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Artificial Blood: A Futuristic Dimension of Modern Day Transfusion Sciences

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Abstract: Artificial blood is an innovative concept of transfusion medicine where specifically designed compounds perform the task of transport and delivery of oxygen in the body to replace this function of allogenic human blood transfusion. Several molecules have been developed in the past few decades to achieve this objective and continous refinements are being continuously made in the quest of the ideal blood substitute. Currently, available technology manufactures artificial blood from haemoglobin obtained from outdated human/bovine blood (Haemoglobin Based Oxygen Carriers) or utilizing Perfluorocarbons. These synthetic blood substitutes are advantageous in that they do not require compatibility testing, are free from blood borne infections, have prolonged shelf life and do not require refrigeration. Artificial blood is projected to have a significant impact on the development of medical care in the future. It can complement the current blood products for transfusion and create a stable supply of safe and effective products. It is likely to reduce the requirements of blood transfusions drastically especially in settings of trauma and surgery thereby reducing the reliance on banked donated blood.

Keywords: Anaemia, blood, erythrocytes, haemoglobin, hypersensitivity, liposomes, resuscitation, transfusion.

1. INTRODUCTION

With the phenomenal increase in the number of surgical procedures (both elective and emergency) and trauma cases, the demand for human blood for transfusion has seen an overwhelming rise. The number of units collected from blood donors is insufficient to cope up with the increasing requirements of human blood which modern day medicine and surgery demands. Additionally, donated human blood is fraught with concerns related to short storage life, possibility of transmission of blood borne infections, allergic reactions and increasing costs of collecting, processing and cross-matching. To mitigate this ever expanding disparity between the demand and supply of blood and the difficulties associated with stored human blood, artificial blood has emerged as a promising option. Artificial blood serves to provide a substitute of conventional blood transfusion where blood or blood products derived from one person is infused into another. The term artificial blood is often used interchangeably with blood surrogate or blood substitutes and all of them are actually misnomers as artificial blood lacks numerous essential attributes of human blood like haemostatic processes, typing and immunologic defense of the body, nonetheless it serves to carry out the important function of transporting oxygen and carbon dioxide throughout the body. Thus, the appropriate terms for these substances can be Red Blood Cell (RBC) substitutes or Artificial Oxygen Carriers (AOC) [1].

2. HISTORICAL PERSPECTIVE

The quest for a suitable alternative for human blood dates back to the 17th century when Sir Christopher Wren suggested the use of ale, wine and opium as blood substitutes [2]. Various other substances like urine, plant resins, sheep blood, milk (for treatment of Asiatic cholera) and salt solutions were also tried previously. Following the path breaking research of Landsteiner on the various blood groups, blood transfusion became a safer and established medical procedure. As the understanding of oxygen transport and delivery of RBC's gradually improved and the necessity of type specific allogenic transfusion was recognized, the foundations of development of artificial blood started being laid in the early 1900's. Amberson *et al.* in 1949 reported the first infusion of cell free haemoglobin in a patient with postpartum haemmorhage for resuscitation [3]. Animal experiments where free haemoglobin was collected by lysing the red blood cells and transfusing the unmodified products in animals resulted in renal failure, coagulopathy, complement activation, antigenicity, histamine release, iron deposition and vasoconstriction. The toxicity was later attributed to the presence of red cell stroma in the product [4, 5]. Also the

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strong affinity of free haemoglobin for nitric oxide (a potent vasodilator) caused unopposed vasoconstriction and pressor response due to its nitric oxide scavenging effect [6].

3. DEVELOPMENT OF BLOOD SUBSTITUTES

Focused research and development in this field received an impetus in the mid 1980's following the apprehensions caused by the possibility of HIV infected blood [7-9]. Association of other infectious diseases like Hepatitis B, Hepatitis C, West Nile virus encephalitis, coronavirus, human T cell leukemia virus and bacterial infections with blood transfusions became increasingly recognized. Allogenic blood transfusions also resulted in certain non infectious complications like haemolytic transfusion reaction, transfusion related acute lung injury, graft *versus* host rejection, anaphylaxis and post transfusion purpura. The amount of donated stored blood was gradually being unable to cope up with the ever increasing demand and thus a deficiency is projected in the years to come. The incurred expenditure of collecting, storing and processing blood and products is also gradually rising [10]. Cumulative effects of these factors provided a major boost to the development of artificial blood in the past few decades.

