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# BLOOD SUBSTITUENTS AN OVERVIEW

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**ABSTRACT**

Blood is a special type of connective tissue that is composed of white cells, red cells, platelets and plasma. A blood substitute is a substance used to mimic and fulfill some functions of biological blood. It aims to provide an alternative to blood transfusion, which is transferring blood or blood based products from one person into another. Artificial blood is a product made to act as a substitute for red blood cells. The main categories of oxygen carrying blood substitutes being hemoglobin based oxygen carriers and perfluoro carbon based oxygen carriers. Depending on the type of artificial blood, it can be produced in different ways using synthetic production, chemical isolation, or recombinant biochemical technology. To obtain hemoglobin, a strain of *E. coli* bacteria that has the ability to produce human hemoglobin is used. Synthetic oxygen carriers may also show potential for cancer treatment, as their reduced size allows them to diffuse more effectively through poorly vasculated tumour tissue, increasing the effectiveness of treatments like photodynamic therapy and chemotherapy.

## INTRODUCTION

### Blood

Blood is a special type of connective tissue that is composed of white cells, red cells, platelets, and plasma shown in figure 1. It has a variety of functions in the body. Plasma is the extracellular material made up of water, salts various proteins that along with platelets, encourages blood to clot. Proteins in the plasma react with air and harden to prevent further bleeding. The white blood cells are responsible for the immune defense. They seek out invading organisms or materials and minimize their effect in the body. The red cells in blood create the bright red color. As little as two drops of blood contain about one billion red blood cells. These cells are responsible for the transportation of oxygen and carbon dioxide throughout the body are also responsible for the typing phenomena.

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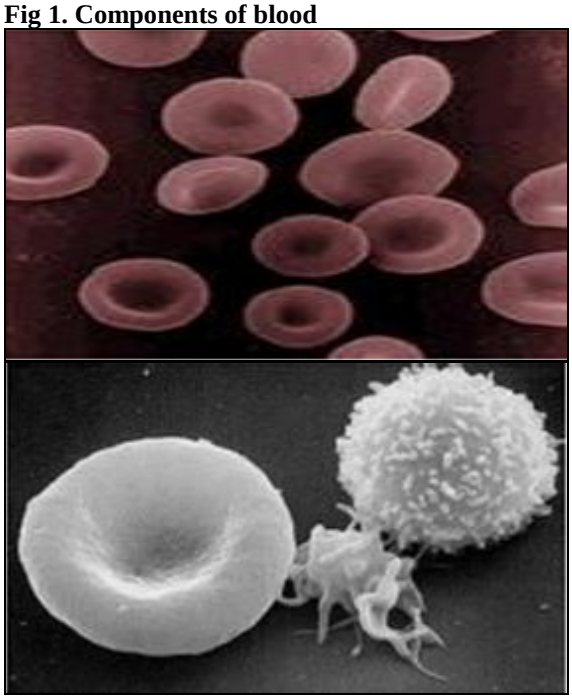
On the membranes of these cells are proteins that the body recognizes as its own. For this reason, a person can use only blood that is compatible with her type. Currently, artificial blood products are only designed to replace the function of red blood cells. It might even be better to call [1, 2].

### Blood Substituent

A blood substitute is a substance used to mimic and fulfill some functions of biological blood. It aims to provide an alternative to blood transfusion, which is transferring blood or blood based products from one person into another. Thus far, there is no well accepted oxygen carrying blood substitutes, which are the typical objective of a red blood cell transfusion; however there are widely available non blood volume expanders for cases where only volume restoration is required. These are helping doctors and surgeons avoid the risks of disease transmission and immune suppression, address the chronic blood donor shortage, and address the concerns of Jehovah's Witnesses and others who have religious objections to receiving transfused blood. The main

categories of oxygen carrying blood substitutes being hemoglobin based oxygen carriers and perfluorocarbon based oxygen carrier [3].

