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Review Article

ARTIFICIAL BLOOD: A TOOL FOR SURVIVAL OF HUMANS

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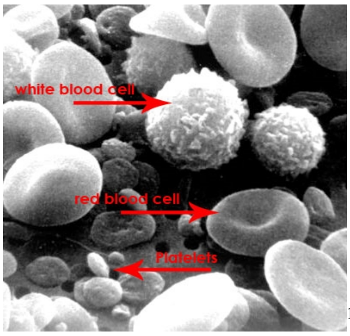
ABSTRACT
Blood is a specialized body fluid that delivers necessary substances to the body cells such as nutrients and oxygen and transports waste products away from those cells. Artificial blood is supposed to fulfil some functions of biological blood, especially in humans. The initial goal of oxygen carrying blood substitutes is merely to mimic blood's oxygen transport capacity. There is additional longer range research on true artificial RBCs and WBCs which could theoretically compose a blood substitute with higher fidelity to human blood. Unfortunately, oxygen transport has been very difficult to reproduce. There are two basic approaches to construct oxygen therapeutic: perflurocarbons (PFCs), a chemical compound which can carry and release oxygen. The specific PFC usually used is perfluorodecalin. Perfluorochemicals will not mix with blood; therefore emulsions must be made by dispersing small drops of PFC in water. This liquid is then mixed with antibiotics, vitamins, nutrients and salts, producing a mixture that contains about 80 different components, and performs many of the vital functions of natural blood. This can benefit damaged, blood-starved tissue, which conventional RBC cannot reach. Therefore the artificial blood is a good tool for the survival of patients at the time of surgery when blood loss is higher. By providing a PFC solution we can maintain the circulating blood volume as well as the need of the patients. PFC solutions can carry oxygen so well that mammals and humans can survive breathing liquid PFC solution, called liquid breathing.
Keywords- Blood, Perflurocarbons, Antibiotics, WBC, RBC

INTRODUCTION

It is very amazing, that an artificial substance could replace something that is so central to human life. To understand the process, it helps to know a little about how real blood works. Blood has two main components -- plasma and formed elements. Nearly everything that blood carries, like nutrients, hormones and waste, is dissolved in the plasma, which is mostly water. Formed elements, which are cells and parts of cells, also float in the plasma. Formed elements include white blood cells (WBCs), which are part of the immune system, and platelets, which help forming clots. Red blood cells (RBCs) are responsible for one of the blood's most important tasks -- carrying oxygen and carbon dioxide.

RBCs are numerous. They make up more than 90 percent of the formed elements in the blood. Virtually everything about them helps them carry oxygen more efficiently. A RBC is shaped like a disc that's concave on both sides, so it has lots of surface area for oxygen absorption and release. Its membrane is very flexible and has no nucleus, so it can fit through tiny capillaries without rupturing. A red blood cells lack of nucleus also gives it more room for haemoglobin (Hb), a complex molecule that carries oxygen. It's made of a protein component called a globin and four pigments called heme. The hemes use iron to bond to oxygen. Inside each RBC are about 280 million haemoglobin molecules.

If you lose a lot of blood, you lose a lot of your oxygen delivery system. The immune cells, nutrients and proteins that blood carries are important, too, but doctors are generally most concerned with whether your cells are getting enough oxygen. In an emergency situation, doctors will often give patients volume expanders, like saline, to make up for lost blood volume. This helps restore normal blood pressure and lets the remaining red blood cells continue to carry oxygen. Sometimes, this is enough to keep the body going until it can produce new blood cells and other blood elements. If not, doctors can give patents blood transfusions to replace some of the lost blood. Blood transfusions are also fairly common during some surgical procedures.



