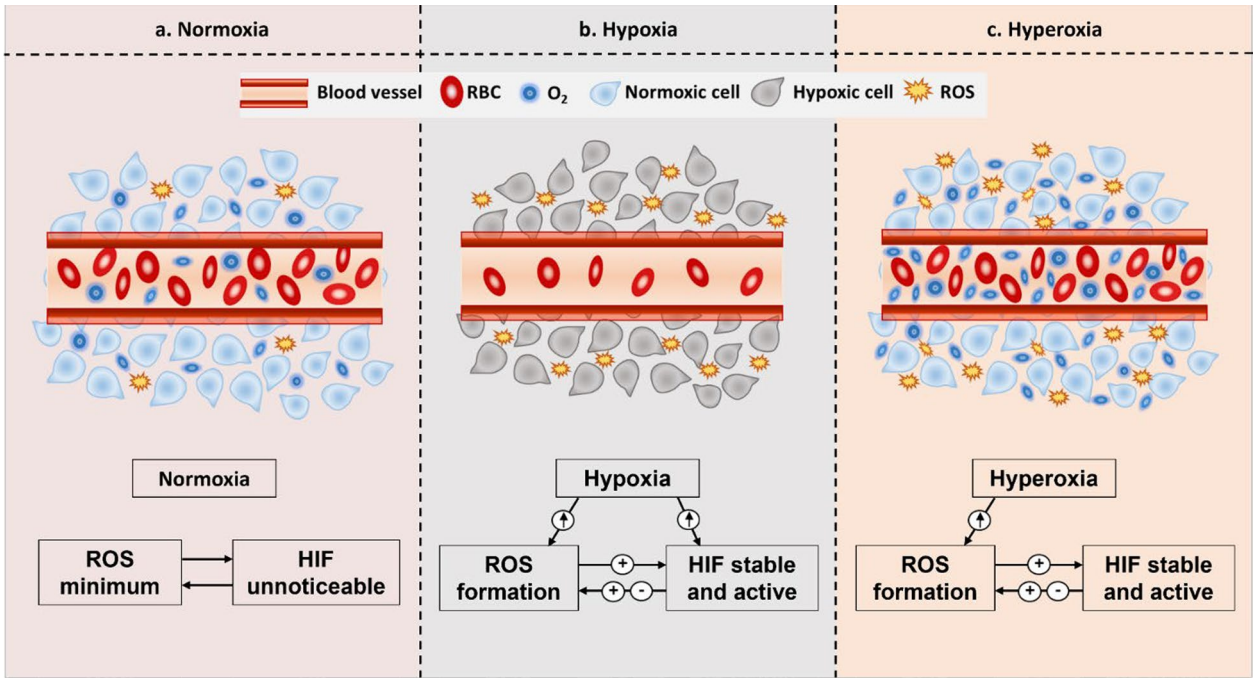


Fig. 8 Correlation between ROS formation and HIF activation shifts with changing oxygen levels. **a** During Normoxia, HIF is consistently synthesized but rapidly degraded, resulting in undetectable levels of HIF, while ROS formation remains minimal; **b** During hypoxia, HIF is stabilized and stable, leading to an increase in ROS formation. Elevated ROS levels can both reinforce HIF stabilization and either diminish or intensify ROS production; **c** During hyperoxia, ROS levels are elevated due to PHD inhibition, preventing HIF stabilization. However, HIF stabilization can be triggered by ROS, while in turn, HIF stabilization may lead to either a decrease or increase in ROS formation

activate inflammation and immune responses that further challenging therapeutic outcomes. AOCs dose needs to be precisely calibrated to prevent oversaturating tissue with oxygen or unintentionally producing ROS surges. To achieve effective dosing, it is essential to understand the specific oxygen demands and redox states of target tissues, which can differ greatly between individuals and disease contexts. Certain AOCs, especially HBOCs, have been linked to inflammatory responses. These immune reactions can heighten oxidative stress, increase ROS burden, and ultimately complicate outcomes by triggering undesired tissue responses. In addition, while downregulating HIF-1α can mitigate detrimental overactivation, it may also prevent advantageous hypoxia-driven reactions like angiogenesis in ischemic tissues [47].