The main purpose of these substances is to provide temporary support to the circulatory system till the time when the body's bone marrow has regenerated sufficient RBC's. They concentrate on one of the important function of blood which is oxygen transportation to the cells and tissues. The main clinical indications of administration of artificial blood include:

1) Trauma: For volume replacement and stabilization.
2) Elective Surgery: Preoperative blood conservation in form of acute normovolemic haemodilution and perioperative volume replacement following massive blood loss.
3) Cardiovascular Surgery: For pump priming, deep hypothermia and intraoperative replacement.
4) Perfusion of Ischaemic Tissues: In sickle cell disease, strokes, peripheral vascular diseases.
5) Oxygenation of Solid Tumours: For increasing susceptibility to radiotherapy and chemotherapy.
6) Preservation of Organs: During transport for transplantation or as cardioplegia.
7) Drug Carrier: In the form of drug conjugated haemoglobin and perfluorocarbons.
8) Miscellaneous: Anaerobic infections, gas embolism, CO poisoning.
9) Contrast Agent: Perfluoro octyl bromide is used as a contrast agent with oxygen carrying capacity in ultrasound, CT scan, MRI, angiography, liver, spleen and tumour imaging.

Apart from being able to transport oxygen the desirable qualities in artificial blood are : (1) No prerequisite of blood grouping, cross matching and compatibility tests. (2) Long shelf life (preferably at room temperatures). (3) Survival in circulation for a substantial period (the intravascular "dwell" time before being cleared from kidneys. (4) Absence of pathogens and adverse effects. (5) Able to effectively deliver oxygen to tissue in addition to transportation.

Current strategies to produce artificial blood includes synthetic production, chemical isolation and recombinant biochemical technologies [11]. Conventional blood substitutes belong to either of the two classes: Haemoglobin Based Oxygen Carriers (HBOC's) or Perfluoro Carbons (PFC's).

4. HAEMOGLOBIN BASED OXYGEN CARRIERS (HBOC's)

HBOC's are derived from haemoglobin which is isolated or synthetically manufactured (Fig. **1**). Oxygen binds covalently to these compounds as they do to naturally occurring haemoglobin. They were designed to fulfill the following purposes : (1) inherent decreased oxygen affinity to increase tissue unloading (2) prolonged intravascular retention (3) decreased colloidal osmotic activity (4) absence of renal toxicity. The source of haemoglobin is either human, obtained from outdated stored blood or bovine blood or genetically engineered [10, 12-14]. Blood group substances, proteins and viruses (if any) are removed by heating and filtration through the process of haemoglobin purification from erythrocytes. The isolated haemoglobin is subjected to molecular modification and reconstitution in an artificial blood formula. Haemoglobin in its natural form consists of 2 α and 2β chains which are bound to an iron haem group which binds oxygen. Manufacturing processes of HBOC involves extraction of haemoglobin and thereafter stabilisation with cross-linking as tetramers or polymerization (using glutaraldehyde or o-raffinose), or conjugation with polyethylene glycol or encapsulation in phospholipid vesicles before mixing into an electrolyte solution [13]. Stabilisation of HBOC using cross linking or polymerization with larger molecules like polyethylene glycol, dextran or polyoxyethylene also aids in a substantial increase in the intravascular "dwell" time (24-48 hours). Newer molecules are being developed which have antioxidants like superoxide dismutase or catalase cross linked to the haemoglobin structure. The incorporation of antioxidants help in reducing the severity of ischaemic reperfusion injury in conditions like stroke, myocardial infarction or organ transplantation [15]. Based on the techniques of improving stability, the following groups of haemoglobin solutions are available:

i) Surface Modified Haemoglobin (PEG Hb, PHP, Haemospan): They are produced by attachment of large molecules like polyethylene glycol to surface lysine groups. This modification increases the viscosity and oncotic pressure of the solution. These solutions have the propensity to cause moderate vasoconstriction. The small size of the haemoglobin molecules allows them to pass through small vessels and oxygenate areas which cannot be reached by RBC's. Thus, they are useful in the treatment of patients with stroke, for increasing susceptibility of tumour cells to radiation or chemotherapy and also the vaso-

pressor effects can be utilized to treat hypotension following septic shock. They also have a lower incidence of antibody production

ii) Intramolecular Cross Linked Haemoglobin (Hemassist, r-Hb1-1,r-Hb 2-0):The tetrameric stabilization and prevention of renal filteration is accomplished by intermolecular cross linking between the two α and the two β subunits using a site specific cross-linker which cross links the two α or the two β chains. Cross linking also reduces the affinity of haemoglobin for oxygen. Commonly used cross linkers include 3,5-dibromosalicyl fumarate (DBBF) and nor-2-formylpyridoxal 5-phosphate (NFPLP).

iii) Polymerised Haemoglobin (Polyheme, Hemopure, Hemolink): Here, the surface amino acid groups are linked by reagents like glutaraldehyde. Negligible side effects have been noted with this compound. This product has been transfused several times on compassionate use basis [16]. Administration of polymerized haemoglobin in patients undergoing infrarenal aortic reconstruction have shown an avoidance of allogenic blood transfusion in 27% of the patients [17].

iv) Liposomes Encapsulated Haemoglobin: The purified haemoglobin is re-encapsulated in a stable lipid membrane. The liposomes are made of phospholipid bilayer with cholesterol molecules added for increasing the rigidity and mechanical stability and enclose a stroma free haemoglobin solution and 2,3 diphosphoglycerate or inositol hexaphosphate as a gelatinous fluid. For added stabilization, ultraviolet radiations or redox inhibitors are used which causes polymerization of unsaturated phospholipids. Stabilisation can also be achieved by coating the liposomes with polymers. Encapsulated haemoglobin (often called haemosomes) allows manipulation of their physiochemical properties and circulation lifetime [18, 19]. The P_{50} of the modified haemoglobin can be suitably altered through modifications of the liposomes which allows for easier oxygen unloading. Oxygen affinity can be adjusted by co encapsulating an allosteric effector like pyridoxal 5'phosphate [20]. They often have negatively charged lipid in their capsules which limits their aggregation. Encapsulation also prevents denaturation of haemoglobin and enhances biodistribution. Further modification with polyethylene glycol increases their half life, makes them water soluble, lowers antigenicity and increases site specific targeting [21, 22]. Since these vesicles are made up of purified haemoglobin or lipids, they are compatible with the immune system. Nanocapsules of haemoglobin made of biodegradable polymer like polylactide have been developed [23]. Polylactide gets converted to water and carbon dioxide in the body and hence does not accumulates in the reticuloendothelial system.

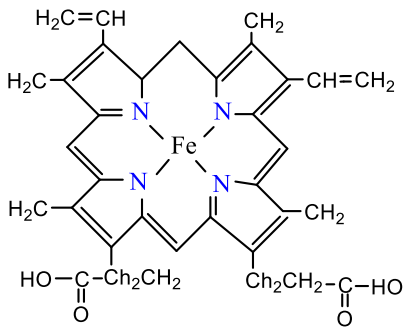

Fig. (1). Synthetically manufactured HBOC.

5. LIMITATIONS OF HBOC's

Certain factors are needed to be considered before widespread advocacy of HBOC's. RBC's do not exert any colloidal osmotic pressure whereas haemoglobin (like other plasma proteins) exerts the same. As a result a cellular haemoglobin can alter the intravascular volume and act as a plasma expander. HBOC's circulation half life is shorter than normal RBC's. Majority of HBOC remain in circulation for around 20-30 hours whereas whole blood transfusion lasts 34 days. They release free radicals inside the body from free haemoglobin and the breakdown products like haem and iron. Methaemoglobin concentrations also increase due to the oxidative properties of HBOC's [7, 8, 24, 25]. The first choice for obtaining haemoglobin is outdated human blood which has a limited supply. Thus bovine blood has to be utilized for procurement of haemoglobin. Bovine haemoglobin has the potential of harbouring the prion pathogen responsible for causing bovine spongiform encephalopathy (Creutzfeldt-Jakob disease). To overcome this problem and also to ensure a steady supply of haemoglobin in future, genetically engineering bacteria to produce a recombinant source of human haemoglobin was attempted.