[1]

Fig. 1 A scanning electron microscope image from normal circulating human blood

This process works pretty well, but there are several challenges that can make it difficult or impossible to get patients the blood they need:

- Human blood has to be kept cool, and it has a shelf life of 42 days. This makes it impractical for emergency crews to carry it in ambulances or for medical staff to carry it onto the battlefield. Volume expanders alone may not be enough to keep a badly bleeding patient alive until he reaches the hospital.
- Doctors must make sure the blood is the right type -- A, B, AB or O -- before giving it to a patient. If a person receives the wrong type of blood, a deadly reaction can result.
- The number of people who need blood is growing faster than the number of people who donate blood.
- Viruses like HIV and hepatitis can contaminate the blood supply, although improved testing methods have made contamination less likely in most developed countries.

Artificial blood doesn't do all the work of real blood. Sometimes, it can't even replace lost blood volume. Instead, it carries oxygen in situations where a person's red blood cells can't do it on their own. For this reason, artificial blood is often called oxygen therapeutic**.** Unlike real blood, artificial blood can be sterilized to kill bacteria and viruses. Doctors can also give it to patients regardless of blood type. Many current types have a shelf life of more than a year and don't need to be refrigerated, making them ideal for use in emergency and battlefield situations. So even though it doesn't actually replace human blood, artificial blood is still pretty amazing.[1]

ARTIFICIAL BLOOD CELLS

Artificial blood or blood surrogates is a substance used to mimic and fulfil some functions of biological blood, usually in the oxygen-carrying sense. The main aim is to provide an alternative to blood transfusion, which is transferring blood or blood-based products from one person into another.[2] Unfortunately, oxygen transport has proven very difficult to achieve artificially, and all efforts as of 2011 have failed to overcome the challenges.[3] Artificial blood does not contain the plasma, red and white cells, or platelets of human blood, but functions to transport and deliver oxygen to the body's tissues until the recipient's bone marrow has regenerated the missing red blood cells.[4] Artificial blood can be produced in different ways using synthetic production, chemical isolation, or recombinant biochemical technology.[5] Current blood substitutes are either haemoglobin-based oxygen carriers (HBOCs) or perfluorocarbons (PFCs). While HBOCs utilize haemoglobin, an actual component of red blood cells, PFCs rely solely on synthetic chemical processes. Other novel products are in very early stages of development. None of the products perform all the functions of blood; neither do they persist in the circulation as long as human red blood cells. However, they all carry and transport oxygen to tissues and can support life temporarily until patients can either regenerate their own red cells or can be transfused with banked blood. In the short term, the prospective benefits of a blood substitute overshadow the shortcomings. In addition to carrying oxygen, such compounds can be sterilized against infectious diseases and used in patients whose religious beliefs prevent them from accepting blood transfusions. [6]

COMPOSITION OF ARTIFICIAL BLOOD

Perfluoro-octyl bromide	-	28%
FO-9982	-	12%
Yolk lecithin	-	2.4%
DSPE-50 H	-	0.12%
Distilled water	-	57.48% [7]

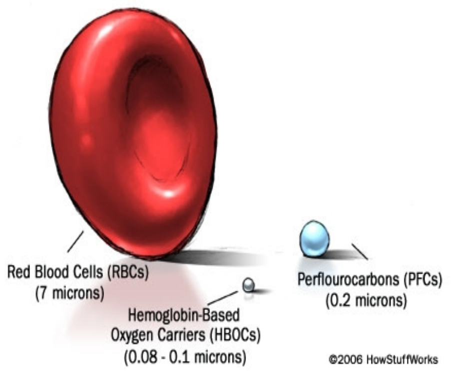


Fig. 2 Both HBOCs and PFCs are considerably smaller than red blood cells. [1]

PFBOCs

In 1966, scientists synthesized “oxygen therapeutic” that survived in mice. PFBOCs achieve oxygen delivery by using organic chemicals with high gas solubility. The perfluorinated carbons are chemically and biologically inert but are able to dissolve a large amount of gas. One of the problems with perfluorocarbons is that they are an oil-like fluid that does not mix well with water and cannot carry water-soluble salts and metabolic substrates. Now a day’s most of the PFBOCs are mixtures of perfluorocarbons with emulsifying agents. Emulsifying agents are substances that help stabilize two seemingly unbendable things. PFBOCs utilize Puronic-68, egg yolk phospholipids and triglycerides as emulsifying agents.

