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Artificial Blood Substitutes: First Steps on the Long Route to Clinical Utility

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Abstract

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The 21st century is challenging for human beings. Increased population growth, population aging, generation of new infectious agents, and natural disasters are some threatening factors for the current state of blood transfusion. However, it seems that science and technology not only could overcome these challenges but also would turn many human dreams to reality in this regard. Scientists believe that one of the future evolutionary innovations could be artificial blood substitutes that might pave the way to a new era in transfusion medicine. In this review, recent status and progresses in artificial blood substitutes, focusing on red blood cells substitutes, are summarized. In addition, steps taken toward the development of artificial blood technology and some of their promises and hurdles will be highlighted. However, it must be noted that artificial blood is still at the preliminary stages of development, and to fulfill this dream, ie, to routinely transfuse artificial blood into human vessels, we still have to strengthen our knowledge and be patient.

Keywords: artificial blood, oxygen carrier, hemoglobin, red blood cells

Introduction

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Being alive is impossible without blood, the complex liquid containing millions of chemicals and cells. When William Harvey for the first time described blood circulation, scientists started to think about a proper replacement such as an artificial blood.[1] However, none of the products developed since then meets all blood functions. In fact, production of a liquid that mimics all blood functions is still a dream, although it may come true in future. Some kind of blood substitutes developed over time, including simple liquids such as, urine, beer, and milk and plant resins,[1] with least similarity to the blood constituents, and cells originated from stem cells with maximum similarity to blood.[2] Artificial blood was highlighted after emergence of HIV in 1980 due to the risk of its transmission by blood transfusion,[3] which imposes higher costs due to the necessary detection tests. In addition, low blood supplies especially in developing countries, lower number of donors due to the aging of population and consequently increased demand for blood products, short storage period, and urgent needs for blood

supplies during wartime and natural disasters are other important reasons that make the development of a suitable blood substitute indispensable.[4] Among the mentioned factors, the safety issue of blood products is the most important one, especially due to emerging of novel infectious agents such as Ebola and H1N1 (a kind of flu virus also called swine flu due to its similarity to those found in pigs) viruses, whose screening also imposes further costs.[5] Today, there is a considerable inadequacy of blood supplies in developing countries, which are inhabited by 80% of the world's population but collect less than 32% of total worlds' blood supplies with low safety standards.[6] Therefore, artificial blood would be of great value for developing countries. Understanding the blood behavior at the microcirculation level where blood and tissues come into contact is a key step in the development and application of blood substitutes. In other words, a blood substitute must be designed in a way that it behaves similar to natural blood in microcirculation.[7] Development of an agent properly mimicking the oxygen-carrying capability of blood among its various functions has been of great interest, and many products have been established based on this property. Here, we mainly discuss and review the blood substitutes that mimic the oxygen-carrying capability of blood. In addition, potential challenges and obstacles against routine application of artificial blood in human will be discussed.

Red Blood Cell Substitutes

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Red blood cells (RBCs) isolated from donated blood are an important component widely used to save patients' lives via oxygen-carrying capacity owing to hemoglobin (Hb).[8] However, there are complications associated with transfusion of RBCs to patients. These complications can be divided into noninfectious and infectious and are the most important concerns for the application of RBCs.[9] Furthermore, crossmatch and blood group typing are needed before transfusion, which is challenging in case of emergencies and when rare blood group types are needed. Hence, it is essential to develop efficient RBC substitutes capable of active oxygen and carbon dioxide transfer.[10,11] The most important features of an RBC substitute include its ability to transport oxygen and carbon dioxide, low cost, no need for crossmatching and blood typing, lack of contamination and infectious agents, easy access, trouble-free storage conditions, extended half-life in circulation, full excretion from body and being not accumulated in various tissues, lack of toxicity, nonimmunogenicity, nonantigenicity, and noncarcinogenicity.[10]

RBC substitutes or synthetic oxygen transporters studied so far are of mainly two types: perfluorocarbon and Hb-based substitutes.