**Fig 1. Components of blood**



## HISTORY OF BLOOD SUBSTITUENT

There has been a need for blood replacements for as long as patients have been bleeding to death because of a serious injury. According to medical folklore, the ancient Incas were responsible for the first recorded blood transfusions. No real progress was made in the development of a blood substitute until 1616, when William Harvey described how blood is circulated throughout the body. In the years to follow, medical practitioners tried numerous substances such as beer, urine, milk, plant resins, and sheep blood as a substitute for blood. They had hoped that changing a person's blood could have different beneficial effects such as curing diseases or even changing a personality. The first successful human blood transfusions were done in 1667. Unfortunately, the practice was halted because patients who received subsequent transfusions died. Of the different materials that were tried as blood substitutes over the years, only a few met with minimal success [4, 5].

In the 1980's with the explosion of HIV, concerns about the safely of the blood supply stimulated renewed interest in the search for an artificial blood substitute. Experiments in the 1960's shown that perfluorocarbon solutions could be used to deliver oxygen to mice these experiments paved the way for developing PFCs as red blood cell substitutes .The first blood substitute to be approved by the FDA was Fluosol-DA, a PFC made by the green cross crop of japan. It was withdrawn just five years later, probably because it was cumbersome to store and prepare as well as the fact that there was no clear clinical benefit. Another PFC-based oxygen carrier, perfloran, was approved for use in Russian in 1996, although there is not much information about it readily available. Hemoglobin- based oxygen carrier, hemo-pure was approved for use in South Africa in 2001[6].

After FDA approval, major restrictions in the area of blood substitutes are cost and effectiveness. While cost may be an issue, Dr. Kim remarks that if we wait another ten to twenty years, HBOCs should have become an effective therapy and be approved by the FDA. At that point larger quantities may be produced, and the cost will have been lowered. Also significant is the rising cost of transfused blood. Because screening techniques are becoming more advanced, the cost of screening one unit of transfused blood is also rising. However, assuming hemoglobin based oxygen carriers were licensed, 70000 kilograms of hemoglobin would be required to replace only twenty percent of the US red cell transfusions in one year, and manufacturing of this large amount of raw materials presents a great challenge. It is estimated that the price of on allogeneic unit of blood lies between 100 to 150 US dollars, whereas HBOC prices are estimated to range between 400 to 800 US dollars. Because of these and other factors there is no simple way to predict to what scale blood substitutes will succeed as an industry, even after FDA approval, although doctors seem enthusiastic. Blood substitutes are not however expected to completely replace allogeneic transfusions unless their spectrum of clinical application of blood oxygen carriers is brought closer to the level of allogeneic blood. A current projection estimates that a decrease of allogeneic red cell use of up to twenty percent may occur once some products are FDA approved, and this may pose some economic problems for blood clinics [7].

Reaching into the unforeseeable future, it is only possible to hope that eventually blood substitutes will be able to cover worldwide shortages, and be cheap and stable enough to be distributed in third world countries where much of the allogeneic supplies are contaminated, or wherever else a need occurs.

## ARTIFICIAL BLOOD SUBSTITUENT

Artificial blood is a product made to act as a substitute for red blood cells. While true blood serves many different functions, artificial blood is designed for the sole purpose of transporting oxygen and carbon dioxide throughout the body. Depending on the type of artificial blood, it can be produced in different ways using synthetic production, chemical isolation, or recombinant biochemical technology. Development of the first blood substitutes dates back to the early 1600s, and the search for the ideal blood substitute continues. Various manufacturers have products in clinical trials, however no truly safe and effective artificial blood product is currently marketed. It is

anticipated that when an artificial blood product is available, it will have annual sales of over $7.6 billion in the United States alone [8].

**Red Blood Substitutes**

Two major types of red cell substitutes are under development: hemoglobin based and perfluorocarbon based. PFCs are completely synthetic hydrocarbon based compounds .The hemoglobin based substitutes use hemoglobin from several different sources like human, animal. Human hemoglobin is obtained from donated blood that has reached its expiration date and from the small amount of red cells collected as a byproduct during plasma donation. One unit of hemoglobin solution can be produced for every2 units of discarded blood. There is a concern that the worsening shortage of blood donors will eventually limit the availability of human hemoglobin for processing. The companies that use human hemoglobin are confident in their supply, especially from the plasma centers that use paid donors. Animal hemoglobin is obtained from cows. This source creates some apprehension regarding the possible transmission of animal pathogens, specifically bovine spongiform encephalopathy. The Biopure Corporation, which uses bovine hemoglobin, has an affiliation with a local breeding farm, allowing close monitoring of the health and diet of the animals. The forty unit so hemoglobin solution can be obtained per slaughtered cow. Recombinant hemoglobin is obtained by inserting the gene for human hemoglobin into bacteria and then isolating the hemoglobin from the culture. This process allows for the manipulation of the gene itself to create variant forms of hemoglobin. One unit of hemoglobin solution can be produced from 750 L of Escherichia coli culture.

