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Yapay Oksijen Taşıyıcılarının Gelişim Süreci ve Klinik Kullanımı
Development Process and Clinical Application of Artificial Oxygen Carriers

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ÖZET

Yapay oksijen taşıyıcıları; travma, cerrahi girişimler, doğum, mide ülserleri, hemorajik şok, vasküler rüptür ve ani kan hacmi kaybına neden olan diğer durumlarda, eritrositlere alternatif olarak geliştirilmiştir. Bu taşıyıcılar aracılığıyla terapötik oksijen iletimi, çeşitli hastalık modellerinin tedavisinde umut verici bir yaklaşım sunmaktadır. Ancak stabil, toksik olmayan ve immünolojik olarak inert yapay oksijen taşıyıcılarının geliştirilmesi hâlâ kritik bir ihtiyaçtır. Geliştirme süreci boyunca birçok zorlukla karşılaşılmış olmasına rağmen, bu taşıyıcıların elektif ve kardiyovasküler cerrahiler, hemorajik şok, dekompresyon hastalığı, akut inme, miyokard enfarktüsü, orak hücre krizi ve serebral hipoksi gibi çeşitli klinik durumlarda kullanımı gösterilmiştir. Bu derleme, terapötik oksijen iletiminde kullanılan hemoglobin bazlı, perflorokarbon bazlı, kök hücre kaynaklı ve oksijen mikro/nano kabarcıklarına dayalı yapay oksijen taşıyıcılarının rollerini kapsamlı bir şekilde incelemektedir. Ayrıca bu yaklaşımlarla ilişkili olası yan etkiler ve sınırlılıklar, tamamlanmış ve devam eden çalışmalar ile güncel klinik gelişmeler ışığında değerlendirilmiştir. Ek olarak, ideal bir yapay oksijen taşıyıcısının geliştirilmesi için gerekli temel özellikler ve geleceğe yönelik çözüm önerileri sunularak mevcut literatüre katkı sağlanması ve alandaki ileri düzey araştırmalara rehberlik edilmesi amaçlanmaktadır.

Anahtar Kelimeler: Yapay oksijen taşıyıcılar, Hemoglobin bazlı taşıyıcılar, Perflorokarbon bazlı oksijen taşıyıcılar, Kök hücre tabanlı oksijen taşıyıcıları, O_2 mikro kabarcıkları.

ABSTRACT

Artificial oxygen carriers have been developed as alternatives to erythrocytes in situations involving trauma, surgical interventions, childbirth, gastric ulcers, hemorrhagic shock, vascular rupture, and other conditions that result in sudden blood volume loss. Through these carriers, therapeutic oxygen delivery presents a promising approach for the treatment of various disease models. However, the development of stable, non-toxic, and immunologically inert artificial oxygen carriers remains a critical need. Despite numerous challenges encountered during their development, the application of these carriers has been demonstrated in a wide range of clinical settings, including elective and cardiovascular surgeries, hemorrhagic shock, decompression sickness, acute stroke, myocardial infarction, sickle cell crisis, and cerebral hypoxia.

This review comprehensively examines the roles of various types of artificial oxygen carriers used in therapeutic oxygen delivery, including hemoglobin-based, perfluorocarbon-based, stem cell-derived, and oxygen micro/nanobubble systems. In addition, potential side effects and limitations associated with these approaches are evaluated in the context of completed and ongoing studies as well as current clinical advancements. Furthermore, the review aims to contribute to the existing literature and guide future research by outlining the essential requirements for the development of ideal artificial oxygen carriers and proposing prospective solutions.

Keywords: Artificial oxygen carriers, Hemoglobin based carriers, Perfluorocarbon based oxygen carriers, Stem cell based oxygen carriers, O_2 microbubbles.

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INTRODUCTION

Blood is a substance vital for the sustenance of human life. One of the primary functions of blood is to transport oxygen from the lungs to all body tissues, thereby meeting the metabolic demands of these tissues (1). Numerous etiologies can lead to blood loss in humans, necessitating blood replacement therapy. In earlier times, physicians attempted to substitute human blood with various materials such as plant resins, milk, beer, chicken or sheep blood, and even human urine (2). In modern medicine, the demand for allogeneic blood transfusions in patients with various potentially life-threatening conditions is extremely high; however, in many regions worldwide, the shortage of human blood for transfusion has reached critical levels. There is a clear need for alternatives to human blood, currently referred to as oxygen therapeutic agents, that can perform the oxygen transport function to body tissues as normal blood does (3, 4). Under normal conditions, oxygen is carried by hemoglobin (Hb) within red blood cells; however, it remains controversial whether Hb molecules can transport oxygen in the bloodstream without the protective effect of the red blood cell membrane. A study conducted in 1933 demonstrated that bovine hemolysates were able to transport oxygen in experimental animals (5). Subsequently the use of cell-free Hb solutions was explored as an oxygen carrier alternative to human blood.

There is an undeniable need for oxygen therapeutic agents, which are novel alternatives capable of replicating the oxygen-carrying function of human blood and maintaining tissue oxygenation. However, allogeneic blood transfusion presents significant challenges, including special storage requirements, risk of contamination during storage, potential transmission of infectious diseases (such as HIV and hepatitis), hemolytic and immunogenic complications, limited shelf life, and poor portability (6).

The clinical application of blood transfusion is generally performed in the form of component therapy. In this context, red blood cell (RBC) transfusion is the most commonly and prominently used component in cases of severe hematological disorders. The primary physiological function of RBCs is their oxygen carrying capacity. These cells can adaptively respond to changes in oxygen concentration within organic tissues, a characteristic that significantly improves survival rates. However, there are various limitations and inherent risks associated with the transfusion of red blood cells obtained from donor blood. These risks include the potential transmission of infectious diseases, non-hemolytic febrile reactions, and reperfusion injury (7). An ideal blood substitute should possess the following fundamental characteristics: stable oxygen-carrying capacity, low immunogenicity, long biological half-life, biocompatibility with the renal system, absence of risk for transmission of infectious diseases,

and the ability to be easily metabolized and eliminated by the body. Additionally, it should be capable of long-term storage, scalable production, and consequently be both readily accessible and practically portable. Due to various risks and notable limitations associated with blood transfusion, researchers have shown significant interest in developing alternative oxygen carrier systems (8).