Perfluorochemical-based RBC substitutes

Perfluorochemicals (PFCs) are colorless, inert, and apparently nontoxic liquids with low boiling point temperatures and are insoluble in water and alcohol.[12] The capacity of PFCs in carrying oxygen was demonstrated for the first time by Clark in 1966. PFCs include straight or cyclic hydrocarbon chains[13] with a general chemical formula of C_nF_{2n+2},[14] and the straight form is a better carrier for oxygen than the cyclic one.[10] The level of oxygen dissolved in PFCs has a direct linear relationship with oxygen pressure, and therefore, high oxygen pressure is necessary for maximum oxygen-carrying capacity.[14] Since hydrogen atoms are replaced by fluorine atoms in PFCs, these compounds are not metabolized due to the strong bond between carbon and fluorine atoms.[13] PFCs are insoluble in aqueous phase, and in case of their clinical application, they are solubilized using an emulsifying agent.[15] One main advantage of PFCs is for people refusing blood or proteins derived from humans or animals.[16] Oxygen is dissolved in PFCs at a concentration of about 40%–50%, which is 20 times higher than the capacity of water and 2 times higher than plasma. In addition, PFCs dissolve 130–160 mL carbon dioxide, which is two to three times higher than the corresponding water capacity.[17] Moreover, there are PFCs with oxygen-dissolving capacities higher than erythrocytes, including FC-80 (a liquid form of PFCs), which

is 10% stronger in this regard.[12] The fundamental difference in O_2 transfer by Hb and PFC is that the former binds O_2, while the latter dissolves it.[18] PFCs are heat resistant and can withstand 300°C and higher temperatures without any change, which makes them easily amenable to heat sterilization.[5] Their small sizes enable them to easily pass through the vessels occluded in some diseases, where RBCs cannot pass; hence, their application helps improving the oxygenation rate. An in vitro study showed that use of PFCs as artificial blood is considerably advantageous in occluded coronary artery to maintain myocardial function.[19] Moreover, Chen et al showed the usefulness of PFCs as artificial blood substitute for oxygen carrying and expanding the plasma volume during surgeries done for the treatment of traumatic and hemorrhagic shocks even in war casualties.[17]

Fluosol-DA was the first accepted PFC-based RBC substitute, which is an emulsion of perfluorodecaline and perfluorotripropylamine.[20] Oxygen-carrying capacity of Fluosol-DA is only 7.2% at 37°C, which is lower than RBCs.[20] The use of this product entails complications such as pulmonary reactions supposedly due to complement activation by the emulsifying agent in Fluosol-DA and can be prevented by steroid injection.[21] Hence, the efficacy of Fluosol-DA was not demonstrated in a prospective clinical trial,[14] and its clinical application was stopped. Perflubron and perfluorodecalin have been extensively studied among PFCs.[22] OxyFlour™ (Hemagen, Inc.) and Oxygent™ (Aka perflubron, Alliance Pharmaceutical, Inc.) are among the second-generation PFC-based blood substitutes, which are rejected by clinical trials due to some side effects such as complications in determining the effective dose for OxyFlour™ administration and also increased risk of stroke following administration of Oxygent™.[4] However, except some changes in clotting factors, no specific interaction between blood components and administered PFCs has been reported.[9] Administration of PFC-based products can result in mild thrombocytopenia (10%–15% reduction in platelet count) as well as flu-like syndrome.[23]

Hb-based RBC substitutes

Human Hb derived from expired RBC bags is the main source of Hb for the production of Hb-based RBC substitutes. Other sources for this purpose include cord blood RBCs and animal (bovine) and recombinant Hb.

Transmission of infection is an important concern in the application of Hbs with human or animal origins. In particular, the assessment of transmission risk of infectious agents causing bovine spongiform encephalitis is very important.[24] However, animal-derived Hb has some advantages over human Hb, including unlimited access, higher resistance to degradation of heme, and the use of chloride ion instead of 2,3-DPG as an allosteric effector present in plasma.[25] Production of genetically modified Hb in microorganisms and plants can address several problems associated with these products. Furthermore, Hb can be genetically manipulated by this approach to overcome some side effects associated with these products, for example, the affinity of Hb for NO and O_2 can be changed.[10] High-level production of recombinant Hb using simple *Escherichia coli* expression system has been reported by Hoffman et al[26] in 1990.

The half-life of Hb is equal inside and outside the RBCs; however, outside the RBCs, the natural tetramer molecule of Hb rapidly converts to dimer and monomer Hb species, which cause severe complications such as kidney damage. Furthermore, outside the RBCs, Hb and 2,3-DPG are separated, resulting in higher affinity for oxygen.[4,16] On the other hand, it has been shown that Hb scavenges the existing NO molecules by its heme groups. NO is also involved in relaxation of smooth muscles of blood vessels, and this property is responsible for the vasoactivity of Hb-based products.[27] Overall, this type of Hb must be modified before its application as an oxygen carrier.[4,16] The Hb-based oxygen carriers (HBOCs) are divided into the following two groups: acellular and cellular HBOCs.

