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MANNERED BLOOD: A REVIEW

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Abstract

We live in a day and age where accidents are becoming more viable and people experience severe trauma. One of the most common effects is blood loss. Blood loss doesn't occur only because of accidents there are various other causes of draining of blood like anaemia, excessive menstrual bleeding etcetera. To make up for the lost blood from the body blood transfusions are performed. Though blood transfusions are safe, there are some complications like allergic reactions, there are chances for hives to occur, and fever may also be accompanied in some cases. It is very important to make sure that we get the blood in every emergency. Stability should be taken with utmost care. We cannot be incontrovertible that we can get blood in all emergencies. So, experts have made efforts to formulate artificial blood to overcome some major cons of biological blood transfusion. This literature review aims to elucidate the methods attempted to articulate blood substitutes and tell us which method is preferred and what is the current status of these substitutes. In addition to that, this paper makes us understand the present and future prospects of artificial blood and also talks about the pros and cons of artificial blood.

Keywords: *Blood loss, allergic reactions, blood transfusion, artificial blood, blood substitute*.

INTRODUCTION

Nearly humans are made of 1.2 to 1.5 gallons of blood. Blood is a fluid that aids in picking up toxic compounds like carbon dioxide from tissues and cells and helps in dispensing oxygen and essential nutrients to the same cells and tissues. Blood is categorically classified into solid and liquid. The liquid part of the blood is the plasma which is further made of water, sodium chloride, and some proteins. The solid part is the erythrocytes, thrombocytes, and leucocytes. Erythrocytes also termed red blood cells act as the carrier of oxygen, leucocytes also known as white blood cells help fight against foreign bodies, and thrombocytes known as platelets help in blood clotting.

In the developing world, people are very much prone to severe injuries caused by rash riding or some health complications that ultimately lead to one main intricacy: blood extravasates. Though

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there are many transfusions performed to meet the blood drain there is still a problem in obtaining the blood at the right time.[2] The increase in demand for blood to perform transfusions are skyrocketed. A person can contribute 2 units of blood in one donation which is not the right proportion for the blood loss existing nowadays. The units collected from humans cannot address the needs in the current medical world. Moreover, collected blood has to be processed and it must be stored in a safer place.[2]

Artificial blood or blood substitutes do not cover all biological blood properties. It can perform one of the most significant functions, which is the carrier of oxygen. Because of this imperative function, man-made blood is often referred to as an oxygen therapeutic agent. It is believed that artificial blood can provide oxygen at a rate faster than biological blood. Additionally, it can also reduce tissue damage in case of cardiac arrest.[3]

Artificial blood has covered all the cons of using biological blood. Artificial blood has grown as a favourable option in the current world. When it comes to man-made blood it must be able to serve all the works and purposes of biological blood. The artificial blood can perform one of the imperative functions which are distributing oxygen throughout the body and at the same time picking up carbon dioxide. In short, it can perform the activity of RBC.[3]

ARTIFICIAL BLOOD CLASSIFICATION

The four major elements of blood are RBCs, WBCs, plasma, and platelets. The advancing need made to evolve alternatives for all these components of blood. As studies point out that alternatives for blood are efficient in performing one therapeutic function of blood which is O_2 carrying i.e., the function of red blood cells.

This paper concentrates on the subclasses of 'RBC replacements' also called 'Artificial O_2carriers'.The three major subclasses of RBC replacements emerged and it includes:

- Perfluorocarbon-base
- Hemoglobin-base
- Stem cell-base

Also, there are other oxygen carriers which include:

- PEGylated hemoglobin – a HBOC
- Oxygen microbubbles
- Biomimetics
- De – novo designed proteins
- Polyactide – polyethylene membrane nano encapsulated polyhemoglobinsuperoxide dismutase – catalase – carbonic Anhydrase: Nano Artificial RBCs that act as oxygen and carbon dioxide carriers with Enhanced antioxidant activity

Perfluorocarbon (PFC) – based (Abiotic)

These are the first group of artificial blood products. PFCs also called organ fluorine compounds are colourless, chemically inert, synthetic molecules composed of carbon and fluorine atoms. Their ability to be used as oxygen-carrying agents was first described by Clark and Gollan in 1966. The interaction between these atoms forms a strong bond that is unaffected by chemical degradation [1].

