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Artificial Blood: A Life Defending Tool

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ABSTRACT

Blood is one of the most essential components of the body. It carries out various functions that are life sustaining. Situations may arise when the requirement of blood quantity cannot be met as in case of intense injury or surgical conditions where volume of blood may not be sufficient enough to support the survival of life. In such cases there is an inevitable requirement of the source from where the requirement can be met. So, the present review focuses on such products which can be used in place of blood for supporting the life. These products are designated as Artificial Blood. Artificial blood is a product made in order to substitute Red Blood Corpuscles, with the main function of transportation of respiratory gases, Oxygen and Carbon-dioxide throughout the body as well as fill fluid volume. But the product lacks other blood related objectives including absence of cells, coagulation properties and defence mechanisms. Artificial blood products can be broadly classified into two categories: Perflourocarbon based and Hemoglobin Based Oxygen Carriers products, each being associated with its specific advantages and disadvantages. The concept of artificial blood is not only theoretical but the products have been developed and are undergoing commercial development, with some being marketed and others being undergoing clinical and preclinical trials. Besides this, other blood replacement alternatives like antigen camouflage, transgenic therapeutic proteins, platelet substitute for cancer, etc. are also employed, while stem cells, dendrimers, biodegradable micelles and blood from placental umbilical cord may serve for future investigation of blood alternatives.

Keywords: Artificial blood, perfluorocarbon (PFC), oxygen (O_2), carbon dioxide (CO_2), hemoglobin (Hb), hemoglobin based oxygen carrier (HBOC).

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INTRODUCTION

Blood is a fluid connective tissue making about 7% of the body weight. It performs variety of functions such as, transports respiratory gases O_2 and CO_2 throughout the body, carries hormones secreted by endocrine glands to their target location, blood clotting, fills of body volume and serves as a medium for many other molecules such as nutrients, antibodies and waste products.[1] It is composed of plasma and blood corpuscles.

Plasma:

It is the extracellular fluid, of blood constituting about 55% of blood volume. It is a faint yellow colored, viscous fluid having an alkaline nature. It is composed of 91-92% water, 7% proteins, 0.9% inorganic constituents, and 0.1% glucose.

Blood corpuscles:

Blood consists of about 45% of blood corpuscles/ blood cells. Blood corpuscles are of three types:

1. Erythrocytes/RBC's: Erythrocytes are biconcave disc shaped structures having a diameter of 7-8μm. Human RBC's lack nucleus and have a life span of about 120 days. RBC's are red in colour due to the presence of hemoglobin, which is responsible for transportation of respiratory gases, O_2 and CO_2.
2. Leucocytes/WBC's: They are concerned with immune and defense mechanisms of the body. They are colourless as they lack any pigment.
3. Thrombocytes/Platelets: They are round or oval cells. They are involved in blood coagulation[2]

ARTIFICIAL BLOOD

Artificial blood is a product which is made in order to act as an alternative or substitute for RBC's. They are designed to transport O_2 and CO_2 throughout the body. But the artificial blood is unable to perform other vital functions of blood like, it does not contains cells, antibodies or coagulation factors. This means, they are neither involved in the body's defence mechanisms nor they participate in blood coagulation. Hence they are currently labeled as 'oxygen carriers' or 'oxygen therapeutics'.[3-5]

Ideal Blood Substitutes

An ideal blood substitute can be defined based on the following attributes:

1. Oxygen carrying capacity
2. Volume expansion

3. Universal compatibility that eliminates the need of cross matching
4. Pathogen free
5. Minimum side effects
6. Long shelf life
7. Cost effective.[4]
8. Non antigenic
9. Sufficient circulation half life
10. Easy availability
11. Easy administration.[6]
12. Shelf stable
13. Sterilizable.

Advantages of Artificial Blood over Human Blood

1. Large scale production is possible hence providing easy availability.
2. Less chances of spread of disease as is encountered with the real blood transfusion.
3. Artificial blood can be stored for longer time, around 1-3years.[7]
4. Amenable to sterilization to remove infectious pathogens.
5. Blood substitutes do not contain any of the antigen that determines blood type hence can be transfused without cross matching, as is required in human blood. [8]
6. Human blood needs refrigeration but artificial blood can be kept at room temperature.
7. This also proves beneficial for those patients that refuse blood transfusions for religious reasons.