Acellular HBOCs Acellular HBOCs have been developed to increase Hb performance and decrease its side effects (Table 1). These are now in various phases of clinical trials and belong to three categories including cross-linked HBOC, polymerized HBOC, and conjugated HBOC (Fig. 1). However, among different modifications of Hb, only nanotechnology-based polyhemoglobin (PolyHb) and conjugated Hb are effective.28 However, due to their short blood half-lives and side effects, a majority of these products did not achieve required criteria in clinical trials.4

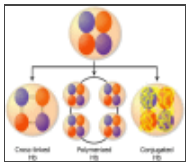

Figure 1

Three major classes of cellular HBOCs are polymerized, cross-linked, and conjugated Hbs. Spontaneous separation of Hb chains is prevented by various modifications. For example, in the cross-linked type, Hb chains are bound by intermolecular covalent bonds, ...

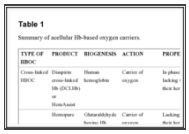

Table 1

Summary of acellular Hb-based oxygen carriers.

Summary of acellular Hb-based oxygen carriers.

TYPE OF HBOC	PRODUCT	BIOGENESIS	ACTION	PROPE
Cross-linked HBOC	Diaspirin cross-linked Hb (DCLHb) or HemAssist	Human hemoglobin	Carrier of oxygen	In plasm lacking their hal
	Hemopure	Glutaraldehyde bovine Hb	Carrier of oxygen	Lacking their hal

One of the main problems limiting the application of these products is their inability to convert Fe^{3+} to Fe^{2+}, which is an important function of RBCs. As a result, Met-Hb with low oxygen-carrying capacity was produced, showing that such complications can be avoided by attaching reducing agents to Hb surface in this product series.4

First, acellular HBOCs were cross-linked HBOC in which intramolecular covalent bonds were formed between globin chains in order to prevent their detachment. Diaspirin cross-linked Hb or HemAssist in which human Hb molecule has been intramolecularly cross-linked between alpha chains has been subjected to extensive investigation. This product has been used for various purposes, such as reduction of neurological damage in a mouse model,29 intraoperative anemia in sheep,30 cardiopulmonary resuscitation in pigs,31 and postcardiac bypass surgery to avoid allogeneic blood transfusion.32 However, the efficiency of diaspirin cross-linked Hb in the above-mentioned studies has been challenging.

Polymerized HBOCs are another acellular HBOC in which Hb molecules are cross-linked intermolecularly to increase the molecular size. Each PolyHb molecule is composed of 4–5 Hb molecules, and various kinds of PolyHb molecules have been produced so far. For example, Hemopure is a polymerized bovine Hb developed by OPK Biotech, which is currently in phase III clinical trial.5 PolyHeme is another product of this family consisted of pyridoxylated human Hb. However, its administration has been rejected by the United States Food and Drug Administration (US FDA) due to its adverse effects5 including hypertension, safety and toxicity issues attributed to vasoactivity, and oxidative stress.5

Oxyglobin is another product developed by OPK biotech in which bovine Hb has been used. This product contains 2% α2β2 Hb, which comparing to Hemopure that contains 31% α2β2 Hb, fewer hypertensive complications associated with free α2β2 is observed following its administration.5,33 In fact, it is the first product approved by the US FDA and the European commission for veterinary use.

In a study conducted by Bian and Chang,34 superoxide dismutase (SOD), catalase (CAT), and carbonic anhydrase (CA) were cross-linked to Hb and soluble nanobiotechnology-based product of PolyHb–SOD–CAT–CA RBC was created, whose efficiency was shown in a mouse model. This product could be stored at room temperature for 320 days and its lyophilized form can be sterilized.