Because of their hydrophobic nature, PFCs are emulsified for intravenous use which can dissolve gases better than other liquids which are due to fluorine's low polarizability which decreases Van der Waals interactions between PFC molecules where these interactions help to dissolve oxygen. PFCs in turn also have strong intramolecular bonds making them stable and weak intermolecular bonds allowing them to behave as gas-like liquids. PFCs are 100 times smaller than RBCs and are capable of carrying oxygen and carbon dioxide without binding to these gases [2].

The rate of intake and release of PFCs is not affected by temperature and environmental factors as it follows Henry's law that dissolved oxygen concentration at equilibrium at a given temperature is directly proportional to the gas partial pressure [10], which results in expeditious and extensive extraction of oxygen whenever needed. Thus, oxygenation is related to the partial pressure of oxygen that is in contact with PFCs. Therefore, better results are obtained if the patient is breathing 100%

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oxygen at the time of infusion (PaO2 > 350 mm Hg). The molecules after infusion are exhaled via the lungs over days [2].

Productsdeveloped

a- Fluosol-DA (Green Cross Corp., Osaka, Japan) was the first generation of PFC emulsions to be produced over 30 years ago. Fluosol was made up of a 7 to 3 ratio of perfluorodecalin and perfluorotripropylamine, with the emulsifier pluronic F-68 (a combination of short-chain linear polymers; Wyandotte Chemicals Corp., Wyandotte, MI). It was offered as a 20% solution capable of transporting a 7.2 percent volume of 100% oxygen at 37°C. At a hemoglobin level of 14 g/dl12, this molecule could also carry 34% of the oxygen content of whole blood. The first known usage of this commercially accessible medicine was in Japan, where 500 mL and 1000 mL infusions were administered to patients with severe gastrointestinal bleeding [1].

b- Oxygent is a second-generation concentrated PFC that is stable. Perfluorooctyl bromide and perflubrodec were the active components in the original formulation, which had 60 grams PFC/dL. The latter was supplied in tiny amounts to keep particle growth under control during storage15. An egg-yolk phospholipid was utilized as an emulsifier for PFCs in a buffered electrolyte solution to create this product. When stored between 2 and 8 degrees Celsius, the finished product had a shelf life of about 24 months, but it was stable enough to withstand a few weeks at ambient temperature. Before moving on to research that established efficacy in humans, over 250 preclinical investigations were conducted to understand the safety of Oxygent in diverse animal species [1].

Current third-generation PFCs include Oxycyte and Perftoran**.**

c- Oxycyte is a 60% emulsion of perfluoro-tert-butyl cyclohexane with purified egg yolk phospholipids. Preclinical trials of oxycyte in a spinal cord and traumatic brain injury were encouraging, but due to difficulty with patient involvement, a secondary US clinical trial was terminated in the year 2014 [10].

d- Perftoranis an emulsion in a nonionic surfactant (Proxanol 268) of perfluorodecalin and perfluoromethylcyclopiperidine. This offers an advantage of even smaller particulate sizes (70 mm) due to non-phospholipid surfactant. Theoretically, this property should result in the reduction of side effects caused by previous generations. This can be stored in the frozen state for years and should be defrozen carefully and can survive at 4 degrees Celsius for two weeks. This product has not undergone any US clinical trials and is not FDA approved [10].

Drawbacks

First-generation PFCs were responsible for complement activation. It is also known to cause flu-like symptoms which are a result of opsonization and phagocytosis of PFC emulsion by the receiver immune system [2].

- Both first and second-generation PFCs had their fall with oxygen supplements, as patients had to receive supplemental oxygen intake as the supply was less than 30% of normal blood [1].
- As the earliest clinical trials showed a lack of beneficial results, no current trials are being performed with PFCs. This ultimately results in denied approval from FDA, unlikely that PFCs are not productive blood substitutes [1].
- But, Toxicology investigations have demonstrated that the emulsion is well tolerated and does not cause serious side effects when administered at the correct and appropriate clinical dose (1.0 – 6.0 ml/kg) [1].