A comprehensive literature search was conducted in the Google Scholar and PubMed databases for the period between 2013 and 2024 using the keywords Artificial Oxygen Carriers, Hemoglobin-Based Carriers, Perfluorocarbon-Based Oxygen Carriers, Stem Cell-Based Oxygen Carriers, and O_2 Microbubbles. A total of 162 studies were identified, of which 78 full-text articles were reviewed after screening titles and abstracts. Ultimately, 40 studies meeting the predefined inclusion and exclusion criteria were included in the review. Thus, the literature search process was performed in a transparent and reproducible manner.

Oxygen Transport Systems

Systemic circulation delivers oxygen and nutrients to cells while simultaneously removing carbon dioxide and waste products from them. Oxygenated blood flow begins from the left ventricle and is transported to body tissues through the arteries. Deoxygenated blood returns from the tissue capillaries and reaches the heart's right atrium via the veins. Oxygen in the blood is transported via two mechanisms: dissolved oxygen in plasma (~1.5%) and oxygen bound Hb (~98.5%) (9). Hb is a tetrameric protein molecule responsible for oxygen transport, located within red blood cells. It consists of two alpha and two beta subunits and has a molecular weight of approximately 64,400 Da. Each peptide subunit contains a heme group within its globin structure, which harbors a centrally located iron atom.

This heme group can bind one oxygen molecule; therefore, one Hb molecule can carry a total of four oxygen molecules. The iron atom is typically in the ferrous (Fe^{2+}) redox state, enabling oxygen binding. Following oxidation (electron loss), the Fe^{2+} form converts to ferric (Fe^{3+}) form; this form is referred to as methemoglobin or ferrihemoglobin. Methemoglobin is incapable of binding oxygen. The affinity of hemoglobin for oxygen increases cooperatively depending on the number of oxygen molecules bound. Deoxygenated hemoglobin, which has low affinity, exists in the T (tense) state, and upon oxygen binding, it transitions to the high-affinity R (relaxed) state. In tissue environments with low partial pressure of oxygen (pO_2), oxygen is released, whereas in the lungs, high pO_2 facilitates greater oxygen binding to Hb. This process is represented by a sigmoidal oxygen dissociation curve (10).

Factors Affecting Oxygen Binding Capacity

Oxygen transport capacity depends on the oxygen concentration in the blood. As described below, various parameters such as environmental factors, temperature, modulatory molecules like 2,3-diphosphoglycerate (2,3-DPG), and diseases can influence the ability of Hb to bind oxygen (11).

Blood pH and CO_2 Levels

During cellular respiration, many biochemical reactions produce CO_2 as a result of increased metabolic activity in tissues. CO_2 reacts with water via the enzyme carbonic anhydrase, facilitating the removal of metabolic CO_2 waste from the blood; this reaction results in the formation of bicarbonate (HCO_3^-) and hydrogen ions (H^+). As blood CO_2 levels increase, the concentration of H^+ ions also rises, leading to a decrease in the pH of the surrounding peripheral tissues. This change enhances oxygen release in peripheral tissues while facilitating oxygen uptake in the lungs. This alteration in oxygen affinity is visually represented in Figure 1 by the oxygen dissociation curve demonstrating the Bohr effect. When pH decreases, hemoglobin responds to the change by releasing oxygen, and its affinity for oxygen decreases; conversely, when pH increases, its affinity rises. This phenomenon was discovered by Bohr and colleagues in 1904 and is known as the "Bohr effect" (12).

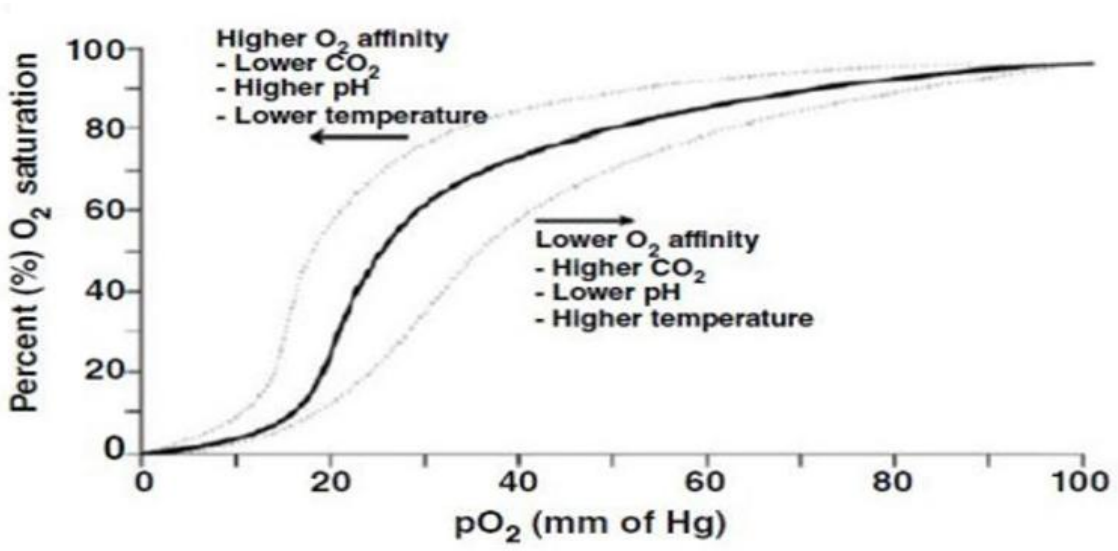

Figure 1. Oxygen dissociation curve showing the Bohr effect (12).