Furthermore, when Hb is released from RBC, it can catalyze the production of ROS, which can damage the hemoglobin. For instances, to protect HBOCs from ROS oxidation, researchers encapsulate Hb with nanozymes that deplete reactive oxygen species like superoxide and hydrogen peroxide and demonstrated the membrane-coated nanocarriers have shown potential as novel oxygen carriers with antioxidant and stealth properties [47]. Conversely, while PFOCs improve oxygen solubility, their accelerated oxygen release can also lead to oxidative stress and subsequent HIF-1α stabilization, which can complicate tissue responses. Moreover, combining HBOCs with antioxidant therapy offers a promising approach in reducing ROS and improving therapeutic outcomes [194]. Antioxidants like N-acetylcysteine (NAC) or vitamin C can directly neutralize ROS generated by HBOC metabolism, helping to minimize oxidative damage [195]. In summary, while AOCs have great potential for treating hypoxia-related conditions, balancing oxygenation, ROS control, and HIF-1α activity within a safe therapeutic window remains a significant challenge, highlighting the need for advanced, context-sensitive AOC delivery systems tailored to specific tissue requirements and disease states.

Demands for novel classes of AOCs

In systemic circulation, RBC play several roles, such as enabling the transport of oxygen and carbon dioxide between the tissues and the lungs, detoxifying and reducing Hb through methemoglobin reductase and small molecule reductants, modulating vascular flow through shear-stress-induced signaling [196], and participating in nitrate reactions [197]. A perfect RBC substitute should possess material properties that faithfully mimic these