Shortcomings of Artificial Blood

1) These products are costly.
2) They increase the risk of heart attack.[7]
3) They are meant just for transportation of respiratory gases i.e. O_2 and CO_2.
4) They do not provide defense to the body against infections.
5) They do not participate in blood coagulation process.

CLASSFICATION OF ARTIFICIAL BLOOD

Artificial blood can be divided into two types depending on the mode of synthesis as well as basic structure.

1. Perflourocarbon(PFC) Based
2. Haemoglobin Based

PFC Based Artificial Blood

They are like hydrocarbons in which fluorine is present instead of carbon. PFC's are clear, colourless liquids and are entirely synthetic. They are chemically inert and have an extremely good capacity to carry dissolved gases. PFC's are extremely small and can fit into spaces that are inaccessible to RBC's.[4,5,9,10]

PFC's are generally insoluble in aqueous solutions. Hence they are prepared as emulsions, in which liquid PFC is emulsified in water and salt. They are generally combined with emulsifiers such as lipids that suspend PFC's in blood.[4]

Perflourocarbon emulsions (PFCE) particles are spherical, having an average diameter of 0.1-0.2µm, with PFC core coated by a thin film of egg yolk phospholipids. Their half life in the circulation is in the range of 6 to 9 h.[11]

The particles are not metabolized in the body and are eliminated unchanged from the circulation by reticuloendothelial system (RES). They are stored in liver and spleen and subsequently exhaled through lungs[5,9,10]

First Generation PFC's

First generation PFC's included Fluosol DA 20% (Green Cross, Osaka, Japan). In this Pluronic F-68 was used as an emulsifying agent. It consisted of two PFC's, perfluorodecalin (PFD) and perfluorotrypropylamine (FTPA). PFD was meant for carrying oxygen and FTPA provided stability.

Second Generation PFC's

When compared to first generation PFC's, these have following features: large O_2 dissolving capacity; faster excretion and less tissue retention; lack of side effects; increased purity. It included three candidates, perfluorodecalin, perfluoro octyl bromide and bis (perfluorobutyl) ethylene.[10]

Advantages

1) They are chemically inert.
2) They do not react with O_2 or other gases.[10]
3) Their composition can be controlled.
4) Large scale production is feasible.
5) They have long shelf life.[12]
6) They do not require typing and cross matching prior to use.
7) They are sterilizable.
8) Can be stored at room temperature.[13]

Problems with PFC's

1) Decreased platelet count due to opsonization of platelets by PFC's and subsquent sequestration and elimination by reticuloendothelial system (RES).[4]
2) Anaphylaxis due to emulgent.[5]
3) Platelet aggregation.
4) Transient thrombocytopenia.[10]
5) PFCE's have been reported to produce flu-like syndrome, back pain, malaise and flushing.
6) They are required to be stabilized and emulsified.
7) They have rapid plasma clearance.[12]

PFC Products and Their Current Status:

1) Fluosol DA

   Sponsored by: Green Cross Corp. of Osaka, Japan.

   Description:

   It is an emulsion mixture of perfluoro-decalin and perfluoro-tripropylamine. It was the first blood substitute to reach clinical trials. Its performance in phase II clinical trials in acutely haemorrhaging patients was disappointing due to low O_2 carrying capacity and severe illness of patients. It was then licensed for percutaneous transluminal coronary angioplasty but was eventually removed from market because of inconvenience encountered during use.[12,14]

2) Oxygent™

   Sponsored by: Alliance Pharmaceutical Corp., USA.

   Description:

   It is a second generation PFCE with a mean particle diameter of 0.16-0.18µm and optimal storage temperature of 2-8^{o}C and PFC content of 60%. Oxygent has reached an agreement with FDA on Phase III trial 9/99 and has entered US Phase III trial 12/99.[15,16]

3) Oxycyte™:

   Sponsored by: Synthetic Blood International, California.

