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Blood Substitutes Current Options, Scope and Future Prospects: A Brief Review

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SUMMARY

According to a study conducted in 2011 approximately 50% patients that walk in to emergency department of hospitals need blood transfusion. This usually includes the patients ending up in Intensive Care Unit (ICU). Usually, purpose of blood transfusion is to improve the oxygen. There are many significant studies stating that there is no efficient improvement in oxygen delivering capacity of patients even after transfusions. Some studies also associate blood transfusions with increased mortality and morbidity. Most of OTAs developed had some level of toxicity to human being so they were not approved but new products that are in line are well researched and a special attention has been given to the optimization of those products.

Keywords: Intensive care unit; Transfusions; Morbidity; Mortality

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INTRODUCTION

Blood is an important fluid performing immense amount of physiological functions for the body. It is so precious that often termed as Red Gold. Red Blood Cells (RBCs) constitute a major portion, 40-45% total blood volume [1]. RBCs are responsible for oxygen transportation to tissues across the body and they are very ideal for this particular function [2]. According to a study conducted in 2011 approximately 50% patients that walk in to emergency department of hospitals need blood transfusion. This usually includes the patients ending up in Intensive Care Unit (ICU). Usually, purpose of blood transfusion is to improve the oxygen [3]. There are many significant studies stating that there is no efficient improvement in oxygen delivering capacity of patients even after transfusions. Some studies also associate blood transfusions with increased mortality and morbidity [4,5].

Allogeneic blood transfusions are very common medical interventions that can in some situations work like an elixir and save lives if blood that is being transfused go through a proper screening [6]. 1980's was a time when human immunodeficiency virus was diagnosed very often to people; this became a matter of concern for public that blood that was being transfused was free of such infections [7]. Although blood transfusion itself is very regulated and standardized procedure but still it accompanied many risks [8]. Infectious (bacterial, viral) and non-infectious (transfusion associated lung injury) risks are most common. Immune response, agglutination reaction due to poor cross matching (ABO, Rh incompatibility) is more to name [9]. Hypersensitivity to transfused blood, non-hemolytic fibril transfusion reactions are also observed in many cases. Contamination of blood can be a result of processing and storage if donor was examined and found healthy at the time of blood donation [10]. Iron overload is also a drawback of allogeneic blood transfusion because on an average RBCs contain 250 mg of iron and repeated transfusions may result in iron build up [11]. Inadequacy of blood is a major issue now a day for developed countries. This compels the health regulating authorities to compromise the safety standards of donated blood [12]. Keeping all these risks and emergency use of blood in mind scientists are always in quest to find out safer replacements for blood e.g, artificial blood [13]. Many tries have been made but in vain. Scientists are not able to produce a liquid that mimics the blood function. In order to qualify as a blood replacement a liquid has to behave same as natural blood. It has to come into contact with every tissue in same way as natural blood do [14]. There has been a difficulty to develop blood replacements but scientists have evaluated certain solutions and compounds for their oxygen carrying ability and some of them gave promising results. So in general when we talk about blood substitutes we are actually referring to RBC substitutes (Oxygen therapeutics) to carry out tissue oxygenation. In this review we will discuss what current option

we have. What are those compounds that qualify as blood substitutes and mimic oxygen carrying function of blood?

LITERATURE REVIEW

Red blood cells substitutes

RBCs have been used in hospitals since ages. They contain a complex of hemoglobin that is responsible for tissue oxygenation in the body so RBCs that are isolated from the blood are in practice [15]. Risks of RBC transfusion are already described above. In order to perfectly qualify for an RBC substitute a compound, molecule or liquid must possess certain qualities, it should be cost effective, able to carry oxygen and carbon dioxide, non-immunogenic, non-antigenic, non-infectious, don't need cross matching, stable and longer halflife, excretable, non-toxic.