Once obtained from any of these sources, the hemoglobin must be purified and modified to decrease its toxicity and increase its effectiveness. This task has not proven to be very easy. Research with hemoglobin based substitutes has actually been under way for over a century. In the 1930s, scientists collected free hemoglobin by lysing red blood [9].

Cells and then transfused the unmodified product into animals after their blood had been drained. Short term survival rates were good, but the animals eventually experienced renal failure, intravascular coagulopathy, and vasoconstriction. Much of the toxicity was later attributed to the presence of residual red cell stromain the product.

Hemoglobin also has been determined to have a strong affinity for a relaxing factor derived from endothelial cells, subsequent hypertension and bradycardia. Hemoglobin normally circulates within red blood cells as a tetramer. When free hemoglobin is transfused, the tetramers rapidly break down into dimers and monomers. These small molecules then freely diffuse into the renal tubules and the subendothelium. To decrease the toxicity of hemoglobin solutions, manufacturers have had to develop methods to stabilize the hemoglobin tetrameric structure and increase its size.

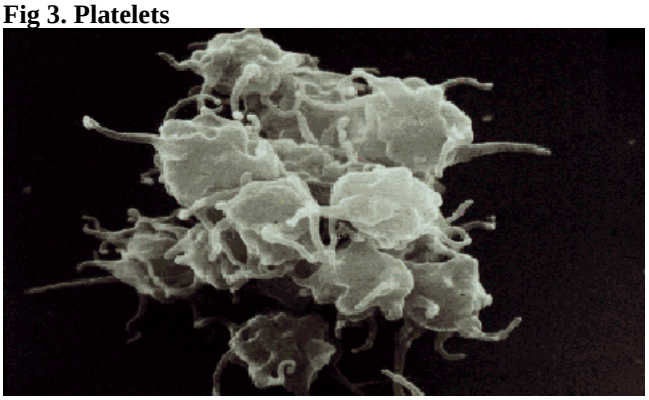
All current red cell substitutes have a short duration of action lasting only about 24 hours in the circulation and are very expensive, with estimates at $500 per unit. Finally, use of these products can interfere with clinical laboratory testing. Hemoglobin solutions will make the patient's blood specimens appear hemolyzed.

PFC solutions can produce lipemia. Both factors can affect the results obtained by some test systems. Close communication between the clinicians using the products and the laboratory will have to occur if reliable test resultsare to be reported [10].

**Platelet substitutes**

The greatest progress in the field of blood substitutes has been with the oxygen carrying solutions. However research on platelet substitutes has been under way since the 1950s. One of the biggest factors pushing the need for platelet alternatives is the 5dayshelf life of the current blood product. This rapid outdate adds additional constraints to an already limited supply. The platelets are also stored at room temperature, thus increasing the risk of bacterial overgrowth. The risk of bacterial contamination of random donor platelets has been estimated to be 1:1500. Ideally, a platelet substitute would have the following properties: effective hemostasis with a significant duration of action, no associated thrombogenicity, no immunogenicity, and sterility, long shelf life with simple storage requirements, and easy preparation and administration [11,12].