Temperature

When body temperature increases, particularly in active skeletal muscles, heat production rises simultaneously, which leads to a decrease in Hbs affinity for oxygen. Conversely, when tissue

metabolism decreases, heat production diminishes, resulting in a lower temperature and an increased affinity of Hb for oxygen (13).

Diseases

Several diseases, such as sickle cell anemia and thalassemia, affect Hb levels and thereby reduce the oxygen-carrying capacity in the body. In sickle cell anemia, the shape of red blood cells changes from the normal biconcave disc to a rigid, elongated crescent form. This abnormal shape prevents the cells from passing through capillaries, causing vascular occlusion (vaso-occlusion) and impairing oxygen transport. Thalassemia is a genetic disorder characterized by an increased number of red blood cells; however, these cells contain less Hb than normal, resulting in reduced oxygen binding and transport capacity (14).

Artificial Oxygen Carriers and Their Benefits

AOCs play a crucial role in managing blood conditions in patients with severe illnesses. AOCs are divided into two main groups: hemoglobin-based oxygen carriers (HBOCs), where oxygen is covalently bound to Hb, and perfluorocarbon-based oxygen carriers (PFOCs), in which oxygen is dissolved within perfluorocarbon (PFC) molecules. Additionally, synthetically produced porphyrin polymers and oxygen micro- and nanobubbles are emerging as effective alternatives among AOCs (15). The most significant advantage of these systems is their ability to immediately deliver oxygen through the circulatory system, thereby saving lives. AOC products can be used in traumatic situations where blood donation is not possible and under challenging environmental conditions. They are also preferable in medical treatments such as elective and cardiovascular surgeries. Furthermore, they are effective in alleviating ischemic conditions such as cerebral hypoxia and fetal ischemia (16). AOCs are also stable, easily accessible, and cost-effective products. They are compatible for use in patients who require blood transfusions but refuse them for religious reasons, such as Jehovah's Witnesses, who believe that receiving blood transfusions contradicts God's will and reject transfusions not only from others but also from their own bodies. Blood management can be challenging in patients with rare blood types, such as the Bombay phenotype (Oh), and in those with severe immune deficiencies like sickle cell anemia. Therefore, AOCs play a vital role as a life-saving and protective system in cases of severe hypoxia (17).

Hemoglobin Based Oxygen Carriers

HBOCs are used as universal oxygen delivery systems in many life-threatening conditions such as hemorrhagic shock, trauma, stroke, myocardial infarction, and acute blood loss. Initially, cell-free Hb was employed for oxygen transport; however, this approach encountered several challenges. The primary issue was the high oxygen affinity of cell-free Hb. While the p50 value

in healthy erythrocytes ranges between 26–28 mmHg, cell-free Hb exhibits a p50 of 10–15 mmHg, causing oxygen to bind tightly to Hb rather than being released to the tissues. During the purification process, 2,3-diphosphoglycerate (2,3-DPG) is lost due to the high oxygen affinity. Furthermore, intravenous administration of cell-free Hb in some patients has led to side effects such as renal toxicity, hypertension, and cardiovascular complications (18). Hb tetramers dissociate into dimers and monomers; these smaller structures can be easily filtered through the kidneys via the glomeruli, potentially resulting in renal toxicity. Additionally, cell free Hb binds and scavenges nitric oxide (NO), a vasodilator, from the circulation; this can adversely affect cardiac function. Furthermore, when circulating NO levels decrease, platelet aggregation which is normally actively inhibited by NO may increase (19).

Hb cannot be used directly as an oxygen carrier because extraction from erythrocytes causes structural degradation and poses toxicity risks. To prevent this, Hb sourced from expired human and animal blood (particularly bovine) or produced via recombinant methods is used as raw material. These hemoglobins are chemically modified and converted into HBOCs using microencapsulation techniques. The extraction of Hb from erythrocytes involves processes such as cell lysis, purification through sterile filtration, chromatography, and sterilization at low temperatures. Initially, Hb binds oxygen slowly; however, binding affinity increases with the second, third, and fourth oxygen molecules, resulting in a positively sigmoidal oxygen dissociation curve. HBOCs are classified into two main groups: chemically modified HBOCs and encapsulated HBOCs within protective capsules (20).

Chemically Modified Hemoglobin Based Oxygen Carriers

They are developed by stabilizing hemoglobin molecules through intermolecular and intramolecular cross-linking. These cross-linking processes, especially through agents like glutaraldehyde, increase the molecular size, thereby prolonging the half-life and improving pharmacokinetic properties. For example, polymerized HBOCs such as bovine-derived Hemopure and Polyheme are used as oxygen carriers in hemorrhagic shock and surgical applications. Products developed with PEGylation modifications also hold significant importance; agents like Hemospan (MP4OX) have shown efficacy in sickle cell anemia, organ protection, and oxygen therapy for COVID-19 (21). PEGylated and carboxyhemoglobin-containing products such as Sanguinate are preferred in sickle cell anemia crises due to their anti-inflammatory and anti-apoptotic effects. Moreover, naturally derived HBOCs such as HemO2Life/Hemarina-M101 obtained from the worm species *Arenicola marina* exhibit significantly higher oxygen-binding capacity and inherent antioxidant properties compared to vertebrate hemoglobins. In general, chemical modifications enhance the molecular stability of

hemoglobin-based oxygen carriers, prolong their half-life, and expand their clinical applications

Chemically modified HBOCs have been associated with several clinically significant adverse effects, particularly renal toxicity. Free hemoglobin released from these carriers can induce oxidative stress and scavenge nitric oxide, leading to vasoconstriction and tubular injury in the kidneys. Therefore, thorough pre-treatment evaluation of renal function, including measurements of serum creatinine, blood urea nitrogen (BUN), and glomerular filtration rate (GFR), is critical to identify patients at higher risk. During therapy, maintaining adequate hydration and considering antioxidant supplementation can help mitigate renal damage. Moreover, careful individualization of HBOC dosing is necessary to minimize toxicity, avoiding unnecessarily high doses. Continuous monitoring of renal function throughout treatment is essential for the early detection of impairment, and in cases of significant renal dysfunction, HBOC administration should be promptly discontinued, followed by appropriate supportive care (22).