PolyHb–fibrinogen is a product showing both oxygen-carrying and blood coagulation properties, which can be used in massive bleedings where the oxygen-carrying capacity is not sufficient by itself.[35]

Winslow and other researchers have shown that the modified Hbs with low P50 (partial pressure of oxygen required to achieve 50% saturation of Hb) and macromolecular diffusivities have an oxygen delivery profile similar to RBCs.[25] Therefore, they used oxidized mono-, di-, tri-, and polysaccharides as cross-linking agents. Their study showed that application of larger polysaccharides led to higher Hb cross-linking and polymerization, and *O*-methylglucopyranoside is the best polysaccharide to achieve this goal.[25]

Conjugated HBOCs are other acellular HBOCs in which inert polymers are attached to the surface of Hb molecules. Due to unique characteristics, low toxicity, and lack of immunogenicity or antigenicity in body, PEG can be the best polymer for conjugation.[36]

Hemospan is a PEG-conjugated Hb, which is under clinical trial as an oxygen carrier. This modification has been shown to increase the circulation half-life of the product.[37]

MP4 is another PEG–Hb conjugate designed as an oxygen carrier. This product did not cause vasoconstriction in animal models, and its efficacy to deliver oxygen to hypoxic tissues was demonstrated; MP4 is now under human clinical trials.[38]

In addition to PEG, other polymers have been used to conjugate Hb, including benzene tetracarboxylate dextran,[39] hydroxyethyl starch (called HRC 101),[40] and albumin.[41]

Recombinant Hb

The highest Hb activity is seen when it is within RBCs. Therefore, when Hb is going to be used in a cell-free format, it must be subjected to various modifications for increasing its half-life in circulation and preventing related complications in the body. In fact, recombinant production of Hb makes it easier to be modified specially through site-directed mutagenesis.[42]

In 1980s, human Hb was produced in large quantities in transgenic organisms. Since then, investigations for higher quality and more efficient production of recombinant Hb were started. It should be noted that most studies have focused on the production of Hb by *E. coli* expression system.[43] However, it has been also expressed in other bacterial systems and also by transgenic mouse and pig.[43] Nevertheless, the most important problems on the way of recombinant production of Hb are low expression yields and expensive production processes,[42] in addition to difficulties in obtaining desired purity.[43]

There have been several attempts to increase the production yield of recombinant Hb. For example, in a study, E. coli expression system was used to produce high quantities of human α-globin and bovine β-globin for therapeutic purposes. Then, the produced Hbs (Hb minotaur) were polymerized using intermolecular disulfide bonds and designated as Hb Polytaur. Animal studies revealed some advantages for this product over the other products of this type.[44]

It has been stated that mutating the Hb chains (βGly16 to Ala and αGly15 to Ala) might increase its production yield by preventing degradation and aggregation of the expressed chains. In addition, to increase the production yield of heme for the generation of functional Hb, the strategy of simultaneous heme transporter generation in bacterial membrane can be used, which might increase heme uptake by the bacterial host and subsequently increase the production yield.[45]

Targeted mutations of Hb might also cause several improvements in its functions outside RBCs. For example, targeted mutations could result in increased oxygen affinity, reduced capacity of Hb to

scavenge NO, reduced autooxidation, decreased rate of heme loss, preventing the detachment of subunits, and decreased irreversible denaturation of subunits.[43]

In a study, in order to improve Hb vasoactivity, and also to prevent its tetramer to dimer conversions and associated complications, rHbβG83C was generated using targeted mutation in which according to Hb Ta-Li, the Cys amino acid substituted for Gly in position 83 of β-chain (β83 Gly → Cys). This polymerizes using intertetramer disulfide bonds, and the molecular size of this product was shown to be stable in fresh frozen plasma.[46]

In another study, rHb (βN108Q) was produced by an E. coli expression system. In this Hb version, N → Q mutation occurred in position 108 of β-chain, which is the α1β1 subunit interface and also the central cavity of Hb. This mutation causes reduced oxygen affinity, increased cooperativity, and decreased autooxidation of the product.[47]

These are only a few cases of successful efforts for the production of recombinant HBOCs being able to be used as a favorable blood substitute. However, none of the products have received therapeutic licensure in the USA.[43]

Cellular HBOCs Other types of Hb-based products as artificial blood are cellular HBOCs, in which Hb is encapsulated in a cell-like structure. In this way, some products with highest similarity to RBCs were produced, which do not cause vasoactivation due to scavenging of NO. A summary of products corresponding to the cellular-based Hb is shown in Table 2.

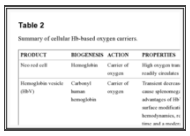

Table 2

Summary of cellular Hb-based oxygen carriers.