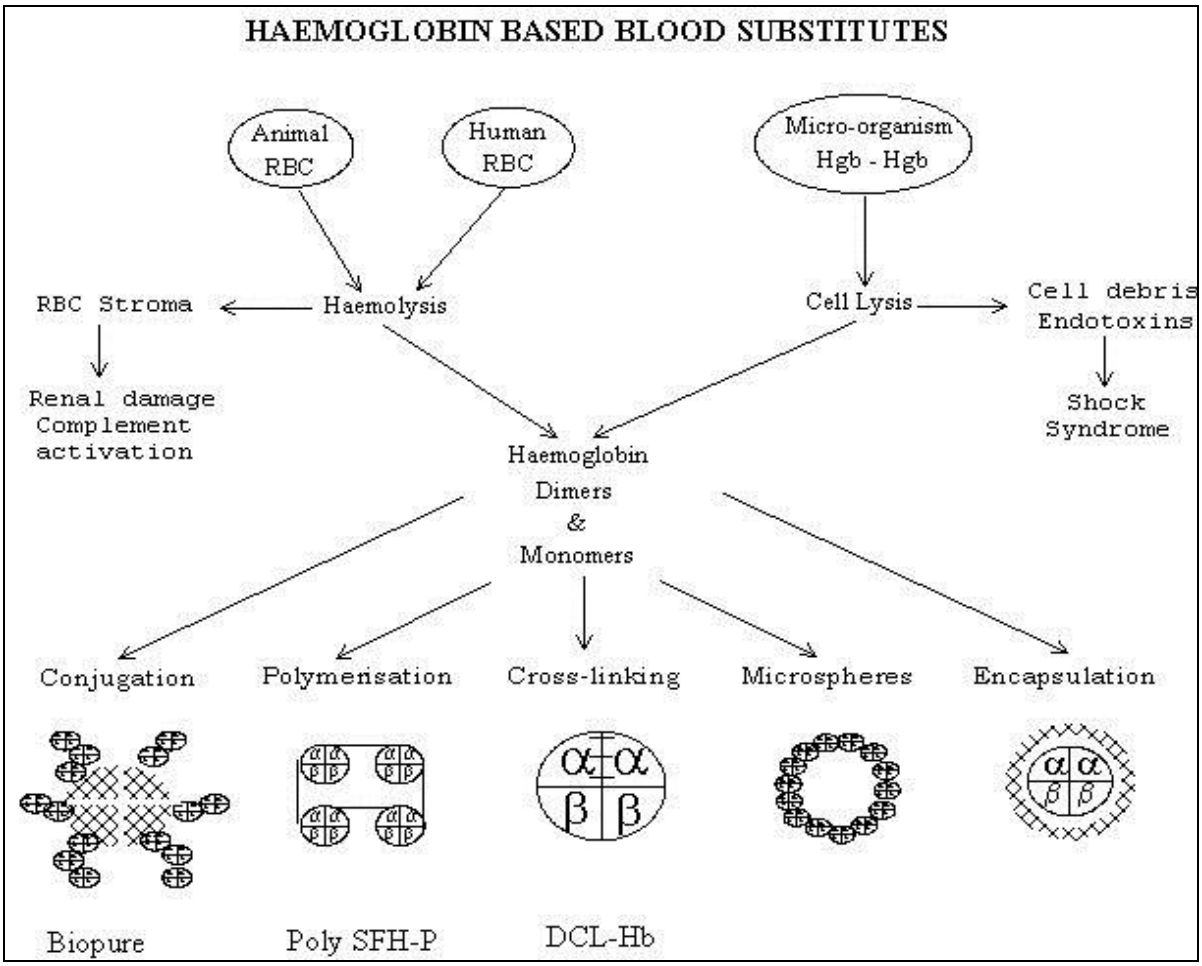
**Fig 3. Platelets**



Several different forms of platelet substitute are now under development of infusible platelet membranes, thrombospheres, and lyophilized human platelets. Only one product, IPM, is currently in clinical trials in the USA.A lyophilized platelet product has been under development since the late 1950s. The current process involves briefly fixing human platelets in paraformaldehyde prior to freeze drying in an albumin solution.

The fixation step kills microbial organisms, and the freeze drying greatly increases the shelf life. Theadhesive properties of the platelets appear to be maintained. This product is currently in animal trials [13].

**THE MANUFACTURING PROCESS**




The production of artificial blood can be done in a variety of ways. For hemoglobin based products, this involves isolation or synthesization of hemoglobin, molecular modification then reconstitution in an artificial blood formula. Perfluorocarbon products involve a polymerization reaction.