   Description:

   It is a perfluoro-tertbutyl cyclohexane intravenous oxygen carrier that contains only carbon and fluorine atoms. It is emulsified with purified egg yolk phospholipids in isotonic aqueous medium. It is a colorless, odorless liquid at room temperature. It has shown to be a safe blood alternative in Phase I clinical trials. Oxycyte has entered two small Phase II studies in patients with traumatic brain injury and sickle cell anemia.[17-19]

4) Perftoran:

Sponsored by: Perftoran, Russia.

Description:

It is a first generation PFCE with a diameter of 0.07µm. It can be stored for 3years at (-4) - (-18)^{o} and for 2weeks at 4^{o}. It is under development for 17years from 1980-1997. In 1997, it was approved in Russia for clinical applications in medicines.[15,20]

5) PHER-O_2:

Sponsored by: Sanguine Corp., USA.

Description:

It is second generation PFCE which was FDA approved in 1989 for angioplasty but was withdrawn in 1994.[6]

HEMOGLOBIN(Hb) BASED ARTIFICIAL BLOOD

Hemoglobin based products are called hemoglobin based oxygen carriers (HBOC's). The molecules of HBOC float in plasma and pick up O_2 from lungs and drop it off in capillaries. The molecules are smaller than RBC's so they can fit into spaces that RBC's cannot.

The sole O_2 carrier in RBC's is hemoglobin (Hb), hence Hb seems to be an obvious candidate as a blood substitute. Hb has a high capacity for O_2 and CO_2 transport. Also absence of RBC membrane and its antigens eliminate the need for compatibility testing also it can be sterilized by ultrafiltration and low heat. But despite these advantages the use of cell-free Hb is hampered by certain drawbacks which includes: short plasma half life, renal toxicities and hypertensive effects.

Hb are associated with erythrocytic membrane stroma lipids that are contaminated with endotoxins, resulting in nephrotoxicities. Hence Hb solutions have to be prepared free of stromal lipids and endotoxins. But stroma free Hb has a very high affinity for O_2 and very short half life. This high O_2 affinity was due to the loss of 2,3-DPG (diphosphoglycerate) during purification process which ultimately leads to inefficient O_2 offloading in tissues.

When this cell free Hb is present in solution, this tetrameric molecule spontaneously dissociates into dimers and monomers that are cleared by kidney, resulting in short plasma half life. As a consequence, chemical modification of cell free Hb becomes necessary. In order to achieve this, three strategies can be employed: Modification of surface of Hb; Polymerization of Hb; and Cross linking of Hb.[8,11,12]

Accordingly, HBOC's are of following types:

1) **Polymerized Hb:** It includes the formation of Hb oligomers through polymerization. In this,

the multiple Hb proteins are linked by aldehydes such as glutaraldehyde and glycoaldehyde which increases the size of oligomers which inturn prevents the rapid excretion of molecule, prolonging its plasma half life.

2) **Polymer conjugated Hb:** It involves bonding of Hb to water soluble polymers in order to increase molecular weight of Hb. Water soluble polymer used is a biocompatible polymer such as polysaccharide most commonly polyethylene glycol, hence it is also known as pegylation.
3) **Intramolecular cross linked Hb:** It involves cross linking of two α or two β subunits to stabilize α-β dimer association. It is mostly achieved by cross linking agents such as, Diaspirin (DBBF, 3,5-dibromosalicyl fumarate) and NFPLP, nor-2-formyl pyridoxal 5-phosphate.
4) **Recombinant Hb:** It involves replacement of few amino acid sequence of human Hb by modifying Hb produced from micro organisms like *E.coli* and yeast to prevent dimer dissociation.
5) **Encapsulated Hb:** It involves encapsulation of Hb within lipid vesicles of phospholipids, fatty acid and cholesterol. These encapsulated Hb are referred to as 'hemosome'.[4,10]

Advantages

1) Immediately offloads O_2.
2) Longer shelf life.
3) Universal compatibility.
4) Immediate availability.
5) No refrigeration required.[4]
6) Are easily sterilizable.[21]

Disadvantages

1) They possess a very increased circulation half life.[4]
2) Reactions similar to foreign protein administration like allergy, headache, blood pressure transitions, fever and chills are encountered.[5]
3) Renal toxicity.
4) Anaphylaxis.[11]

Manufacturing Process Of HBOC'S

HBOC's can be obtained either by isolation or synthesis of Hb, followed by modification and then reconstitution in an artificial blood formula. There are 3 major sources of hemoglobin for the HBOC's; Human hemoglobin, bovine hemoglobin, and recombinant human hemoglobin.