Haemoglobin - based blood substitutes (Biotic)

Hemoglobin (Hb) is the iron-containing oxygen-carrying component of red blood cells. Hb has 2alpha and 2beta chains which are bonded with the iron haem group that binds to oxygen.

Hemoglobin-based oxygen carrier (HBOC) agents are derived from hemoglobin which is isolated or synthetically manufactured. This act as a better alternative that would efficiently deliver oxygen to tissues without any toxicity. Currently, developed products are not successful in getting FDA approval under clinical trials but HBOC are approved and are being used in two countries- Russia and South Africa outside of the US [1].

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In HBOCs oxygen binds covalently as they do to natural hemoglobin. The source of hemoglobin for HBOC can be either human, obtained from outdated stored blood or bovine, or genetically engineered. The blood substances, viruses, and proteins present if any are removed by heating and filtration. Isolated hemoglobin is subjected to molecular modification and reconstitution in an artificial blood formula.

Manufacturing process

Extraction of hemoglobin – stabilization 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[2].

The following are the available hemoglobin solutions based on the improving stability:

a- Surface modified haemoglobin (PEG Hb, PHP, Haemospan)

Produced by the attachment of large molecules like polyethylene glycol to the surface lysine group. This increases the viscosity and oncotic pressure of the solution. The small size of these haemoglobin molecules allows them to pass through smaller vessels that cannot be reached by RBCs.

Useful in the treating – patients with stroke, increased susceptibility of tumour cells to radiation and chemotherapy,and vasopressor effects – used to treat hypotension followed by septic shock[2].

b- Intramolecular cross linked haemoglobin (HemAssist, r-Hb-1, r-Hb- 2-0)

Intermolecular cross-linking between 2alpha and 2 beta subunits using a point-specific crosslinker that cross-links these chains is performed for the tetrameric stabilization and prevention of renal filtration. Commonly used cross-linkers include 3,5-dibromosalicyl fumarate (DBBF) and nor-2-formylpyridoxal 5-phosphate (NFPLP).But cross-linking decreases the affinity of haemoglobin to oxygen[2].

c- Polymerised haemoglobin (Polyheme, Hemopure, Hemolink)

Amino acid groups on the surface are linked by reagents like glutaraldehyde. This has shown a negligible amount of side effects. Patients undergoing infrarenal aortic reconstruction infused with polymerized haemoglobin have shown avoidance of allogeneic blood transfusion in 27% of patients[2].

d- Liposomes encapsulated haemoglobin (Haemosomes)

Haemoglobin after purification is re encapsulated with a stable lipid membrane.

Liposomes are made of a phospholipid bilayer with cholesterol molecules added for enhancing mechanical stability and rigidity and enclose a stroma-free hemoglobin solution and 2,3 disphosphoglycerate or inositol hexaphosphate is a gelatinous fluid. Ultraviolet radiations or redox inhibitors are used for added stabilization that results in the polymerization of unsaturated phospholipids. Also, coating liposomes with polymers results in stabilization.

The oxygen affinity of these chromosomes can be increased by co encapsulating an allosteric effector like pyridoxal 5' phosphate.

Also, encapsulation prevents denaturation of Hb and enhances biodistribution. Modification with polyethylene glycol increases half-life makes them water-soluble decreases antigenicity and improves site-specific targeting[2].