We can divide the substances that are studied for their oxygen carrying ability and other parameters into two groups:

- Hemoglobin based
- Perflourocarbon based

Hemoglobin based oxygen therapeutics

As the name suggests, they are based on hemoglobin. In blood banks when an RBC blood bag is expired it cannot be transfused as whole so it's a waste. Hemoglobin from such expired RBC bags is isolated and is used to produce Hb based oxygen therapeutics. This is not the only source used to obtain the hemoglobin. Blood from umbilical cord, bovine hemoglobin and Hb that is produced using recombinant technology are some other sources.

Like there are risks with blood transfusions, Hb applications too have risks of various infections. Hb coming from animal source is specially screened for spongiform encephalitis [16]. Hemoglobin from animals sources is superior to human Hemoglobin in many aspects. It has longer half life, it's supply can be maintained and it uses Cl ion as allosteric factor instead of 2,3-DPG (2,3-diphosphoglyceric acid) [17]. Recombinant technology can be utilized to produce quality Hb and it enables us to modify Hb as per our desires for example we can increase its affinity for oxygen *via* genetic manipulation [18]. This process of producing recombinant hemoglobin has been successfully used by scientists in 1999 to produce robust and authentic Hb [19]. There are some benefits of Hb isolated from RBCs but it's use isn't devoid of complications and side effects. Affinity of hemoglobin is much higher when it's not with RBC because 2,3-DPG separates from hemoglobin molecule because 2,3-DGP is the one responsible for liberation of oxygen from hemoglobin so when it's not there hemoglobin has high affinity for oxygen and it holds it tight and oxygen supply gets is inefficient, kidney damage is reported because hemoglobin isolated from RBCs which is in tetramer converts to monomer [20,21]. Vasoactivity of hemoglobin based products should be monitored as well because higher or lower affinity of Hb can lead to disturbance in activity of smooth muscles and can cause vasoconstriction. No is required for relaxation of smooth muscles [22]. Since this effort has started to introduce a safe Hb based blood substitute (oxygen carrier) there's not a single substance made which got FDA approval to be used in clinical practice because of unnerving experiences during clinical trials. Although Russia and South Africa have granted the approval to use HBOC (Hemoglobin Based Oxygen Carriers) as treatment options [23]. Right now, all the substances that are under research are Hemoglobin solutions but scientists are focusing on producing neo red cells (micro-encapsulated hemoglobin) in future [24]. This group of Hb based blood substitutes is further divided into two categories:

- Acellular Hb
- Cellular Hb

Cellular haemoglobin includes three different types of haemoglobins. Crosslinked, polymerized and conjugated haemoglobin based oxygen carriers (Fig. 1-3). Different modifications have been done to make haemoglobin suitable for transfusion but only polymerized haemoglobin and conjugated haemoglobin have evidence of being well tolerated [25]. But these substances didn't perform upto the mark in clinical trials and many problems e.g, smaller half-lives were reported along with a bunch of side effects do neither of them got approval. In trials it was seen that Hb based oxygen carriers were not able to convert ferric ion to ferrous ion which is characteristic to RBCs thus limiting their use. Hemoglobin carries the oxygen in its ferrous state. When it oxidizes into its feric state it can no longer bind the oxygen (Methemoglobin). Irreversible conversion of ferrous to feric state of iron in hemoglobin can block the oxygen supply. Scientists suggested that a reducing agent attached to surface of Hb can overcome this hurdle [26].

Fig. 1. Types of oxygen carriers.

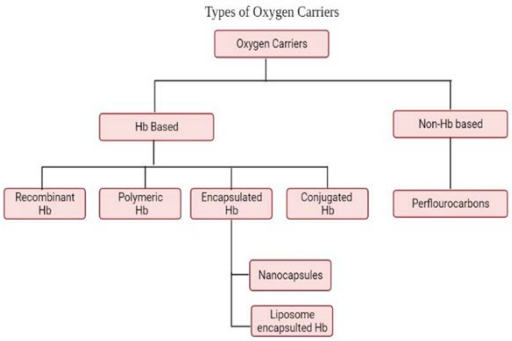


Fig. 2. Heme and globin.