Clinical Trials

Many HBOCs have passed safety tests in clinical trials; however, only some have reached phase III, with a few receiving approval while others were discontinued due to failure. For example, HemAssist advanced to phase III but was terminated in 1999 due to increased mortality rates. Optro showed positive results in phase II for elective surgery but was discontinued in 2014 due to concerns about NO scavenging. Hemolink halted clinical trials in 2004 after phase II. Hemopure underwent various safety studies from phase I to phase III and was approved as an oxygen carrier in South Africa in 2001 and in Russia in 2012. It is also used in veterinary medicine under the name Oxyglobin. In the USA, it has been granted emergency use authorization by the FDA but has not received full approval. Nevertheless, it can be used in severe anemia cases within clinical trials and expanded access programs. Although Polyheme completed phase III, its licensing application was denied in 2009, and its use was discontinued. PHP, or Hemoximer, failed in phase III due to increased mortality risk and was canceled in 2014. Hemotech contains purified bovine Hb cross-linked with ATP, adenosine, and glutathione and has been used in patients with acute blood loss, completing phase I trials. Hemospan, a PEGylated modified hemoglobin, passed phases IIa and IIb in 2012 but was discontinued in 2013 after phase IIc was canceled. Sanguinate completed phase II in 2017 (23).

Encapsulated Hemoglobin Based Oxygen Carrier Systems

Encapsulated Hb products are designed to closely mimic RBCs by preventing vasoconstriction caused by NO scavenging. These systems were first developed by Chang and colleagues in the 1950s and 1960s. To imitate the natural erythrocyte structure, encapsulation processes involving various effector molecules or reducing enzymes have been implemented. Compared to acellular hemoglobin products, encapsulated Hb offers advantages such as reducing hypertension, extending half-life, and improving shelf life. Typically, polymeric membranes composed of collodion (cellulose nitrate), PEG-polylactate polymers, phospholipids, and cholesterol are used for encapsulation. Additionally, these artificial Hb cells can preserve erythrocyte-specific enzymatic activities, including catalase (CAT), carbonic anhydrase, and 2,3-DPG (24).

Preclinical Evaluation of the Efficacy and Safety of Oxygen Transport Using Hemoglobin-Based Oxygen Carriers

Various studies have been conducted using cell lines (in vitro) and animal models (in vivo) to evaluate the efficacy and safety of HBOC products. HBOCs stand out as therapeutic agents aimed at reducing NO scavenging and vasoconstriction caused by oxidative tissue damage, due to their oxygen transport and plasma-expanding properties. Hb microparticles (HbMP-700) have reduced the risk of intravascular vasoconstriction by preventing early and excessive oxygen release thanks to their high oxygen affinity. These effects were confirmed in vitro, and no toxicity or clinical symptoms were observed in in vivo mouse models (25). Polyethylene glycol-conjugated bovine-derived carboxyhemoglobin, SANGUINATE®, improved cardiac function and mitral valve competence after myocardial infarction in rats. Additionally, OxyVita C improved systemic blood pressure; this effect prevented pial arteriole constriction and cerebral vasoconstriction in rat brains. Liposome-encapsulated hemoglobin (LEH) contributes to the prevention of hemorrhagic shock and the maintenance of vital organ perfusion by alleviating oxygen deficiency. Studies conducted on cynomolgus monkeys demonstrated that LEH, exhibiting high oxygen affinity, helped reduce histological damage in the cerebral cortex and preserved the brain's oxygen metabolism rate (26). Overall, HBOCs stand out with their high oxygen affinity, improved circulatory response, and low oxidation profile; some products also support the regulation of systemic blood pressure and the prevention of vasoconstriction.

Perfluorocarbon Based Oxygen Carriers

Perfluorocarbon-based oxygen carriers (PFOCs) are hydrocarbons in which hydrogen atoms are completely replaced by fluorine atoms, sometimes along with additional halogens. They are clear, chemically inert, non-toxic liquids with low boiling points. Additionally, they are characterized by their insolubility in water and alcohol. In clinical applications, emulsifying

agents are required to increase the solubility of Perfluorocarbons (PFCs); this feature provides a particular advantage for individuals who reject human or animal-derived blood or proteins (27). PFCs have a high gas-dissolving capacity. The solubility of gases in PFC liquids is proportional to the decrease in molecular volume, following the order CO_2> O_2> CO> N_2. Due to their small particle size, PFC emulsions can easily circulate through occluded blood vessels in certain diseases and contribute to increased oxygenation when erythrocytes are insufficient in oxygen transport. The oxygen solubility of PFCs is approximately 20 times that of water and about twice that of plasma, around 40-50%. Additionally, carbon dioxide solubility ranges between 130–160 mL, which is about two to three times that of water. Linear-structured PFCs, such as perfluorooctyl bromide, exhibit higher oxygen solubility compared to cyclic molecules like perfluorodecalin. In general, oxygen solubility in PFCs is inversely proportional to molecular weight and directly proportional to the number of fluorine atoms (28). Currently, PFOCs are classified into five subclasses based on the primary PFC type used in emulsion products. These include: perfluorodecalin-based PFOCs such as Fluosol-DA and Perftoran; albumin-derived PFC-based AOCs; perfluorooctyl bromide (PFOB)-based PFOCs like Oxygent; perfluorodichloro-octane-based PFOCs such as Oxyfluor; tert-butyl perfluorocyclohexane-based PFOCs like Oxycyte; and dodecafluoropentane (DDFPe)-based PFOCs.