Productsdeveloped

a-The **Stroma-freehaemoglobin** (SFH) products were the first generation of blood replacements. The SFHs were made by lysis of packed RBCs, which produced soluble hemoglobin. After centrifuging the mixture to remove the majority of red cell stroma, SFH was produced. The technique produced 500 ccs of a 7 g/100 ml hemoglobin solution with normal physiologic sodium, potassium, and bicarbonate concentrations. The solution had a pH of 7.1 to 7.2 and an osmolality of 270 to 280 mOsm/kg. The concentration of methemoglobin ranged from 7 to 12 percent of total hemoglobin and did not change significantly over a four-week storage period at 4oC27. Ultrafiltration or crystallization could be used to make SFHs. The ultrafiltration-prepared SFH was studied [1].

b-Chemical modification of SFH produces **Pyridoxilatedhemoglobin-**

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polyoxyethyleneconjugates(PHPCs) which are the second generation HBOCs.These products have been designed mainly to overcome the major disadvantage of SFHs i.e., increases oxygen affinity, short circulatory half-life, and nephrotoxicity. PHPCs are prepared after getting the SFH; free hemoglobin is pyridoxilated (addition of Vit B6) to adjust the oxygen affinity and conjugated later with α-carboxymethyl-ω-carboxymethoxy-polyethylene to increase molecular weight and long circulatory half-life [1].

The second-generation HBOCs developed are:

Polyheme (Northfield laboratories, Evanston, IL)**:** Polyheme - location of clinical use in the United States, not approved by FDA; reached phase 3 US clinical trials, but approved by South Africa; currently phase 2 trials are on hold due to safety issues.

Hemopure also called HBOC-201 (Hemoglobin Oxygen Therapeutics LLC, Souderton, PA):Hemopure - location of clinical use in the United States and Europe, not approved by FDA; but available through FDA expanded (compassionate use) access program- this allows the product to be used for patients with severe life-threatening anemia left with no option to be treated.

Hemolink(Hemosol Inc. Toronto, Canada): Hemolink - location of clinical use North America, not approved by FDA reached phase 3 trials. Biophysical properties include oxygen-carrying characteristics with non-cooperative behavior. Ongoing trials on sickle cell anemia patients and in cardiac surgery.

c- Hemoglobin crosslinked between the α chains with bis(dibromosalicyl) fumarate (DBBF) or αα-hemoglobin forms the third generation HBOCs. This product was developed at Letterman Army Institute of Research (LAIR) in San Fransisco, CA.

HemAssist developed by Baxter International Corporation (Deerfield, IL) is diaspirin-crosslinked hemoglobin (DCLHB). The products developed were more similar to support the toxicological and physiological experiments and had similar oxygen affinity to that of blood. HemAssist - location of clinical use in the US, not approved by FDA reached phase III trials but halted due to low efficacy and safety record.

They are produced from outdated human or bovine blood, with sterile saline washing the RBCs to remove all plasma traces and then subjected to hypertonic lysis. The remaining material is filtered out allowing the purified hemoglobin for crosslinking and the resultant product is purified crosslinked hemoglobin with a total yield of 55% - 58%. To maintain specific crosslinking between lysine 99α residues, an allosteric effector (2,3 DPG pocket) was added that keeps hemoglobin deoxygenated. Finally, the DBBF reactor was added and the mix was heated to remove unreacted hemoglobin and pathogens. The final product resulting is Ringer's lactate or Ringer's acetate that can be stored frozen for up to one year under -20degreeC [1].

Drawbacks

- Clinical testing of the HBOCs has raised other safety concerns related to vasoactivity and cell toxicity, the latter either as a direct effect or onemediated by oxidative product.
- One of the most feared limitations of HBOCs is blood vessel constriction which leads to hypo-oxygenation and subsequently leads to an increase (high level) in systemic and pulmonary blood pressure. Studies show that free Hb scrounging nitric oxide (NO) is the major mediating factor for vasoconstriction. Compared with RBCs, HBOCs lack the free-radical scavenging system that includes superoxide dismutase and Hb reductase.
- Fe (III) Hb produced during Hb autoxidation releases heme which is an additional source of oxidative stress and reactions in the plasma.
- Methemoglobin is a degradation product, whose making can be held by the enzyme methemoglobin reductase in erythrocytes.
- HBOCs have the natural ability to produce oxidative-free radicals, capable of inducing cell damage and terminating biochemically essential processes.
- Also, HBOCs cause reperfusion because of their high oxygen content [1].