Encapsulation of Hb by a phospholipid layer (liposome-encapsulated Hb [LEH]) prolonged its half-life and shelf-life comparing to acellular products. LEH particles are much smaller than RBCs (1:30). This small size enables their entry into areas of body that are not accessible for RBCs. Hence, they can pass through clots and blockages causing more oxygenation during stroke.[3] However, this product has a short circulation half-life, which can be solved by a number of approaches for example by PEGylation of the particles' surface.[3]

In a study, liposome-encapsulated Hbs known as neo red cells were developed, and their efficiency as artificial RBCs was demonstrated in total cardiopulmonary bypass in an animal study, which showed even higher oxygen delivery capacity than RBC.[48]

Modifying the surface of these liposomes, including PEGylation, can result in products with higher half-life, stability, and solubility, as well as lower antigenicity and immunogenicity. Hb vesicle is a PEGylated product with increased serum half-life and decreased recognition by the immune system.[49,50]

In addition to the removal by reticuloendothelial system, another reason for low serum half-life of LEHs is shear-induced liposome destruction in bloodstream. Hence, to address this issue, an actin matrix was introduced into the aqueous core of the submicron liposomes to increase their mechanical strength. This strategy caused increased half-life of the product known as LEAcHb.[35]

Another series of products used as RBC imitators are biodegradable Hb-loaded polymeric nanoparticle (HbPNP). However, the most important problem with their application is rapid clearance by phagocytes either directly or through opsonins. To address this problem, several studies have been conducted. In one study, changing the surface charge by cationized cetyltrimethylammonium bromide increased half-

life of the PEGylated HbPNP. It was demonstrated that surface charge of products used as artificial blood substitutes has a profound effect on their circulation time, where anionized HbPNPs are rapidly cleared from bloodstream and cationized HbPNPs have high circulation half-life.[51]

Other cellular-based biocompatible Hb products with repetitively branched molecular structures are dendrimers. Poly(propylene), poly(amide amine), and polyether are among synthetic dendrimer products.[52] Fe(II) porphyrin-loaded dendrimers were prepared in some studies to simulate Hb, playing its role. The shape and size of these products are similar to Hb, and they are able to bind and release oxygen. However, their production is time consuming and costly. Therefore, a kind of dendrimer known as hyperbranched polymer has been developed, with reduced problems, which can be used as oxygen carrier by some adaptations.[53,54] Dendrimers are also used for encapsulation in drug delivery. Therefore, it has been suggested that dendrimers could be used as artificial oxygen carriers by encapsulating Hb.[55]

As an Hb-based RBC substitute, nanocapsules bearing a membrane made of ultrathin polyethylene glycol–polylactic acid (PEG–PLA), containing polymerized Hb and all RBC enzymes, were also studied.[56] The advantage of this method compared to the previous product in which only PLA membranes have been used was a significant increase in the half-life of the product due to reduction of phagocytosis shown in mice.[56,57] High circulation half-life and presence of enzymatic systems, including reductase, are the most important advantages of this system. The presence of enzyme system, especially the reductase, results in prevention of the accumulation of methemoglobin.[56]

A new class of materials for encapsulation of Hb is lipogel and NHPs (nanoscale hydrogel particles). In case of NHPs, nonbiodegradable but biocompatible hydrophilic polymers are used to enclose Hb within solid and spherical nanoscale hydrogel particles. Various complications caused by Hb release subsequent to the degradation of biodegradable polymers resulted in higher interests for application of nonbiodegradable polymers. Lipogels are NHPs enclosed within a lipid bilayer that is actually a hydrogel–liposome. They are made by photopolymerization of poly(*N*-isopropylacrylamide) and polyacrylamide monomers. This approach in turn enhances the mechanical strength of liposomal system. In fact, this product has the advantages of hydrogel and liposome together. These products have high Hb-loading capacity and low susceptibility that can be recognized by the reticuloendothelial system.[58]

An increased Hb-loading capacity even higher than that of lipogel and NHP is observed in polymersome-encapsulated Hb (PEH), which is a product made up of biodegradable, biocompatible, amphiphilic diblock copolymers and is used to enclose human or animal Hb. PEHs can be easily produced in large quantities, and their oxygen affinity is comparable to human erythrocytes. The mechanical resistance and possibility of changing the size and thickness is higher in PEH than in liposomes, and they can withstand temperatures up to 60°C. These products are stable in saline solution and blood for several months and at least five days, respectively. According to accumulating evidence, these products could herald the emergence of an ideal artificial oxygen.[59,60]

In single-protein nanocapsules, a protein molecule is encapsulated with a thin polymer layer and the resulting product has mechanical, heat, and pH resistance. This method has been used for Hb (Fig. 2).[61]