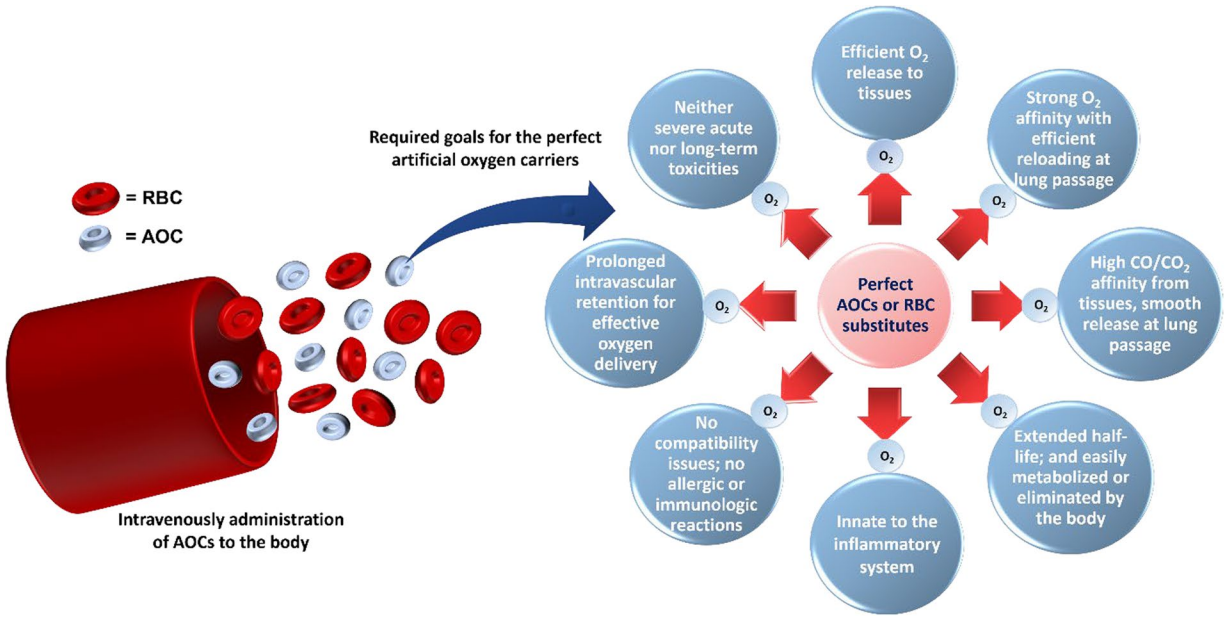


Fig. 9 Required goals for the perfect AOCs

essential functions. The pioneer focus was primarily on oxygen transport in the development of RBC substitutes, recent advancements have seen the incorporation of features related to both carbon dioxide transport and hemoglobin detoxification in newly developed materials [198].

An ideal RBC substitute should have some essential characteristics which are depicted in Fig. 9. The important goals include achieving a high affinity to oxygen with smooth release at the tissue and a high affinity to carbon monoxide /carbon dioxide with smooth release at lung passage like RBC functions [13]. Additionally, a perfect RBC substitute must possess a reasonable circulatory half-life and be readily metabolized or eliminated by the body. The primary challenges associated with AOCs involve triggering inflammatory responses within the body, as well as the potential for inducing hypotension and hypertension, which should be prevented [56]. Over and above, a perfect RBC substitute also has the following characteristics: Preservation for extended periods under ambient conditions, absence of incompatibility issues like allergic or immunologic reactions during transfusion, extended intravascular retention for effective delivery of oxygen to tissues, neither severe acute nor long-term toxicities, eliminated risk of pathogen transmission, cost-effective production, and physiological characteristics for the uptake and release of oxygen [3, 13, 14, 199]. These materials must also match or surpass the human blood's ex vivo shelf-life used in a clinical setting.

Future perspectives in the field of oxygen carriers

Despite considerable attempts to resolve issues related to oxygen delivery using HBOCs and PFOCs, their clinical application has been impeded by their toxicity. Genetic alterations and chemical modifications significantly influence the effectiveness of HBOCs, impacting both their efficiency and potential side effects. Polymerized and crosslinked HBOCs have effectively mitigated the negative effects of cell-free Hb. Further exploration of diverse polymerization techniques holds promise for enhancing the oxygen-carrying capabilities of polymeric Hb [200]. It is important to note that the site of polymerization significantly impacts the oxygen-carrying capacity of polymerized Hb. Therefore, a major challenge lies in identifying the optimal site for polymerization that does not compromise the oxygen-binding abilities of the Hb complex. An alternative strategy involves creating recombinant hemoglobin for HBOCs. Recombinant Hb-based HBOCs provide advantages including a natural origin for transfusion, improved shelf-life, lower disease transmission risk, standardized final product, and worldwide recognition. However, the main challenge ahead is selecting mutations to create recombinant Hb with reduced oxidation, heme loss, and NO scavenging, while preserving core Hb properties [52]. Currently, the use of recombinant Hb offers a limitless source of Hb. Human Hb function is influenced by compounds like 2,3-DPG, which bind deoxygenated Hb more strongly than oxygenated Hb [201]. In contrast, effective management of metHb levels is essential to maintain optimal oxygen-carrying capacity.

Additionally, bovine Hb demonstrates superior stability at higher temperatures during isolation and processing. Thus, in terms of availability, stability, and oxygen transport capacity, bovine Hb presents numerous advantages over human Hb [202].