Green Cross Corp. in Osaka Japan developed the first PFBOC with a mixture of perfluorodecalin and perfluorotripropylamine and using egg yolk phospholipids and Pluronic-68 as emulsifying agents. The product was called Fluosol-DA. The problem with Fluosal-DA was that they dissolve less oxygen than pure liquids. It could only deliver 0.4 ml oxygen per 100 ml. In order to meet metabolic oxygen demand, the patients would have had to breathe in gas that was 100% oxygen which would lead to adverse effects due to oxygen toxicity. More recently, Alliance Corp developed a mixture of Perfluoro-octyl bromide and egg yolk phospholipids as the emulsifying agent. Oxygent, their product, could deliver up to 1.3 ml oxygen per 100 ml, but this is still much lower than normal blood which could deliver blood at 5 ml oxygen per 100 ml. The advantage of Oxygent over Fluosal-DA is that it has a longer circulation time, and it can be excreted from the body in 4 days compared to Fluosal –DA which could take months.[8]

ADVANTAGES OF PERFLUOROCARBONS (PFC) EMULSIONS-

- PFCs do not react with oxygen.
- PFCs allow easy transportation of the oxygen to the body.
- They allow increased solubility of oxygen in plasma.
- PFCs minimize the effects of factors like pH and temperature in blood circulation.

DISADVANTAGES OF PERFLUOROCARBONS (PFC) EMULSIONS-

- Often causes flu-like symptoms.
- This is often caused by phagocytosis of the perfluorocarbons emulsion by the recipient organism’s immune system
- Unable to remain mixed as aqueous solutions – thus, they must be prepared as emulsions for use in patients.
- A decrease in blood platelet count.
- PFC products cannot be used by the human body, and must be discarded.
- This takes approximately 18-24 months.
- PFCs absorb oxygen passively, patients must breathe at a linear rate to ensure oxygenation of tissues.[9]

HBOCs

HBOCs are oxygen carriers that use purified human, animal or recombinant haemoglobin. The first major clinical study with purified haemoglobin resulted in nephrotoxicites, poisonous effects on the kidney. The haemoglobin used was found to have ethrocyte membrane stromal lipids as well as bacterial endotoxins. To side-step these problems, stroma-free haemoglobin were developed that also was free of endotoxins and it did reduce nephrotoxicity but new problems arose. The stroma-free haemoglobin was found to have too high of an affinity for oxygen which affected oxygen being delivered to tissue. Today, many new processes have been developed that fix these problems.[7] In 1898 haemoglobim solution was firstly used for the treatment of anaemia. Various researchers modified Hb solution to form purified Hb solution. Currently, HBOCs represent an interesting class of blood substitutes, which are undergoing advanced clinical trials. The therapeutic goal of these compounds is to avoid or reduce blood transfusion in different surgical and medical situations of acute Hb deficiency. The main advantages of artificial blood include availability in large volumes, storage for prolonged periods, rapid administration (without typing and cross matching) and sterilisation by pasteurisation. Their main known disadvantages are, reduced circulation half-life, hemodynamic and gastrointestinal perturbations, probably related to nitric oxide (NO) scavenging, free radical induction, and alterations of biochemical and haematological parameters (increase in liver enzyme levels, platelet aggregation).[10]

Synthetic haemoglobin based products are produced from haemoglobin harvested from an E. coli bacteria strain. The haemoglobin is grown in a seed tank and then fermented.

ADVANTAGES OF HEMOGLOBIN-BASED OXYGEN CARRIERS (HBOCS)-

- Available in much larger quantities.
- Can be stored for long durations.
- Can be administered rapidly without typing or cross-matching blood types.
- Can be sterilized via pasteurization.