Figure 2

Structure of a typical cellular Hb-based oxygen carrier in a single-protein nanocapsule. In this product, Hb is covered by a thin layer of acrylamide and bisacrylamide monomers. This thin layer increases the thermal and pH stability of Hb and also protects **...**

An oxygen carrier has been developed by a research group, in which Hb is conjugated to biodegradable polymer micelles.17 The micelle is a PEG–PMPC–PLA triblock copolymer in which PEG acts as shell, PMPC (PLA-b-poly (2-methacryloyloxyethyl phosphorylcholine)) bearing propargyl groups as the middle layer and PLA as core layer. Following the production of these micelles in an aqueous solution, Hb groups bind micelles via propargyl groups in which Hb is surrounded and protected by PEG chains (Fig. 3).17

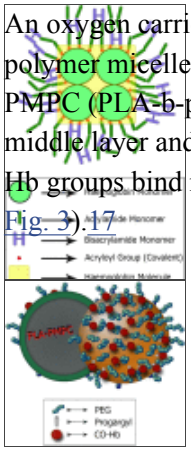

Figure 3

Structure of a micelle formed from triblock copolymers. In this product, Hb is conjugated to biodegradable polymer micelles. It contains a triblock copolymer made up of PEG (as external layer), PMPC consisting of propargyl groups (as middle layer), and **...**

RBCs Differentiated From Stem Cells

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Perhaps, the real RBCs are RBCs differentiated from stem cells. These cells can be used as an ideal product for injection to patients requiring chronic blood transfusion as well as patients with rare blood groups or autoantibodies. Stem cells derived from various sources including bone morrow, cord blood, embryonic stem cells, and induced pluripotent stem cells (iPSCs) have been used for this purpose.62 RBCs were derived from iPSCs from fetal and adult human fibroblasts using a two-step protocol for the first time by Lapillonne et al.13 This suggests that iPSCs could represent an unlimited supply for the production of RBCs for clinical application. Mass production of RBCs in laboratory for their application in transfusion have been performed by adjusting various production conditions such as providing optimal culture conditions for cord blood-derived hematopoietic stem cells and subsequent coculture of erythroid progenitors with human fetal liver stromal cells. This resulted in improved terminal erythroid maturation process and plenty of cells were obtained, which were comparable with natural RBCs in many aspects.63 Immortalized erythrocyte progenitor cells are obtained through the introduction of C-MYC and BCL-XL into multipotent hematopoietic progenitor cells derived from pluripotent stem cells. Overexpression of these proteins led to erythroblasts with consistently high self-replication capacity. Elimination of overexpression of these genes induced the differentiation of these erythroblasts to mature erythrocytes.64 Exposing $CD34^{+}$ cells to a short pulse of cytokines favorable for erythroid differentiation before their expansion and their subsequent expansion is another approach in this respect.65

Mass production and high production costs for clinical applications are the most important problems in this field. It would be expected to overcome these obstacles in near future to make an unlimited source with maximum similarity and minimum complications to replace RBCs derived from donated blood.62

Conclusion

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Due to the increased demand for blood transfusion and concerns about blood-borne pathogens, development of artificial blood substitutes, especially HBOCs, is under intensive focus. However, although many important steps have been taken to date, no oxygen-carrying blood substitutes are approved for use by the US FDA. Side effects and short half-life are the two main reasons that they did not met criteria for being approved. The fact of having no approved product in this field shows that there is an important challenge against formulation and application of promising and effective blood substitutes. In addition, it indicates the immense potential that exists in this field. However, being optimistic, it seems that science and technology would facilitate developing real blood substitutes, at least oxygen-carrying blood substitutes, whose production will substantially alleviate the worldwide shortage of blood needed for transfusion. It seems that future studies on artificial blood substitutes

would focus on real blood substitutes, ie, RBCs obtained through differentiation of stem cells. Hence, by considering the first steps that have been taken successfully toward this ideal product, it would be expected in the near future to have a source of required RBCs with minimum complications and maximum similarity to replace RBCs derived from donated blood.

Acknowledgments

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Footnotes

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Author Contributions

Analyzed the data: MHR. Wrote the first draft of the manuscript: SM. Contributed to the writing of the manuscript: AJ-N. Agree with manuscript results and conclusions: MHR, SM, AJ-N. Jointly developed the structure and arguments for the paper: AJ-N. Made critical revisions and approved final version: MHR. All authors reviewed and approved of the final manuscript.

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