Developing new formulations of PFCs is essential to mimic the desirable characteristics of an ideal oxygen carrier. It is crucial to focus on enhancing the emulsifying agent and addressing other formulation conditions in order to pursue clinical approval. As earlier mentioned, existing PFOCs face primary issues such as large particle size, prolonged persistence in the body, low storage stability, extensive tissue distribution, and high PFC content in certain formulations. Large particle size and high PFC content are common issues that contribute to prolonged residence times in the body. Large particle size results in accumulation in the liver via uptake in the reticuloendothelial system (RES), while extended residence in the body can induce side effects such as complement activation-related pseudo allergy (CARPA) reactions [203]. Particles smaller than 1000 nm, have the ability to traverse all capillaries upon injection into the bloodstream, and are typically swiftly absorbed by macrophages of the RES. Following intravenous injection, PFOCs are stored in the phagocytes of the RES, resulting in the removal of perfluorinated compound particles from the blood. Large PFC particle size in PFOCs prompts RES uptake, resulting in liver accumulation and intrahepatic toxicity, underscoring the importance of nanoparticles being < 100 nm to evade liver RES uptake. A prolonged residence time is essential to facilitate in vitro excretion by employing high vapor pressure PFCs, while ensuring emulsion stability through the use of a biocompatible stabilizer. In addition, other essential prerequisites for developing PFOCs include a short half-life and residence time in the body, as well as ensuring perfect storage conditions and a rapid in vivo disappearance rate [204].

The distinct characteristics of oxygen carriers, including their transport properties, administration rates, and dosages, play a crucial role in patient safety and treatment efficacy. Patient safety is paramount, and these factors should be central to guiding product development. We summarized key considerations for developing safe and effective oxygen carriers, such as HBOCs and PFOCs, in Table 10.

Conclusions

Blood donations and transfusions are currently experiencing a global decline. This trend poses significant challenges for healthcare systems and patients alike. In the midst of the 2020 COVID-19 pandemic, shortages in blood supply were observed globally, which is still going on. Not only COVID-19, whenever various pandemic conditions arise like HIV, MERS-CoV, there is a significant and drastic drop in blood donations. Apart from pandemic situations, recently there is a requirement to stockpile medical blood reserves in anticipation of potential casualties arising from the conflict in each affected country. Ensuring an adequate blood

Table 10 Key considerations for product development

AOCs	Critical considerations	References
HBOCs	Effective management of metHb levels is essential to maintain optimal oxygen-carrying capacity	[3, 23, 199–202]
	Ensuring sufficient Hb content, including encaps enhances stability and efficacy	
	Modulating NO production and extending the half-life can improve therapeutic effects by reducing vasoconstriction and enhancing oxygen delivery	
	Stability during storage and reconstitution is critical, especially for freeze-dried products	
	Selection of safe Hb sources (e.g., bovine or human)	
	Adherence to GMP standards for raw material supply are essential for reducing risks associated with biological variability	
	Complete removal of free Hb is crucial to avoid toxicity and adverse immune responses	
PFOCs	PFOCs require high doses for effective oxygen transport; as they are excreted mainly through the lungs, formulations must allow for rapid clearance to minimize accumulation and related risks	[14, 22, 58, 66, 204]
	Emulsions often face stability issues, necessitating long-term refrigeration to ensure consistent performance and prevent degradation	
	Secure sourcing and compliance with GMP standards are critical to maintain product safety and minimize contaminants	
	Addressing ROS production is important for stability and safety, as ROS can cause oxidative damage and limit efficacy	
	Ensuring optimal particle size and controlled PFC content is essential for PFOCs, as large particles and high concentrations can lead to prolonged residence times in the body, impacting clearance	

transfusion supply is vital not only for civilian healthcare but also for military operations. In this scenario, AOCs offer diverse benefits beyond allogeneic blood transfusions, potentially providing morbidity and mortality advantages for patients facing significant distress. Over the years, research studies have shown notable progress in the development of AOCs such as HBOCs, PFOCs, SCOCs, and OMNBs. However, progress in development has stagnated due to the emergence of side effects, including toxicity within the body. The development of HBOCs has encountered numerous hurdles in the past, with the most significant being the severe side effects of acellular Hb, including nephrotoxicity due to the dissociated tetramer, hypertension mediated by NO scavenging, and inflammation. Utilizing recombinant Hb eliminates infection risks and offers the capability to modify the globin protein, facilitating research into the structural basis of oxygen-binding. Perfluorocarbons (PFCs), although subject to multiple clinical trials and recommended for clinical use in some studies and FDA-approved cases, are not widely utilized due to formulation issues, despite their known capacity to capture and transport oxygen and other gases. However, PFOCs require smaller particle sizes (< 100 nm) to avoid prolonged residence in the body and potential toxicity due to absorption by the RES, otherwise, it faces struggle to pass through the liver, leading to prolonged body residence and potential toxicity. Furthermore, creating personalized RBC concentrates from adult stem cells could emerge as a significant advancement, particularly if cost-effective, high-volume production under clinical standards is achieved. Over the past four decades, AOC development has been challenging, with numerous unsuccessful tests and failed projects. However, recent innovative advancements suggest promising results on the horizon within a reasonable timeframe.