DISADVANTAGES OF HEMOGLOBIN-BASED OXYGEN CARRIERS (HBOCS)-

- Reduced circulation half-life
- Disrupts certain physiological structures, especially the gastrointestinal tract and normal red blood cell haemoglobin.
- They release free radicals into the body.[9]

IDEAL CHARACTERISTICS OF ARTIFICIAL BLOOD-

1. Safe to use.
2. Compatible in human body.
3. Able to transport and release oxygen where needed.
4. Storable and durable for longer time periods.
5. Is free of pathogens and toxins which would produce an immune system response in the human body.

USES OF BLOOD SUBSTITUTES-

1. Donations are increasing by about 2–3% annually in the United States, but demand is climbing by between 6–8% as an aging population requires more operations that often involve blood transfusion.
2. The blood supply in many countries is very safe but this is not the case for all regions of the world. Blood transfusion is the second largest source of new HIV infections in Nigeria. In certain regions of southern Africa, it is believed that as much as 40% of the population has HIV/AIDS, although testing is not financially feasible. A disease-free source of blood substitutes would be incredibly beneficial in these regions.
3. In battlefield scenarios, it is often impossible to administer rapid blood transfusions. Medical care in the armed services would benefit from a safe, easy way to manage blood supply.
4. Great benefit could be derived from the rapid treatment of patients in trauma situations. Because these blood substitutes do not contain any of the antigens that determine blood type, they can be used across all types without immunologic reactions.
5. Transfused blood is currently more cost effective, but there are reasons to believe this may change. For example, the cost of blood substitutes may fall as manufacturing becomes refined.
6. Artificial Blood can be stored for much longer period than transfusable blood, and can be kept at room temperature. Most haemoglobin-based oxygen carriers in trials today carry a shelf life of between 1 and 3 years, compared to 42 days for donated blood, which needs to be kept refrigerated.
7. Blood substitutes allow for immediate full capacity oxygen transport, as opposed to transfused blood which can require about 24 hours to reach full oxygen transport capacity due to 2,3-diphosphoglycerate depletion. Also, in comparison, natural replenishment of lost red blood cells usually takes months, so an oxygen-carrying blood substitute can perform this function until blood is naturally replenished**.**
8. Oxygen carrying blood substitutes also would become an alternative for those patients that refuse blood transfusions for religious or cultural reasons, such as Jehovah's Witnesses.

Hemoglobin-based blood substitutes boost the odds of death and heart attack for recipients. [11] Analysis showed that an artificial blood substitute increased risk for mortality more than usual care in patients who had surgery, trauma, or stroke.[12]

CURRENT THERAPEUTICS-

Perfluorocarbons (PFC) based-

Perfluorochemicals cannot mix with blood, therefore emulsions must be made by dispersing small drops of PFC in water. This liquid is then mixed with antibiotics, vitamins, nutrients and salts, producing a mixture that contains about 80 different components, and performs many of the vital functions of natural blood. PFC particles are about 1/40 the size of the diameter of a red blood cell (RBC). This small size can enable PFC particles to traverse capillaries through which no RBCs are flowing. This can benefit damaged, blood-starved tissue, where conventional red cells cannot reach. PFC solutions can carry oxygen so well in mammals, including humans, can survive breathing liquid PFC solution, called liquid breathing.

Fig. 3 PFC-based artificial blood made by Oxygent

Perfluorocarbons-based blood substitutes are completely man-made; this provides advantages over blood substitutes that rely on modified haemoglobin, such as unlimited manufacturing capabilities, ability to be heat-sterilized, and PFCs' efficient oxygen delivery and carbon dioxide removal. PFCs in solution act as an intravascular oxygen carrier to temporarily augment oxygen delivery to tissues. PFCs are removed from the bloodstream within 48 hours by the body's normal clearance procedure. PFC particles in solution can carry several times more oxygen per cubic centimetre (cc) than blood, while being 40 to 50 times smaller than haemoglobin.