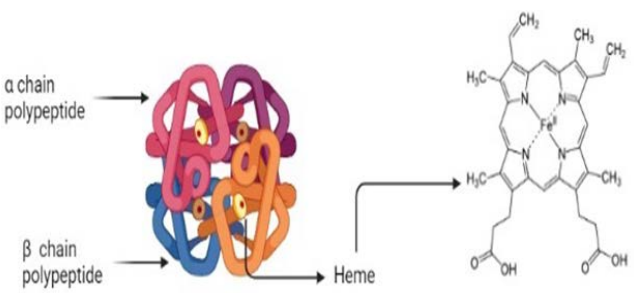
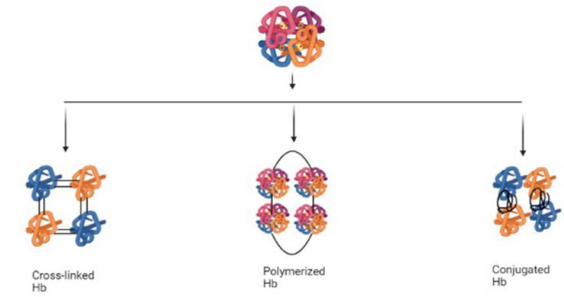


Fig. 3. Types of cellular haemoglobin based oxygen carriers.



1st generation blood substitutes

Stroma free hemoglobins were the first Hb based RBC substitutes developed. Solute hemoglobin was produced by loysis of RBCs and resulting mix was centrifuged to separate RBC stroma which leaves SFH (Stroma Free Hemoglobins) behind. SFH could be prepared using two basic methods [27]. Ultrafiltration and crystalization. Ultrafiltration yields more pure hemoglobin solutions which are virtually free of proteins and phospholipids which make its storage safe and during experiments it showed no vasoconstriction so it's better than prepared by crystallization.

Second generation blood substitutes

Production of stroma free hemoglobin gave scientists a basic framework to develop safe blood substitutes so this lead to development of Pyridoxilated Hemoglobin-Polyoxyethylena Conjugated products (PHPCs). They were produced to fulfill the shortcomings of SFH e.g, shorter half-life, NO scavenging, higher oxygen affinity and kidney damage [28]. The name suggests the components of this complex. It is stroma free hemoglobin attached to vitamin B_6 to improve its oxygen offloading capacity α-carboxymethyl-ω-car-boxymethoxy-polyethylene which increases its half-life in circulation system [29]. There are multiple PHPCs made (**Tab. 1**) but none of them us approved except hemopure that too in South Africa and not by FDA [30].

Third generation blood substitutes

LAIR (Letterman Army Institute of Research cross) in San Francisco prepared a compound in which hemoglobin was linked to two α chains using DBBF, bis (dibromosalicyl) fumarate or αα-hemoglobin instead. Sometime later only Baxter international corporation introduced similar cross linked hemoglobin using dispirit and they named it hemassist. Experiments showed they are better than other generations having more similar properties to blood [31,32]. These products have been tested in various animal models e.g, to controls the loss of blood during surgery in sheep [33], during CPR in swine [34] and post the bypass surgery to replace natural blood transfusion [35]. Results of Aspirin cross linked Hb were discouraging though.

Oxyglobin developed by OPK biotech uses bovine hemoglobin contains 2% $\alpha^2\beta^2$ Hb which is pretty less compared to 31% of hemopure which makes it a good candidate. Studies show that less hypertensive complications were observed with its use [36]. These encouraging results made oxyglobin the first substance approved by FDA for use by veterinarians.

A product with fibrinogen linked to polymerized unit of Hb was developed called Liposome-Encapsulated Actin-Hemoglobin (LEAcHb) which showed effective blood coagulation activity so this can be used in patients with bleeding disorders [37].

Tab. 1. Second generation blood substitutes.

Product	Manufacturer	FDA status	Status
PolyHeme	Northfield Laboratories (Evanston, IL)	Not approved	Approved in South Africa
Hemopure	Hemoglobin Oxygen Therapeutics LLC (Souderton, PA)	Not approved: available through	Used in severe life threatening conditions
HemAssist	Baxter International Cooperation (Deerfield, IL)	Not approved	Discontinued
Hemolink	Hemosol Inc. (Missisauga, Canada)	Not approved	Under trials

Conjugated Hb is also a good candidate as an oxygen therapeutic agent. In such complexes, different polymers can be attached on the surface of Hb. Although many polymers have been used to conjugate with Hb like benzene tetra carboxylate dextran, HRC (hydroxyethyl starch) and albumin Polyethylene Glycol (PEG) is so far the best polymer for this purpose because it's inert, non-toxic and non-immunogenic. It's characteristics are also very unique [38-41].