Table 1: Products developed

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Perfluorocarbon - based	Haemoglobin - based
Fluosol- DA	Stroma free hemoglobin (SHF)
Oxygen	Pyridoxilatedhemoglobin-Polyoxyethylene conjugates
Oxycyte	Polyheme (Northfield laboratories, Evanston,IL)
Perftoran	Hemopure also called HBOC-201
	Hemolink HemAssist

Stem cell based – Laboratory grown RBC's

RBCs whose function is to carry oxygen can be produced directly from stem cells under laboratory conditions.

Red cells are produced from human embryonic stem (hES) cells obtained from blastocysts. A medium containing various growth factors and transcription factors is used for culturing isolated cells. These factors stimulate the cells to differentiate into all the components of blood cells including Red Blood Cells.

But the efficiency and future of transfusing RBCs derived from hES are however complicated by immune rejection, as these could be from an allogeneic graft.

Also, ethical issues exist relating to the origin and the need to destroy human embryos while sourcing.

Noble prize-winning work of Takahashi et al. demonstrated that the donor cells can be reprogrammed back to their pluripotent form and can be developed into RBC. However, the quality and yield of RBCs resulting from both hiPS and hES are merely limited.

In 2017, the work performed by Trakarnsanga et al. changed the field by differentiating a large number of RBCs produced from an immortalized adult erythroid line. These cells were identical to biological RBCs biochemically and morphologically. They captured and transported oxygen and persevered for a 9 days study duration. This novel development is still existing because of its potential for RBC production of any blood group on an industrial scale.

Ongoing studies are being conducted to verify safety, especially for oncogenic potential.

The huge production cost (estimated approximately at the US $8,000-15,000/unit) must be managed. Finally adhering to Good Manufacturing Practices standards must make sure that this methodology is to be adopted for extensive use and is not limited to patients with rare blood groups and genetic/inherent disorders [10].

SATISFACTORY PURPOSE OF BLOOD SUBSTITUTES

● Normally, biological blood requires one day to restore the oxygen levels after transfusion because they are degraded by 2,3 diphosphoglycerate but these substitutes have a quick and quality oxygen dispensation compared to original blood.

● The hoarding period for artificial blood is about 1 to 3 years, which is longer than normal blood. They can be stocked at room temperatures and do not require an extra relegation period.

● It is more compatible than the biological blood because of the excavation of protein molecules and so our body's defensemechanism does not recognize the foreign bodies. So, there is no need for the performance of the compatibility test.

● If biological has to be transfused it has to be checked for any allergies or infections before transfusing. But the risk of infections and anaphylactic reactions can be avoided with artificial blood substitutes.

● Very pious People do not accept blood from other people, they consider it profane and they refuse the blood. Such a condition won't happen with this artificial blood because it is chemically made.

● Some of the other advantages are the non-appearance of renal defacement, dodging of hazardous vestigial stroma, fruitful vascular halflife, and skyrocketed blood and oxygen delivery even to the capillary bed.
● When it comes to cost, blood is costlier because it includes hoarding prices, lancing costs, and the process of donor find but all the above-mentioned costs are not included in blood substitutes.

CURRENT AND FUTURE PROSPECTS OF BLOOD

In terms of clinical practice, there are several potential emerging developments in perioperative transfusion. Among these are shifts in transfusion practice blood bank operations and philosophy, the use of autologous transfusion procedures, and the development of novel artificial blood replacements. Current methods will be fine-tuned, and a variety of factors will play a role. The most significant recent advancements in surgical blood transfusion practice are a decrease in reliance on random transfusion triggers and an increase in the use of various kinds of autologous transfusion. Other therapeutic adjustments, such as changes in blood bank management practice, have been less obvious. The reasons for blood conservation, artificial blood replacements, and oxygen carriers are all the same. Both are going to have an effect.

CONCLUSION

Taking into account the studies on artificial blood, developed products are not successful in getting FDA approval under clinical trials but HBOC are approved and are being used in two countries- Russia and South Africa outside of the US. Keeping this as the only source the complete need for artificial blood cannot be satiating the demand for biological blood transfusion. Currently, experts are developing an easy and feasible manufacturing process for manmade blood and trying to replicate the entire function of biological blood.

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