Recombinant Haemoglobin (Optro): Recombinant DNA technologies can be utilized to produce modified haemoglobin in organisms like *E.coli* and yeast. Here, certain segments of the amino acid sequence of human haemoglobin are replaced to prevent disassociation into dimers and maintain oxygen affinity. The haemoglobin gene is then transferred using a plasmid vector into *E.coli* cells. Expression of these genes leads to the production of haemoglobin proteins. This approach eliminates the concerns related to disease transmission through haemoglobin obtained from human or bovine sources. High costs of this techniques is, however, a major hindrance.

6. PERFLUOROCARBON (PFC) BASED PRODUCTS

PFCs are chemically inert molecules with structural similarity to hydrocarbons and where the hydrogen groups are replaced with fluorine. Their size is about 100 times smaller than RBC's. They are capable of carrying oxygen and carbon dioxide without binding to these gases [26] and had been previously used to provide oxygen to premature infants with respiratory distress syndrome. Due to their insolubility in water, they need to be emulsified by addition of lipids

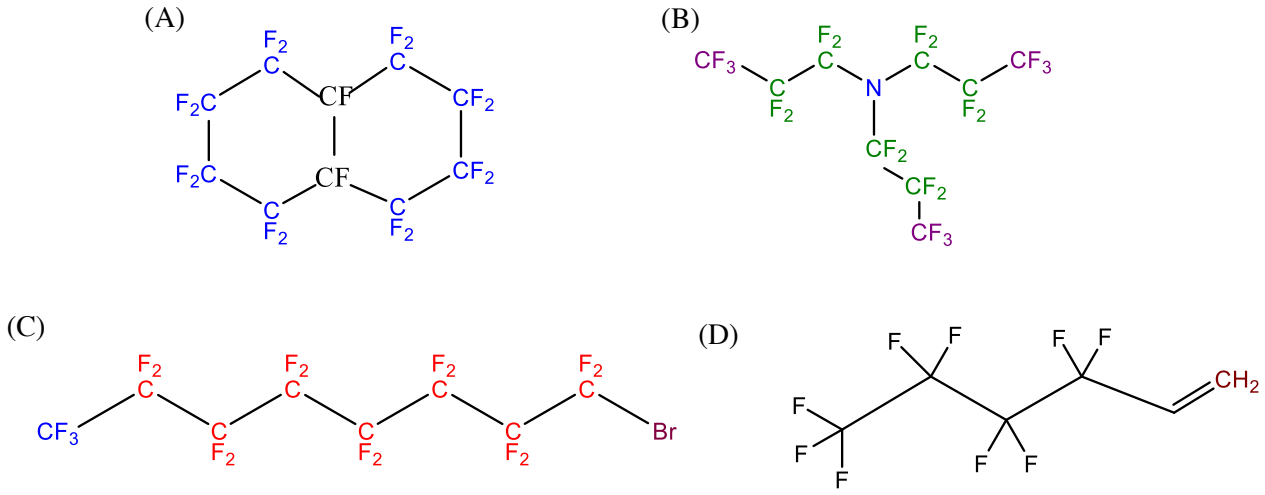

Fig. (2). Perfluorocarbons Classification : Perfluorodecalin (**A**); Perfluorotrypropylamine (**B**); Perfluorooctyl bromide (**C**); and Perflurorobutyl ethylene (**D**).