Fluosol-DA exhibited a significantly low oxygen carrying capacity (%7,2 v/v) compared to human erythrocytes and was therefore insufficient in conditions such as severe anemia. Additionally, due to its poor intravascular stability and difficulties in administration, its development was discontinued. Although Oxyfluor™ and Oxygent™ increased oxygen carrying capacity thanks to their high lipophilicity, they were withdrawn from clinical trials due to serious side effects and stroke risk, respectively. In contrast, Perftoran™, approved in Russia and Mexico, has been safely administered to over 30,000 patients, and studies for FDA approval are ongoing (29). Research continues on safer and more efficient emulsification methods in the development of PFC-based oxygen carriers. Wrobeln and colleagues encapsulated perfluorodecalin using human serum albumin (HSA) to obtain a biocompatible emulsion. The erythrocyte membrane, due to the presence of various glycans and proteins on its surface, can circulate for approximately 120 days; at the same time, its biocompatible, non-immunogenic, and biodegradable properties make it an ideal natural material for long-circulating drug carriers.

PRECLINICAL EVALUATION OF PFOCs

PFC emulsions, with nanoparticles approximately 0.2 μm in size, can reach microcapillary areas inaccessible to erythrocytes (approximately 7 μm), providing an advantage in oxygen transport. Due to these properties, efficacy and safety evaluations have been conducted both in in vitro and in vivo. The newly developed albumin-bound synthetic oxygen carrier (A-AOC) has demonstrated effective oxygen transport in various animal models thanks to its longer circulation half-life and biocompatibility. It was well tolerated in intravenous applications and showed higher oxygen-carrying capacity in rats compared to Perftoran®. Additionally, it maintained stability in body temperature and pH, increased arterial partial oxygen pressure, and decreased CO_2 pressure, resulting in a more efficient oxygenation profile. A-AOC was able to limit hypoxic tissue damage; however, treated rats exhibited increased arterial blood pressure and decreased blood glucose levels. Additionally, this agent has been shown to reduce lesions and mortality associated with decompression sickness (DCS) (30). In recent animal model studies, the development process continues with the aim of achieving safer and more effective use of A-AOC.

Advantages of Perfluorocarbon Based Oxygen Carriers

PFOCs stand out as effective materials capable of physically dissolving various gases, including respiratory gases such as O_2, CO, CO_2, and NO, due to their high gas solubility and physicochemical properties. When present in circulation alongside erythrocytes, PFCs contribute to the preservation of hemoglobin-bound oxygen until reaching hypoxic tissues. Compared to albumin-based oxygen carriers, the oxygen transport capacity of PFCs remains unaffected by changes in pH, temperature, pharmacological, and environmental factors. Additionally, they do not undergo metabolic transformation and exhibit chemical resistance to heat. These characteristics make PFOCs safer alternatives to HBOCs, which are associated with immune reactions, hypertension, and short half-life. Notably, some PEG2K-conjugated liposome-encapsulated hemoglobin (LEH) formulations have demonstrated immunological neutrality and good tolerance upon repeated dosing. Furthermore, since PFCs do not react directly with oxygen, they enhance plasma-level oxygen transport, thereby optimizing oxygenation. One of the most important advantages of PFCs is their ability to remain stable at room temperature for one year or longer, while maintaining oxygen delivery by penetrating microvessels and occluded arteries (31). These carriers exhibit high storage stability due to the chemical stability conferred by carbon-fluorine bonds. Finally, albumin-derivative PFC nanoparticles have demonstrated high oxygen-carrying capacity and reduced hypoxic injury in rat models. Despite their advantages, perfluorocarbon-based oxygen carriers (PFOCs) have been associated with certain adverse effects, particularly due to their tendency to accumulate

in reticuloendothelial system (RES) organs such as the liver and spleen. This accumulation may lead to dose-dependent toxicity and impaired organ function. To minimize these adverse effects, PFOC formulations should be carefully optimized through strategies like reducing particle size and modifying surface characteristics. Surface engineering techniques, including polyethylene glycol (PEG) conjugation, have proven effective in reducing opsonization, thereby decreasing RES uptake and subsequent phagocytic clearance. During treatment, regular monitoring of hepatic enzymes and other relevant biochemical parameters is critical for early detection of organ toxicity. Moreover, accurate dosing and avoidance of prolonged or excessive administration are essential to prevent toxic reactions. Recent advancements in nanotechnology have enabled the development of next-generation PFOC platforms designed for targeted oxygen delivery and controlled release, enhancing biocompatibility and safety. In cases of toxicity, PFOC administration should be immediately discontinued and appropriate supportive care initiated (32).

Stem Cell Based Oxygen Carriers

Stem cells are undifferentiated cells with the ability to transform into various cell types and tissues, possessing high regenerative capacity. Embryonic stem cells, which constitute the foundation of a living organism, first emerge during the embryonic stage and contribute to the formation of tissues and organs (33). Recent advances in stem cell technologies have enabled the production of erythrocyte-like cells from various culture systems, leading to the development of methods that mimic the natural erythropoiesis process in humans.

Stem Cell Based Oxygen Carriers (SCOCs) are produced through induced hematopoietic stem cells derived from different sources and exhibit physicochemical properties and biological functions similar to those of natural erythrocytes. They hold promise for the long-term oxygen delivery needs of patients with chronic anemia, advanced kidney or liver diseases, acquired or congenital erythropoiesis defects, and chronic blood loss. Moreover, they represent suitable candidates for patients with rare blood groups or those who are positive for autoantibodies and require repeated blood transfusions. Erythropoiesis is the process by which multipotent hematopoietic stem/progenitor cells differentiate into mature erythrocytes. In humans, natural erythropoiesis typically begins in the spleen and liver during early fetal development and continues in the bone marrow thereafter. In ex vivo erythrocyte production, human hematopoietic stem/progenitor cells (hSPCs) are subjected to erythroid differentiation through three sequential phases: commitment, proliferation, and maturation. During this process, cytokines such as stem cell factor (SCF) and erythropoietin (EPO) are commonly added to the culture medium to support erythroid differentiation and maturation. In some cases, the cells are

co-cultured with feeder cells derived from murine or human sources. In general, erythrocyte-like cells can be synthesized in vitro through the expansion and differentiation of human embryonic stem cells (hESCs), native hSPCs, or induced pluripotent stem cells (iPSCs) (34).