A study reports that in early 20th century cellular Hb suspended in Ringer's lactate solution was used intravenously to during treatment of 15 patients but most of them unfortunately ended up having renal toxicity and other cardiovascular problems [42].

In 1950's US Navy also attempted to treat many patients using cellular oxygen therapeutics but all of them developed toxicity [43].

Recombinant hemoglobin have been successfully expressed in *E. coli* transgenic mouse, pig and some other bacterial systems but most efficient expression system remains to be the *E. coli.* Difficult downstream processing and low production quantities are matter of concern when it comes to producing recombinant Hb [44,45]. There has always been struggle to produce large quantities of Hb in an expression system. In an experiment scientists were successful to produce large quantities of α-globin and bovine β-globin and link them intramolecularly *via* disulphide bonds to produce a polymer.

Clinical evaluation tests of this substance give promising results [46].

Cellular Hb based products are advantageous because they appear to be very similar to RBCs. They are contained within an artificial cell like membrane. Some of the products with highly similar properties have been produced that doesn't have vasoactivity because they do not scavenge naturally existing NO in cells. Experiments have shown that an artificially produced layer of phospholipids around hemoglobin can increase its shelf life by 30 times. Lipsomal-encapsulated Hb was produced using this technique and it was a very efficient compound for oxygen delivery in terms of micro circulation. They are way smaller in size than RBCs so can access places where RBCs are not able to reach. This property makes them very useful during strokes when a better oxygenation of blocker tissue is much needed and challenging. Studies report a higher oxygen delivering ability of LEAcHb when compared to RHCs [47].

Surface modification of liposomal-encapsulated Hb with suitable agent can increase their half-life and decrease their immunogenicity. PEG (polyethylene glycol) was used for this purpose and Hb vesicles were produced with higher half-life, better circulation, solubility and low immunogenicity [48,49]. All the Hb based cellular products made are listed in **Tab. 2** with their properties [50-59].

Tab. 2. Cellular Hb based oxygen therapeutics.

Parameters	Parameters	Parameters
Neo red cell	Hemoglobin	Oxygen Carrier, high circulation, high clearence by phagocytosis, strong membrane of capsule
Hemoglobin vesicles (HbV)	Carbonyl human Hb	Oxygen carrier, gradual dcrease in phagocytic activity, encapsulated. high metabolism, hemodynamics, circulation due to PEG
Liposome encapsulated actin-hemohlobin (LEAcHb)	Bovine Hb	Oxygen carrier, longer half-life in circulation, disk shaped
Polymeric nanopartical cintaining hemoglobin (PNP)	Hb	Biocompatible, rapid clearence
Cationized HbPNP	Bovine Hb	Biocompatible, no cytotoxicity, longer half life
Fe(II) porphyrin dendrimer	Porphyrin	Similar in shape to RBCs, costly
Membrane of ultrathin PEG-PLA containing hemoglobin and all emzymes of RBCs	Hb	All RBC enzymes, longer half life
Nanoscale Hydrogel Particle (NHP)	Bovine Hb	Hemoglobin releasing character, good affinity to oxygen
Lipogel	Bovine Hb	High affinity to oxygen, low immunogenicity
Polymersome-capsule of Hemoglobin	Human and Bovine Hb	
Single protein nanoparticle (SNP)	Human Hb	Stablized, resistant to heat & mechanical steess.
Conjugated micells of Hb polymers (biodegradable)	Bovine Hb	N/A

Hemoglobin loaded polymeric nano sized particles are another preparation made to replicate the function of RBCs. Their activity as oxygen carriers is good but they have a rapid clearance by phagocytes which make its half-life in circulation very short. An approach to solve this problem is to add a cation on surface which increases its half-life sufficiently. This lead scientists to believe that HbPNPs carrying captions linked to their surface are more stable and their clearance is slow while HbPNPs carrying anionic species liked to their surface are cleared rapidly thus having shorter half-life.