through high pressure homogenization which suspends tiny particles of PFC in the blood. Saturation of PFC occurs passively as molecules of oxygen dissolve into molecular cavities within liquid droplets. Oxygenation of PFC is related to partial pressure of oxygen which is in contact with PFC. Therefore, best results are obtained if the patient is breathing 100% oxygen at the time of infusion (PaO_2 > 350 mm Hg). The reticuloendothelial system is responsible for the systemic removal of PFC's and they are exhaled *via* the lungs resulting in a short dose dependant circulatory half life. After infusion, the molecules vaporize and then are exhaled over days. The PFC's which were used initially had the disadvantages of accumulating in the reticuloendothelial system. They score over haemoglobin based molecules on the following counts (1) no reactivity with oxygen or other gases (2) increases oxygen solubility in the plasma (3) No effect of temperature, pH and 2, 3 diphosphoglycerate on dissolution of oxygen (4) allows easy and faster transfer of oxygen from cells to the tissues (5) Variable carrying capacity dependent upon the FiO_2. PFC's are further classified as (Fig. **2**):

1) First Generation PFC: Fluosol DA 20% was the prototype of this class and consisted of perfluorodecalin and perfluorotrypropylamine. Perfluorodecalin transpor-ted oxygen and perfluorotrypropylamine provided stability to the compound. Fluosol was approved by FDA as a blood substitute for cardiac surgery. Fully oxygenated PFC is infused during coronary angioplasty to provide oxygen delivery during surgery.
2) Second Generation PFC: Perfluorodecalin, perfluorooctyl bromide and bis (perfluorobutyl) ethylene belong to this class. Their oxygen carrying capacity is substantially higher than the first generation PFC and their excretion is faster with lower tissue retention.

7. DRAWBACKS OF PFC

First generation PFC were responsible for complement activation [27]. Especially those which were lecithin based demonstrated cytotoxicity of phagocytic cells like monocytes and granulocytes. PFC is known to cause flu like symptoms which occurs due to opsonisation and phagocytosis of PFC emulsion by the recipient organism's immune system. Exposures to high oxygen concentration during PFC infusion can result in oxygen toxicity. PFC is also implicated in transient reduction in platelet counts which begins 3-4 days after administration and normalizes by 7 to 10 days [7]. Also, PFC products cannot be used by the human body and needs to be removed and this process requires around 18-24 months. They can overload the reticuloendothelial system and suppress its function. As it can be retained in organs, histological effects like appearances and enlargement of vacuolated histiocytes are seen in liver biopsies [27]. A higher rate of neurological complications have been found in human cardiac surgery cases [28].

8. BENEFITS OF BLOOD SUBSTITUTES OVER RBC's

1) Faster and Better Oxygen Distribution: These molecules allow full capacity oxygen transport immediately after transfusion as against stored blood which requires 24 hours to attain full oxygen carrying capacity due to the depletion of 2,3 diphosphoglycerate. Higher extraction rates and ratios of PFC's allows for reaching 90% of the oxygen carrying capacity compared to only 25-30% for haemoglobin. Low affinity for oxygen allows for rapid unloading of oxygen to the tissues. They can ensure adequate oxygen delivery at haemoglobin levels of 2 gm/dl without adverse effects.
2) Longer Shelf Life: They can be stored at room temperatures for prolonged periods (1-3 years) and are ready to be used as compared to stored blood which can be stored for around 35-42 days. There is no necessity of refrigeration of these products.
3) Universal Compatibility: Since all the protein components are removed, the human immune system does not

recognize it as a foreign entity. Hence the necessity of compatibility testing based on blood groups is avoided. The possibility of clerical errors which might result in mismatched transfusions is also evaded.

4) Prevention of Transmission of Infectious/Anaphylactic agents: Products are sterilized and hence chances of viral or disease transmission are alleviated.

5) Reduction in ischaemic, inflammatory and reperfusion injury.

6) Jehovah's Witness: This religious group's belief prohibits the acceptance or donation of blood or blood products. Due to the chemical nature of PFC, it can be an acceptable and practical alternative for this group to fulfill the need for blood transfusions.