O_2 Micro and Nanobubbles

Among oxygen carrier systems O_2 Micro and Nanobubbles (OMNBs) represent an innovative approach that enables the direct delivery of oxygen to tissues under hypoxic conditions. These systems facilitate the transport of oxygen to deoxygenated erythrocytes, hypoxic tissue regions, and vascular beds. OMNBs are small, spherical structures with a gas-filled core surrounded by a shell composed of phospholipids, proteins, or polymers. While microbubbles are typically used in non-invasive imaging techniques (e.g., photoacoustic imaging), nanobubbles are more commonly employed for therapeutic and diagnostic purposes. One of the most significant applications of OMNBs is to provide oxygen support in response to hypoxia, which is frequently observed in solid tumors.

Hypoxic cells exhibit greater resistance to chemotherapy and radiotherapy. OMNBs can enhance the oxygen enhancement ratio (OER), thereby improving tumor responsiveness to treatment. Due to their small size (0.1–20 μm), they can penetrate both large and small blood vessels (35). OMNBs are utilized in various applications, including oxygen delivery, drug transport, gene therapy, and molecular imaging. The lipid, protein, or polymeric materials used in their outer shell facilitate controlled oxygen release and enhance transport safety. Lipid-shelled microbubbles are the most commonly used structures. With a diameter of approximately 2–4 μm and a thin (3 nm) shell composed of phospholipids, these bubbles are both flexible and stable. This structure facilitates the efficient diffusion of oxygen into tissues. Protein-shelled micro/nanobubbles (MNBs) are produced through processes such as denaturation and emulsification. Due to their rigid structures, they exhibit prolonged circulation half-life. Albunex, an FDA-approved formulation, belongs to this category. In contrast, polymer-shelled bubbles are characterized by thicker shell structures (150–200 nm), which confer greater resistance under ultrasound exposure, although they exhibit lower echogenicity. These systems can be fabricated using various techniques including sonication, microfluidic systems, agitation, inkjet printing, and laser ablation (36). Figure 2 provides a schematic overview of artificial oxygen carriers, including HBOCs, PFOCs, SCOCs, and OMNBs (37).

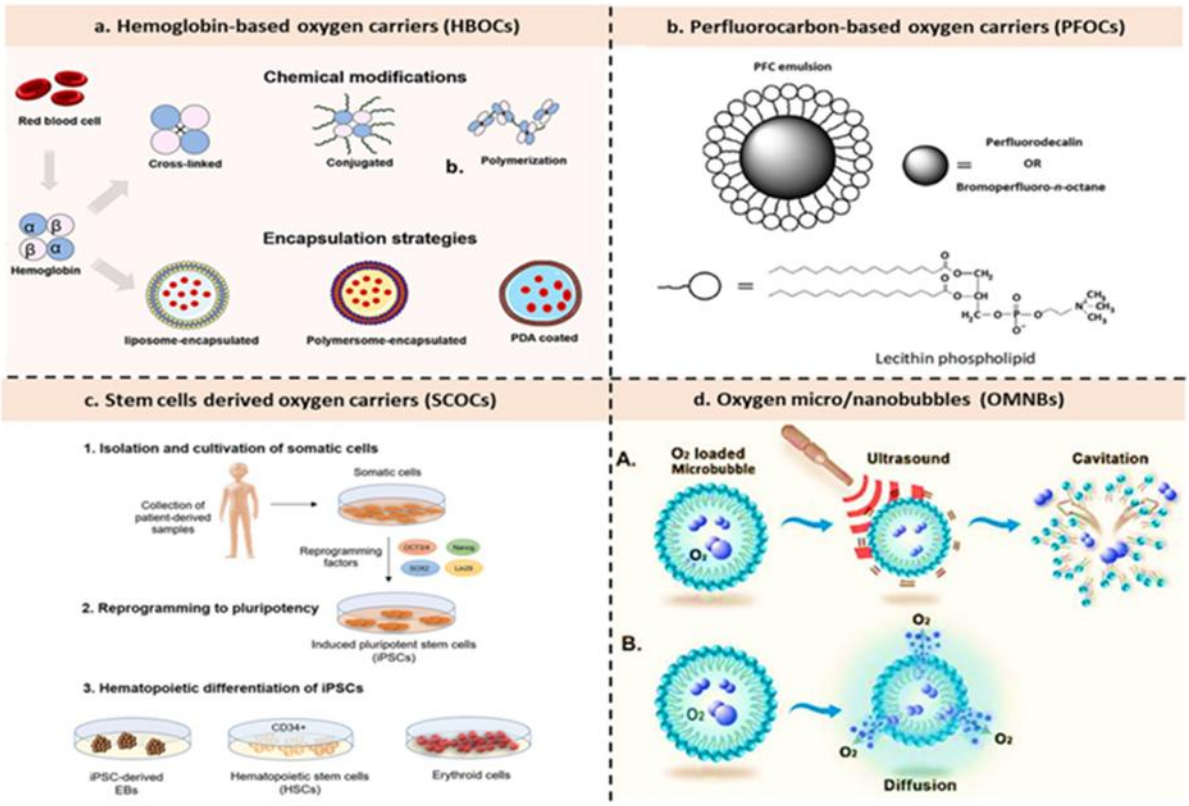

Figure 2. Artificial oxygen carriers: a) HBOC: Hemoglobin modification and encapsulation, b) PFOC: Perfluorocarbon emulsion and encapsulation, c) SCOC: iPSC reprogramming and erythroid differentiation, d) OMNB: Ultrasonic disruption and oxygen release (37).