**Hemoglobin synthesis**

To obtain hemoglobin, a strain of *E. coli* bacteria that has the ability to produce human hemoglobin is used. Over the course of about three days, the protein is harvested and the bacteria are destroyed. To start the fermentation process, a sample of the pure bacteria culture is transferred to a test tube that contains all the nutrients necessary for growth. This initial inoculation causes the bacteria to multiply. When the population is great enough, they are transferred to a seed tank. A seed tank is a large stainless steel kettle that provides an ideal environment for growing bacteria. It is filled with warm water, food, and an ammonia source which are all required for the production of hemoglobin. Other growth factors such as vitamins, amino acids, and minor nutrients are also added. The bacterial solution inside the seed tank is constantly bathed with compressed air and mixed to keep it moving. When enough time has passed, the contents of the seed tank are pumped to the fermentation tank. The fermentation tank is a larger version of the seed tank. It is also filled with a growth media needed for the bacteria to grow and produce hemoglobin. Since pH control is vital for optimal growth, ammonia water is added to the tank as necessary. When enough hemoglobin has been produced, the tank is emptied so isolation can begin. Isolation begins with a centrifugal separator that isolates much of the hemoglobin. It can be furthers egregated and purified using fractional distillation. This standard column separation is based on the principle of boiling a liquid to separate one or more components and utilizes vertical structures called fractionating columns. From this column, the hemoglobin is transferred to a final processing tank [14].

**Final processing**

Here it is mixed with water and other electrolytes

to produce the artificial blood. The artificial blood can then be pasteurized and put into an appropriate packaging. The quality of compounds is checked regularly during the entire process. Particularly important are frequent checks made on the bacterial culture. Also, various physical and chemical properties of the finished product are checked such as pH, melting point, moisture content, etc. This method of production has been shown to be able to produce batches as large as 2,640 gal (10,000 L).

**Blood substitutes are useful for the following reasons**

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. Although the blood supply in many countries is very safe, 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 [15].

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. Synthetic oxygen carriers may also show potential for cancer treatment, as their reduced size allows them to diffuse more effectively through poorly vasculated tumour tissue, increasing the effectiveness of treatments like photodynamic therapy and chemotherapy. The U.S. military is one of the greatest proponents of oxygen therapeutics, mainly because of the vital need and benefits in a combat scenario. Since oxygen therapeutics are not yet widely available, the United States Army is experimenting with varieties of dried blood, which take up less room, weigh less and can be used much longer than blood plasma.[citation needed] Saline has to be added prior to use. These properties make it better for first aid during combat than whole blood or packed red cells [16].

## ADVANTAGES OF BLOOD SUBSTITUENT

- Once available, artificial blood substitutes will allow for rapid treatment of anemic patients.
- Unfortunately, the effects thus far are short lived; so many patients will eventually require allogeneic blood transfusions.
- With the ongoing shortage of blood donors, which is worsening each year, it has become increasingly important to learn and practice blood conservation measures. For example, in the past, a minimum order for a red cell transfusion consisted of 2 units.
- Physicians were questioned if they ordered anything less. Now physicians are being educated about transfusing judiciously.
- It may be possible to maintain the patient by transfusing just 1 unit of blood. That single unit may successfully ameliorate patients' symptoms until their endogenous red cell production increases adequately [17].

## SUMMARY

Medical care in the armed services would benefit from a safe, easy way to manage blood supply. 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. While it is true that receiving a unit of transfused blood in the US does not carry many risks, with only 10 to 20 deaths per million units, blood substitutes could eventually improve on this. 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.

## CONCLUSION

Despite many years of research, the ideal blood substitute continues to elude researchers. Most of the initial attempts at synthesizing blood substitutes failed because of significant adverse effects. However, continued research has helped us better understand the physiology of red blood cells and the interactions of RBCs with their surrounding environment. This has helped in developing newer products that do not have significant vasoactive properties, as did the first generation compounds. Hopefully, as better blood substitutes are developed and enter routine clinical use, the need for blood transfusions in the operative and trauma settings will decrease. Large scale production of blood substitutes would also help to meet the anticipated increase in demand for blood as the population ages and the blood donor pool diminishes.

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**CONFLICT OF INTEREST**:

The authors declare that they have no conflict of interest.

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