Isolation of Hb can be done from human blood i.e. donated blood that has expired before use or from animal blood. As animal's blood Hb is quiet different from that of human's, it has to be modified prior to its use. The problem which exists while using naturally occurring source of Hb such as human or animal Hb is the probability of microbial contamination. Also there is a possibility of prion disease transmission with bovine Hb. The purification, modification and viral inactivation techniques adopted for the production of HBOC's prove hostile to micro organisms, eliminates them and hence prove to be a remedy to the above problem.

In order to produce Hb synthetically, amino acid, bacteria, warm water, glucose, molasses, alcohol, urea, liquid ammonia and acetic acid is used.[3,12]

Hemoglobin Synthesis

To obtain Hb, a strain of E.coli bacteria that is capable of producing human Hb is used. Fermentation process is adopted which consists of following steps:

1) Test tube containing all necessary nutrients which are required for the growth of bacteria is taken and to this, sample of pure bacteria culture is added.
2) This inoculation results in bacterial growth. When the population grows to an appreciable level, it is transferred to a seed tank which is a large stainless steel kettle providing an ideal environment for growing bacteria.
3) Warm water, food, ammonia source, amino acids, vitamins and other minor nutrients which are all required for the Hb production are added to the seed tank.
4) Bacterial solution is constantly stirred and bathed with compressed air.
5) After an appreciable period of time, the contents of seed tank are pumped to the fermentation tank.
6) Fermentation tank is filled with growth media which is required for bacterial growth and Hb production.
7) Ammonia water is added to the tank in adequate amount required for pH control.
8) When enough Hb has been produced, tank is emptied and Hb is isolated using centrifugal separator, which is further segregated and purified by fractional distillation.
9) In the final step, Hb is mixed with water and other electrolytes to produce artificial blood.[3]

HBOC Products and Their Current Status

1) Hemopure:

   Sponsored by: Biopure, Cambridge, Massachussets, USA.

   Description:

It is a glutaraldehyde cross linked polymerization product of bovine Hb, in which two or more tetramers are covalently linked. It was developed by OPK Biotech. It was initially designed by the company biopure, which was brought by OPK Biotech when the company the company went bankrupt. It is stable at room temperature for 3 years. It is currently approved for use in South Africa and has undergone some early clinical trials in the U.K.[22]

2) Hemolink:

Sponsored by: Hemosol Inc., Missisauga, Ontorio, Canada.

Description:

It is a raffinose polymerized HBOC.. It has been studied in phase II clinical trials in dialysis and as an O_2 carrying replacement fluid in acute normovolemic hemodilution. It has completed phase III trial in cardiac surgery in Canada, U.S. and Europe.[12]

3) Polyheme:

Sponsored by: Northfield Laboratories, Chicago, Illinois, USA.

Description:

It is a glutaraldehyde polymerized human Hb. It has been studied as an alternative to RBC's in trauma and surgery. Preliminary studies showed that the patients required fewer banked blood transfusions when transfused with PolyHeme. O_2 carrying replacement fluid in acute normovolemic hemodilution. It has completed phase III trial in cardiac surgery in Canada, U.S. and Europe.[12]

4) Oxyglobin:

Sponsored by: Biopure, Corp., USA.

Description: It consists of chemically stabilized bovine Hb. It has been licensed for veterinary use. It was approved by FDA and European Union for canine anemia in 1997 and 1998 respectively[6,12]

5) Hemospan:

Sponsored by: Sangart, San Diego, Canada.