The table below provides a comparative overview of the four main artificial oxygen carrier systems (HBOC, PFOC, SCOC, and OMNB) discussed in our review. The advantages and limitations of each system in clinical applications are clearly and concisely summarized (Table 1).

Table 1. Advantages, Limitations, and Clinical Applications of Artificial Oxygen Carriers (AOCs)

Artificial Oxygen Carrier System	Advantages	Limitations	Clinical Application Areas
HBOCs (Hemoglobin-Based Oxygen Carriers)	- High oxygen-carrying capacity - Controlled oxygen release - Plasma volume expansion	- Risk of renal toxicity - Vasoconstriction due to free Hb scavenging nitric oxide (NO) - Inflammation and cardiovascular complications	- Trauma - Severe anemia - Surgery - Patients refusing blood transfusion (e.g., Jehovah's Witnesses) - Tumor hypoxia
PFOCs (Perfluorocarbon-Based Oxygen Carriers)	- High solubility for respiratory gases (O_2, CO_2) - Ability to reach microcirculation due to small particle size - Stable storage at room temperature	- Accumulation in reticuloendothelial organs (liver, spleen) - Short circulation half-life - Challenges in stable emulsion formulation	- Respiratory distress (e.g., COVID-19) - Tumor hypoxia - Acute blood loss - Surgical interventions

SCOCs (Stem Cell-Derived Oxygen Carriers)	- Mimic natural erythropoiesis - Potential for long-term oxygen delivery - Emerging scalability for industrial production	- Technically complex and costly production - Still in early experimental phase	- Rare blood types - Autoimmune hemolytic anemia - Chronic transfusion-dependent patients
OMNBs (Oxygen Micro/Nanobubbles)	- Effective oxygen delivery to hypoxic tumor microenvironments - Controlled release capability - Theranostic potential (therapy + imaging)	- Stability issues - Risk of ultrasound-triggered disruption - Limited clinical data (mostly preclinical)	-Tumor hypoxia - Drug/gene delivery - Photoacoustic imaging

Clinical Applications and Therapeutic Uses of Artificial Oxygen Carriers

Artificial oxygen carriers (AOCs) have become an important alternative, especially in cases where blood transfusion is not feasible or appropriate. In clinical practice, AOCs are commonly used in emergency trauma settings to rapidly provide oxygen and prevent hypoxia caused by blood loss. They are also employed during elective surgeries when blood donor availability is limited or when patients carry a high risk of allergic reactions. Additionally, AOCs serve as life-saving options for special patient groups such as Jehovah's Witnesses who refuse blood transfusions and individuals with immunological complications like autoimmune hemolytic anemia. In cancer treatment, AOCs are being explored for their potential to reduce tumor hypoxia and thereby improve the efficacy of chemotherapy and radiotherapy.

Despite these promising applications, the widespread clinical adoption of AOCs faces several challenges. High production costs and the need for cold chain storage limit their accessibility, particularly in low- and middle-income countries. Moreover, their short circulation half-life and the requirement to manage possible side effects contribute to increased overall costs. Consequently, the development of more stable, longer-lasting, and cost-effective formulations remains a critical need. Advances in nanotechnology and the design of biocompatible carriers offer promising avenues to address these issues. Although the clinical applications of artificial oxygen carriers are broad, their therapeutic benefits in various diseases and clinical conditions are particularly noteworthy. Below, the applications of these carriers in some important disease groups are detailed.

Anemia and Sickle Cell Anemia;

Anemia is characterized by a reduction in oxygen transport due to low hemoglobin levels or erythrocyte deficiency. In sickle cell anemia, this condition becomes more complex with recurrent painful crises and multi-organ damage. The primary goal of AOCs is to mitigate hypoxia and thereby prevent these complications. HBOCs are particularly preferred under

"compassionate use" in specific cases such as Jehovah's Witnesses who refuse blood transfusions or autoimmune hemolytic anemia. HBOC-201 has been found effective in providing temporary oxygen support in sickle cell anemia patients with multi-organ failure. Additionally, HBOC-201 provided an effective treatment alternative in a rare case of a leukemia patient with Vel-negative blood type, where a suitable donor could not be found. Similarly, in a Jehovah's Witness patient diagnosed with acute lymphoblastic leukemia, infusions of HBOC-201 maintained stable low hemoglobin levels and prevented ischemic complications. In another study involving 10 patients with severe anemia, all responded positively to HBOC-201 treatment and were discharged without complications. These findings indicate that HBOC-201 may serve as a safe and effective alternative for critically ill patients.

Cancer and Tumor Hypoxia;

Tumors exhibit rapid and irregular growth, leading to increased oxygen consumption and the formation of a hypoxic tumor microenvironment (TME). The reduction of oxygen levels within tumors significantly limits the efficacy of therapies such as chemotherapy, radiotherapy, photodynamic therapy, and sonodynamic therapy. AOCs alleviate this hypoxia by delivering oxygen to tumor tissues, thereby enhancing treatment response. While the oxygen concentration in normal tissues ranges between 30–50 mmHg, it can fall below 5 mmHg in hypoxic tumors. Therefore, improving the hypoxic microenvironment is critical for therapeutic success. HBOCs are also being investigated to enhance the effectiveness of radiotherapy. Xu and colleagues demonstrated in mouse models bearing tumors derived from HeLa and Miapaca-2 cells that fractionated radiotherapy combined with HBOCs reduced hypoxia and suppressed HIF-1α expression (38).