Fe (II) porphyrin loaded on a hyper-branched dendrite is tested for their oxygen carrying capacity which is remarkable. Their production is costly but their size, shape and oxygen carrying function resembles RBCs very much.

Polyethylene glycol-polyactic acid's ultra-thin membrane which contains polymerized hemoglobin are also proposed and studied as a model to replace the blood. They are reported to have a longer half-life due to low rate of phagocytosis in animal models. As Enzymes if RBCs are also included in this preparation, there are many important functions replicated of RBCs e.g, reductase prevents methemoglobin from accumulating in the body. More compounds made for the purpose of evaluation include nano scale hydrogen particles (NHPs) having an envelope which is biocompatible but isn't biodegradable so they are of much interest, polymersome-encapsulated Hb which have even more higher Hb loading capacity than NHPs and have similar oxygen carrying capacity to erythrocytes. They are easy to make, biodegradable and heat resistant, single protein encapsulated Hb which are stable to mechanical stress and heat resistant and PEG–PMPC–PLA triblock copolymer conjugated to hemoglobin called micelles (**Tab. 3**).

Tab. 3. Enlists PFC products made with their status and nature.

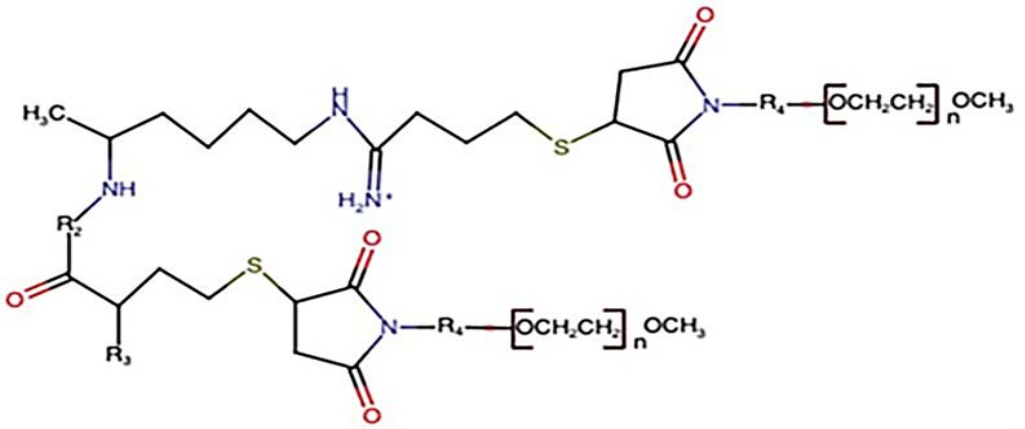
Product	Manufacturer	Location of use	FDA status	Trial status
Flusol-DA-20	Green Cross Corporation (Osaka Japan)	Japan, United States	Approved in 1989	Discontinued due to side effects
Oxygent	Alliance Pharmaceutical Corporation (San Diego, CA)	Europe, China, United States	Not approved reached in phase II trials	Discontinued due to funding
Oxycyte	Synthetic Blood International (Costa Mesa, CA)	United States	Not approved reached in phase IIB trials	Discontinued
Pretorian	Russian Academy of Sciences (Puschino, Russia)	Russia Maxico	Not approved	Rebranded as Vidaphor in US, awaits trials

RBCs from pluripotent stem cells

Stem cells can be used to produce a continuous supply of RBCs. Bone marrow, embryonic stem cells, cord blood and iPSCs are few sources to obtain stem cells and iPSCs (induced Pluripotent Stem Cells) that are usually isolated from fibroblasts can actually be very efficient source as their supply can be maintained [60].

Another approach to maintain the supply of RBCs is to derive the hematopoietic stem cells from cord blood and introducing C-MYC (C-Myelocytomatosis) that is a transcription factor activating pro-proliferating genes and keep the growth signal activated and BCL-XL which is an anti-apoptotic gene to get an immortalized cell kind of erythrocytes. This will lead to continuous replication of those cells which later can be differentiated into RBCs (Fig. 4) [61-63].