Description:

It is prepared by conjugating human Hb tetramer to polyethyleneglycol (PEG). It is of two types; high concentration PEG-conjugated Hb (Hemospan); and low concentration PEG-conjugated Hb containing pentastarch (Hemospan PS). It has been designed to reduce vasoconstrictive effects. It has completed phase I and II trials and phase I trials have been published.[6,12]

6) Hem Assist:

Sponsored by: Baxter Healthcare, Deerfield, Illinois, US.

Description:

It consist of Hb, crosslinked using diaspirin which is then purified, heat inactivated and stored frozen. It reached phase III clinical trials but the trial was haulted in trauma, surgery and acute ischemic stroke in 1998. Mortality rate was high in traumatic and hemorrhagic stroke patients.[12]

7) PHP:

Sponsored by: Apex Bioscience Research Triangle, Park, North California.

Description:

It consists of pyridoxylated human Hb which is further conjugated to polyoxyethylene. It is being studied as nitric oxide scavenger in shock. It is under phase III clinical trial.[12]

8) Optro:

Sponsored by: SOMATOGEN (Baxter) Inc., Boulder, US.

Description:

It is the only recombinant human Hb produced by *E.coli.* In this two α subunits are joined to two β subunits by a short linker peptide. It is under phase I and II clinical trials.[12]

9) Oxyvita:

Sponsored by: OXYVITA, Inc., New York.

Description:

It is a new generation HBOC that is currently under preclinical testing.[6]

OTHER BLOOD REPLACEMENT ALTERNATIVES

1) Antigen Camouflage

It involves engineering the surface of RBCs for camouflaging, which would create universal blood type. The process involves coating the surface of RBC with a biocompatible polymer, PEG. PEG molecule forms permanent bonds on the cell surface. The coating masks the antigenic entity present on the surface of RBC so that they are not recognized as foreign bodies by the recipient's immune system.

2) Transgenic Therapeutic Proteins

It involves insertion of genes of human coagulation factors into the pig embryos, which results in the synthesis of corresponding human proteins in adult pig's milk which can then be purified for therapeutic use.

3) Platelet Substitute For Cancer

One of the major side effects associated with cancer therapy is thrombocytopenia, a clinical condition where platelet count decreases. So the products used in the treatment of thrombocytopenia includes Synthocytes.

These are the microcapsules to which fibrinogen is chemically linked which act as human blood platelet alternative in the bleeding prevention.[13]

APPLICATIONS OF ARTIFICIAL BLOOD

Artificial blood find its applications in the following fields:

1) During disasters or natural calamities to meet the increased demand of blood supply.[5]
2) Used as therapy in following situations: Hemorrhage, hemorrhagic shock, anemia, acute and evolving myocardial infarction, cardiac failure, brain infarction, acute arterial thrombosis and embolism, gas embolism.
3) As an organ recovery aid in acute renal failure, acute hepatic failure and acute pancreatitis.
4) In whole body rinse out in cases of drug intoxication and acute hepatic failure.
5) In cardiopulmonary bypass, deep hypothermia and cardioplegia, as perfusional organ protection during surgery.
6) In preservation of donor organ.
7) In tumor radiotherapy and chemotherapy.
8) As a contrast agent.
9) As culture media for tissues and bacteria.[10]
10) In cases of acute blood loss due to trauma or surgery.
11) In acute blood loss in Jehovah's Witnesses.
12) In erythropoiesis.[12]
13) In restoration of fluid volume.
14) In reduction of tumor hypoxia.
15) In preoperative hemodilution.[23]

CONCLUSION

Substances designed to transport O_2 and CO_2 throughout the body when introduced into the blood stream are called artificial blood products. They lack many blood functions such as immunity and coagulation. As they are meant only for O_2 delivery to vital organs, tissues and cells, they are correctly called as O_2 therapeutics. Two categories of artificial blood products have been developed each associated with benefits as well as risks. There are several companies working on the production of safe and efficacious blood substitute, with some being marketed

and others under clinical and preclinical trials. As the developed products suffer from their own drawbacks and short term blood replacement applications, long lasting products are under research to be developed.

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