Application of Synthetic Oxygen Delivery Systems in COVID-19;

COVID-19 is characterized by respiratory distress and hypoxia resulting from lung injury. In severe cases, HBOCs are utilized to stabilize oxygen levels and support tissue oxygenation. The HBOC known as HEMO2Life® possesses a high oxygen-carrying capacity and antioxidant properties, which may enhance oxygenation and reduce the risk of cytokine storm in COVID-19 patients. Its size, approximately 250 times smaller than human erythrocytes, allows for effective navigation through the microcirculation. Preclinical studies have demonstrated its capacity to increase oxygenation, particularly in brain tissue. During the pandemic, oxygen carriers such as HEMO2Life® have shown promise in reducing the need for intubation, shortening the duration of oxygen supplementation, and alleviating the burden on intensive care units. However, these agents provide temporary support; they do not treat the viral infection nor repair long-term pulmonary damage. Therefore, the use of AOCs as

adjunctive therapy in COVID-19 management is recommended, with further clinical investigations warranted in this area.

β-Thalassemia

A genetic blood disorder, β-thalassemia causes anemia and impaired oxygen transport due to insufficient β-globin chain production. Excess free α-globin chains induce damage and hemolysis in red blood cells. Synthetic oxygen carriers (SCOCs) provide adjunctive support in oxygen therapy and may reduce the need for blood transfusions. Allogeneic hematopoietic stem cell transplantation (HSCT), particularly when performed with HLA-matched sibling donors, increases the likelihood of complication-free survival in thalassemia patients. However, transplants from unrelated donors carry increased risks of morbidity and mortality (39).

Future Perspectives in the Field of Oxygen Carriers

Despite numerous studies aimed at addressing oxygen transport issues using HBOCs and PFOCs, their clinical applications remain limited due to toxicity concerns. Genetic modifications and chemical alterations significantly influence the efficacy, efficiency, and side effect profiles of HBOCs. Polymerized and cross-linked HBOCs have effectively reduced the adverse effects associated with free Hb. Further investigation into various polymerization techniques holds potential to enhance the oxygen-carrying capacity of polymeric Hb. However, since the site of polymerization critically affects the Hbs oxygen-binding capacity, careful selection of this site is of paramount importance. Ensuring that polymerization does not compromise the oxygen-binding function of the Hb complex remains a significant challenge. As an alternative approach, recombinant hemoglobin production has gained attention. Recombinant Hb-based HBOCs offer several advantages, including serving as a natural transfusion source, having a long shelf life, reduced risk of disease transmission, standardized product quality, and global acceptance. However, the foremost challenge ahead is selecting appropriate mutations to reduce oxidation, hemin loss, and NO binding, while simultaneously preserving the fundamental functions of hemoglobin. Currently, recombinant Hb provides an unlimited source of Hb. Human Hb function is influenced by compounds such as 2,3-diphosphoglycerate (2,3-DPG), which binds more strongly to deoxygenated Hb than to oxygenated Hb. Nevertheless, effective management of methemoglobin (metHb) levels is essential to maintain optimal oxygen-carrying capacity. Additionally, bovine hemoglobin is known to exhibit superior stability at higher temperatures during isolation and processing.

The nanoparticle size being smaller than 100 nm is critically important to prevent uptake by the liver's reticuloendothelial system (RES). The use of PFCs with high vapor pressure facilitates rapid clearance in vitro, while the stability of the emulsion is maintained by

biocompatible stabilizers. In the development of PFOCs, short in vivo half-life, appropriate storage conditions, and rapid in vivo elimination are fundamental requirements. From this perspective, bovine Hb offers several advantages over human Hb in terms of accessibility, stability, and oxygen-carrying capacity. The development of new PFC formulations is essential to mimic the ideal characteristics of oxygen carriers. For clinical approval, improvements in emulsifiers and optimization of formulation conditions are necessary. Current PFOCs face significant challenges including large particle size, long residence time in the body, low storage stability, wide tissue distribution, and high PFC content. Large particle size leads to retention in the liver by the RES; prolonged residence can cause adverse effects such as complement activation-related pseudoallergy (CARPA). Although particles smaller than 1000 nm can pass through all capillaries after injection, they are rapidly captured by RES macrophages. After intravenous injection, PFOCs are stored in RES phagocytes, facilitating the clearance of perfluorocarbon particles from the blood. However, large PFC particle sizes increase RES uptake, raising the risk of hepatic accumulation and intrahepatic toxicity (40).

CONCLUSION

In recent years, the decline in blood donations and transfusions, particularly the shortages experienced during pandemic periods such as COVID-19, has posed a significant challenge to healthcare systems. Insufficient blood supplies adversely affect not only routine patient care but also emergency interventions and major surgical procedures. At this point, AOCs have emerged as a promising alternative to allogeneic blood transfusions. Developed in various forms such as HBOCs and PFOCs, these agents provide life saving support by meeting patients' oxygen demands. However, one of the major barriers to the clinical application of AOCs has been their associated toxicity and side effects. HBOCs have been linked to complications such as renal toxicity due to free hemoglobin, inflammation, and elevated intravascular pressure. In contrast, PFOCs face challenges related to particle size, prolonged retention within the body, and organ accumulation. These adverse effects have limited the widespread clinical adoption of AOCs and have resulted in the discontinuation of several development projects. Nevertheless, advances in science and technology offer considerable promise in overcoming these obstacles. Stabilization of hemoglobin through polymerization and chemical modification has contributed to reducing toxicity. Recombinant hemoglobin production eliminates the risk of infection and enables targeted alterations in protein structure. Additionally, reducing nanoparticle size, improving biocompatibility, and enhancing molecular stability have facilitated the safer and more effective systemic circulation of AOCs. Looking ahead, the broader adoption of these technologies in clinical settings will require

detailed toxicity profiling, formulation optimization, and cost effective manufacturing processes. These developments may play a critical role in addressing blood shortages, managing emergency health crises such as pandemics, and treating trauma related cases. In conclusion, although challenges persist in the field of artificial oxygen carriers, ongoing research and technological innovation are driving significant progress toward their safe, effective, and widespread clinical use. It is anticipated that future advancements will transform patient care and significantly reduce dependency on traditional blood transfusions.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Ethics Approval and Consent

Ethical approval was not required since it was a review article.

Conflict of Interest

No conflict of interest was declared by the authors.

Author Contributions

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