Abbreviations

RBC	Red blood cell
WBC	White blood cell
HIV	Human immunodeficiency virus
TA-GVHD	Transfusion-induced graft-versus-host disease
TRALI	Transfusion-associated acute lung injury
AOCs	Artificial oxygen carriers
Hb	Hemoglobin
PFC	Perfluorocarbon
HBOCs	Hemoglobin-based oxygen carriers
DBBF	Bis-(3,5-dibromosalicyl)-fumarate
NFPLP	2-Nor-2-formylpyridoxal phosphate
NO	Nitric oxide
PEG	Polyethylene glycol
metHb	Methemoglobin
ROS	Reactive oxygen species
PLGA	Poly-lactide-co-glycolide
PDA	Polydopamine
PFOCs	Perfluorocarbon-based oxygen carriers
DDFPe	Dodecafluoropentane
PFOB	Perfluorooctylbromide
SCOCs	Stem cell-based oxygen carriers
hSPCs	Hematopoietic stem/progenitor cells
SCF	Stem cell factor
EPO	Erythropoietin
HESCs	Human embryonic stem cells
iPSCs	Induced pluripotent stem cells
BM	Bone marrow
PB	Peripheral blood
G-CSF	Granulocyte colony-stimulating factor
UCB	Umbilical cord blood
PSCs	Pluripotent stem cells
HMSCs	Human mesenchymal stromal cells
OMNBs	Oxygen micro/nano bubbles
HIF-1α	Hypoxia-inducible factor-1α
HS	Hemorrhagic shock
TBI	Traumatic brain injury
DCLHb	Diaspirin cross-linked hemoglobin
MAP	Mean arterial pressure
LEH	Liposome-encapsulated hemoglobin
MOFs	Metal–organic frameworks
AML	Acute myeloid leukemia
MI	Myocardial Infarction
AAR	Area at risk
ANH	Acute normovolemic hemodilution
HSA	Human serum albumin
NMP	Normothermic machine perfusion
DCS	Decompression sickness
GOLD	Glasgow oxygen level dependent
PFE	Perflubron-based fluorocarbon emulsion
OLNB	Oxygen-loaded nanobubbles
MACF	Methacrylamide chitosan hydrogel
LPD	Low-potassium dextran
hDPCs	Hair dermal papilla cells
OALI	Oleic acid-induced injury
EVNP	Normothermic mechanism perfusion
HLA	Human leukocyte antigen
PMO	Peritoneal microbubble oxygenation
TOPLMBs	Oxygen/paclitaxel loaded microbubbles
PHD	Prolyl hydroxylase
RES	Reticuloendothelial system
CARPA	Complement activation-related pseudo allergy

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Author contributions
Conceptualization was carried out by N. Mohanto and J.P. Jee. The investigation was conducted by N. Mohanto, H. Mondal, Y.J. Park and J.P. Jee. Original draft preparation was done by N. Mohanto, H. Mondal, Y.J. Park and J.P. Jee and review and editing were undertaken by J.P. Jee. The visualization was performed by N. Mohanto and J.P. Jee. J.P. Jee provided resources and overall supervision for the study. Funding acquisition was secured by Y.J. Park and J.P. Jee.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Consent for publication
All authors contributed to the article and approved the final manuscript.

Competing interests
The authors declare no competing interests.

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