CONCLUSION

The rapid advancements in screening techniques of donor blood have reduced the incidences of transfusion associated infections like HIV and hepatitis, nevertheless the gap between the increasing demand of human blood and the limited supply continues to widen. Moreover, immunological effects of blood transfusions result in increased wound infections, delayed healing and progression of malignant diseases. Even though the existing varieties of artificial blood only serve for oxygen transport, delivery and volume expansion, their concurrent usage with other blood salvaging techniques can significantly lower (or avoid) the requirement of allogenic blood transfusion during surgical procedures. Hyperoxic ventilation in combination with small boluses of PFC's can maintain adequate tissue oxygenation intraoperatively in surgeries with anticipated major blood loss [13] The clinical implications of artificial blood can be far reaching in specific situations of mass military or civilian casualties (war, natural disasters) and in areas with scarcity of safe blood for transfusion (like South Africa and Nigeria where a large population is HIV infected). Thus, it becomes a strong basis for promoting the development of artificial blood or blood substitutes. The extensive clinical application of these substances is currently impeded by issues related to safety, cost, lower intravascular dwell times, sufficient supply of raw materials, toxicity and prolonged tissue retention.

Additionally, contemporary blood substitutes do not possess immunologic or clotting properties which are essential properties of human blood. Therefore, further developments in the field of preparation of artificial platelets [6], white blood cells and blood proteins are underway to mitigate this shortcoming of the available molecules and to ensure that the future blood substitutes contain the functionality of these elements. Development of lyophilized platelets, infusible platelet membranes and fibrinogen coated albumin microcapsules are encouraging strides in that direction to enhance the procoagulant effect and effectiveness of existing platelets in patients with thrombocytopenia [29]. Upcoming dimensions for further investigations include experimentation with stem cells (production of RBC's of specific and rare groups by the method called as blood pharming) [30], transgenic haemoglobin (from transgenic pigs to reduce rejection) and polyhaemoglobin enzyme complexes (reduction of ischaemic reperfusion injuries in strokes,myocardial infarctions, haemmorhagic shock and transplant surgeries) [31].

Artificial blood substitutes are not devoid of their share of complications. Commonly observed side effects include increase in systemic and pulmonary arterial resistance, decreased cardiac output, jaundice, increased activities of enzymes like amylase, lipase or transaminases [32-35]. The increased levels reflect clinical pancreatitis or is an error of interference with the photoelectric detector is still speculative.

Clinical impact of vasoconstrictive activity of blood substitutes is still unclear which might have deleterious effects on organ blood flow and functions. Thus non vasoactive blood substitutes are preferred. Therefore, the availability of a non-vasoactive HBOC may be desirable. Maleimide-activated polyethylene- glycol-modified haemoglobin (Hemospan^{TM}, Sangart Corp.) represents such an HBOC featuring a low haemoglobin concentration (4 g/dL), a high oxygen affinity (p50 5.9 mmHg) and a high viscosity (2.5 cP) [36].

A sizeable volume of clinical trials and systematic studies on the clinical safety and efficacy is yet needed to be done before artificial blood substitutes find their way into regular practice. Recent literature points towards newer vistas of therapeutic application of blood substitutes in clinical practice ranging from foetal hypoxia in preeclampsia [37], cerebral ishchaemia [38] and liver transplantation [39]. Additionally, they are being utilized as contrast agents [40] or infection tracers [41]. Future research initiatives in this context should, therefore, be directed towards providing adequate quantities of safe, efficacious, commercially viable alternatives with lesser drawbacks which will reduce dependency on donated blood and reduce mortality due to transfusion requirements. A new avenue in the field of transfusion medicine is thus open to immense possibilities.

LIST OF ABBREVIATIONS

AOC	=	Artificial Oxygen Carriers
CT	=	Computerised Tomography
DBBF	=	3,5-Dibromosalicyl Fumarate
HBOC	=	Haemoglobin Based Oxygen Carrier
MRI	=	Magnetic Resonance Imaging
NFPLP	=	nor-2-Formylpyridoxal 5-Phosphate
PEG	=	Polyethylene Glycol
PFC	=	Perfluoro Carbons
RBC	=	Red Blood Cell

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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