Fig. 4. PEGylated haemoglobin.

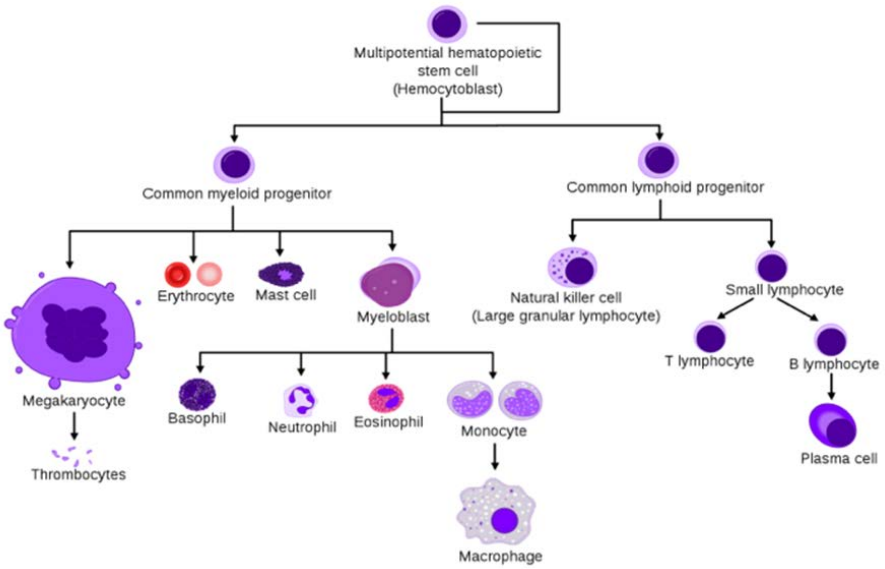


Perfluorocarbon based blood substitutes

Some perfluorocarbon compounds are known to possess certain physical characteristics e.g, viscosity, gas solubility, density, vapour pressure, lipid solubility which contribute to their ability to perform gas exchange [64]. It was in 1966 that oxygen delivering capacity of was first reported by Clark and Gollan [65]. PFCs are inert and stable fluorinated carbon compounds (**Fig. 5**).

They are solely hydrophobic in nature so a stabilized emulsion are made and used intravenously. PFC emulsions are best gas dissolving liquids as for now. Their stability owes to their strong intermolecular bonds and their gas dissolving ability is due to their week intermolecular interactions due to low polarizability of chlorine atoms making a electronegative shield around carbon core. Due this week vander waals attractions between PFC molecules they behave

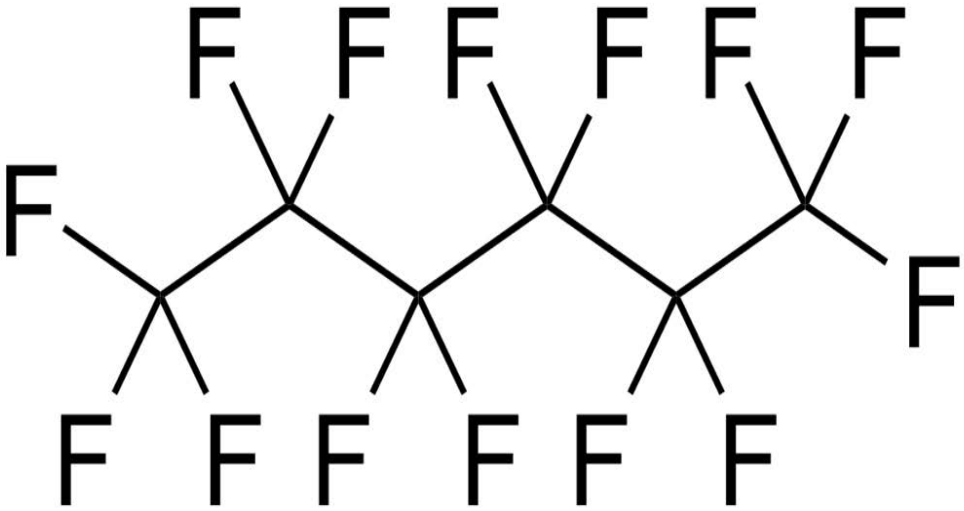
Fig. 5. Erythropoiesis.