Name	Sponsor	Description
Oxygent	Alliance Pharmaceuticals	Oxygent is currently approved for Phase II Trials in US and Europe. It is developed to reduce the need for donor blood during surgery. PFCs are surrounded by a lecithin surfactant in a water-based solution. The lecithin is digested intracellularly. Oxygent has done well overall in most clinical trials, but recently, a cardiac surgery study found participants to be slightly more likely to suffer if treated with Oxygent rather than by standard care.
Oxycyte	Oxygen Biotherapeutics	Oxycyte is currently approved for Phase II-b Trials in the United States. Oxycyte Targeted as an oxygen therapeutic rather than a blood substitute, with successful small scale open label human trials treating traumatic brain injury at Virginia Commonwealth University.[13]
PHER-O_2	Sanguine Corp	In research
Perftoran	Russia	Approved for Russian clinical application in 1996. Registered in Mexico as PERFTEC, distributed by KEM Laboratory (Mexico). Status: Approved an authorized blood substitute in Mexico in 2005.[14] Perftoran facilitates oxygen delivery together with remaining red blood cells at blood replacements and will have wider area for application than just a blood substitute. Its infusion alleviates symptoms of ischemia at different types of occlusion vessels disease, improves grafting in plastic surgery, diminishes inflammation and prevents rejection of transplants, activates detoxication functions of liver, inhibits retro-virus infection development. Local Perftoran applications are able to accelerate wounds and ulcers healing.[15]

Haemoglobin based

Haemoglobin is the main component of red blood cells, comprising about 33% of the cell mass. Haemoglobin-based products are called haemoglobin-based oxygen carriers (HBOCs). However, pure haemoglobin separated from red cells cannot be used, since it causes renal toxicity. It can be treated to avoid this, but it still has incorrect oxygen transport characteristics when separated from red cells. Various other steps are needed to form haemoglobin into useful and safe oxygen therapeutic. These may include cross-linking, polymerization, and encapsulation. These are needed because the red cell is not a simple container for haemoglobin, but a complex entity with many biomolecular features.

Name	Sponsor	Description
Hemopure	Biopure Corp	Hemopure is currently approved for Phase III trials in the United States and was more widely approved in South Africa. It is Biopure's first-in-class product for human use, and is a HBOC solution. It is made of chemically stabilized, cross-linked bovine (cow) haemoglobin in a salt solution. Many safety measures are taken to render free of pathogens, including herd control and monitoring. Its molecules can be up to 1/1,000 the size of RBCs, facilitating oxygen transport and off-loading to the tissues.[16]
Oxyglobin	Biopure Corp	Oxyglobin is currently approved for veterinary use in US and Europe. Oxyglobin solution is the first and only oxygen therapeutic to be both US FDA and European Commission approved for veterinary use. It consists of chemically stabilized bovine haemoglobin in a balanced salt solution and contains no red blood cells. The cross-linked haemoglobin, several tetramers bound together, works by circulation in the plasma and supplying oxygen to tissues. Introduced to veterinary clinics and hospitals in March 1998 and nationally distributed by October 1998, Oxyglobin has been used primarily for blood transfusions and for treatment of anaemia in dogs. Currently, Oxyglobin can only be used in canines and not in humans.
PolyHeme	Northfield Laboratories	PolyHeme is a unique human hemoglobin-based oxygen-carrying blood substitute in development for the treatment of urgent, large volume blood loss in trauma and surgical settings, with a particular focus on settings where blood is not immediately available. It is the only blood substitute that has completed a Phase III trial. It represents the leading technology in this field. Originally it began as a military project following the Vietnam War, and has shown great potential for both military and civilian use. It uses human haemoglobin as the oxygen-carrying molecule in solution, and the extraction and filtration of this haemoglobin from red blood cells is the first step in production. Using a multi-step polymerization process, the purified haemoglobin is associated into tetramers and is incorporated into an electrolyte solution. The polymerization of the haemoglobin represents the critical step in this process because, as demonstrated by failed attempts at blood substitutes, when haemoglobin remains disassociated, it tends to take up nitric oxide, causing vasoconstriction. Also, free haemoglobin can be taken up by the kidney, causing dysfunction and failure, similar to a hemolytic transfusion reaction. [17]
Hemospan	Sangart	Hemospan is currently in Phase II trials in the United States. It is produced by the company Sangart, which was found by Dr. Robert M. Winslow in 1998. It is produced in powder form, which can then be mixed into liquid form and transfused immediately, regardless of a patient's blood type. This technology relies on coupling with polyethylene glycol (PEG) to eliminate the toxicity associated with free haemoglobin. According to researchers haemospan can be stored for a year. The scientist has optimized certain factors which are involved in oxygen delivery in the production of Hemospan. In the past four years, Hemospan has shown promise as a possible commercial product, yielding positive results in both Phase Ib/II and Phase II clinical trials.
Dextran-Haemoglobin	Dextro-Sang Corp	Dextran-Haemoglobin is currently in vetrinary trials. It is created by the Dexto-Sang Corporation. It is a conjugate of the polymer dextran with human haemoglobin molecules. The safety of dextran has already been established, due to its wide use as a plasma volume expander. Conjugation of haemoglobin to dextran increases its half-life inside the body, and prevents tissue damage that occurs with free haemoglobin from processing by the kidneys and exit into the extracellular space.
Hemotech	HemoBiotech	Hemotech is currently approved for Phase I trials. HemoBiotech, based in Dallas, is developing Hemotech, a human blood substitute developed in 1985 by researchers, Mario Feola, MD and Jan Simoni, PHD, DVM from the Texas Tech University Health Sciences Center. It has been able to identify and nullify the source of toxicity issues associated with previous blood substitute candidates. It is expected to be compatible will all blood types and has a shelf life of 180+ days compared to 41 days for donated human blood. Hemotech's lack of toxicity is due to Hemobiotech's proprietary chemical modification of haemoglobin. The company believes the use of bovine blood provides an additional advantage over products developed from outdated human red blood cells or from perfluorochemicals (PFCs), as bovine blood is more readily available and more cost-effective to use. Limited tests have shown it to be clear of the vasoconstriction and inflammatory toxicity issues that have hampered competitors. [18]

ARTIFICIAL BLOOD vs. BLOOD SUBSTITUTES

Artificial Blood	Blood Substitutes
The main purpose of artificial blood is to act as normal blood in the body, providing a long-term solution to blood loss or distortion. No working artificial blood has been created in the status quo	The main purpose of a blood substitute is to provide temporary support to the circulatory system when necessary. Blood substitutes generally are focused on the role of transporting oxygen for short-term cases such as blood transfusions or surgeries. Blood substitutes are generally simpler since they are only focused on one of the several functions of real blood. Several types of blood substitutes have been found including: Hemoglobin-based oxygen carriers Perfluorocarbons emulsion.[9]

Hence we can conclude that the artificial blood is a good tool for the survival of patients at the time of surgery when blood loss is higher. By providing a blood substitute we can maintain the circulating blood volume as well as the need of the patients. The blood substitutes carry and transport oxygen to tissues and can support life temporarily until patients can either regenerate their own red cells or can be transfused with banked blood. PFC solutions can carry oxygen so well that mammals and humans can survive breathing liquid PFC solution, called liquid breathing. In addition to carrying oxygen, such compounds can be sterilized against infectious diseases and used in patients whose religious beliefs prevent them from accepting blood transfusions. In the short term, the prospective benefits of a blood substitute overshadow the shortcomings.

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