like gassed and dissolve gasses. PFCs follow Henry's law of gas distribution so oxygen delivery is much more efficient which makes them an ideal candidate to be used as oxygen carriers in humans. A product developed perflourooctyl bromide (perflubron) has been tested (**Fig. 6**) among many others, reached in phase II and III human models with promising results but studies halted due to lack of funds [66].

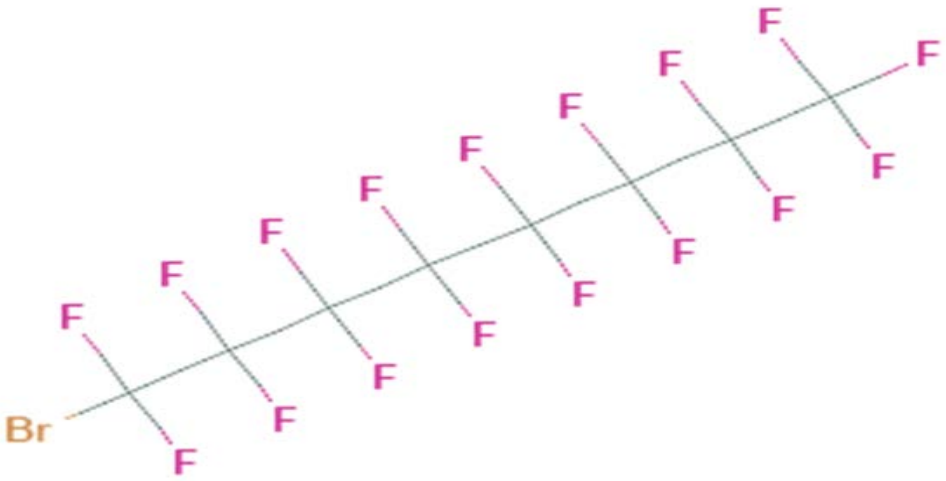
First compound prepared for this application was Fluodol-DA developed by green cross corporation in Osaka, Japan. It contained 7:3 rations of perflourodecalin and perfluorotripropylamine as

Fig. 6. Perfluorocarbon.



main active ingredients Pluronic F-68 was used as an emulsifier in this product (Fig. 7).

Fig. 7. Perfrfluorooctyl bromide.



was used in coronary angioplasty [67]. PFCs have been tested to be used in many conditions e.g, patients with surgical blood loss, gastrointestinal bleeding and oesophageal cancer. It has also been tested in patients who simply refuse blood transfusions on moral or religious grounds. All these trials established Fluosol-DA as principle injectable oxygen therapeutic which paved the way for new products to be developed [68-69].

DISCUSSION

Many products developed had some discouraging results during trials but this hasn't stopped scientists to develop more oxygen therapeutic agents. KaloCyte Inc., Baltimore, MD have designed an OTA named ErythroMer that is under process of development and testing. This product is designed after an intense process of research to mimic the interaction of RBCs with gasses. Doctors hope that phase I A 20% solution was launched in the market which could carry 7.2% volume of 100% oxygen at room temperature of 37ºC. It clinical trials will begin in 2022 [70]. Hemarnina, Morlaix, France also have developed a product a product Hemo2 Life which is based on hemoglobin from lugworm and encapsulated with natural enzymatic activities as well [71]. OxyVita (New York), Hb Vesicles (Tokyo), HemoAct (Tokyo) are some other Hb based products that are under development.

CONCLUSION

There has been a great amount of intensive research to develop and optimize a product that can be used instead of allogeneic transfusions but till date no product has been given a green flag by FDA. Most of OTAs developed had some level of toxicity to human being so they were not approved but new products that are in line are well researched and a special attention has been given to the optimization of those products. Hope is still there.

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