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A REVIEW ON ARTIFICIAL BLOOD

S. Shalini*

*Sree Vidyanikethan College of Pharmacy, Tirupati, Andhra pradesh, India.

ABSTRACT

Artificial blood is a product made to act as a substitute for red blood cells, blood substitutes are used to fill fluid volume and/or carry oxygen and other blood gases in the cardiovascular system. More accurate terms are volume expanders for inert products, and oxygen therapeutics for oxygen-carrying products. Ideal blood substitute should have Universal compatibility: elimination of cross matching, Pathogen free, Minimal side effects, Survivability over a wider range of storage temperatures, Long shelf life, Cost efficient etc. Their main function is replacing lost blood volume and oxygen carrying capacity. Haemoglobin-based oxygen carriers vaguely resemble blood. They are very dark red or burgundy and are made from real, sterilized hemoglobin, which can come from a variety of sources. Unlike haemoglobin-based oxygen carriers, perfluorochemicals are usually white and are entirely synthetic. This review discussed there are several promising techniques like stem cells, dendrimers, biodegradable micelles and from placental umbilical cords (for artificial blood synthesis. Future scope of artificial blood is it is used in case of transfusion transmitted diseases. It's concluded that large-scale production of blood substitutes would also help to meet the anticipated increase in demand for blood.

Keywords: Artificial blood, Haemoglobin-based oxygen carriers, Perfluorochemicals.

INTRODUCTION

Artificial blood is a product made to act as a substitute for red blood cells Blood substitutes (also called artificial blood or blood surrogates) are used to fill fluid volume and/or carry oxygen and other blood gases in the cardiovascular system. Although commonly used, the term is not accurate since human blood performs many important functions which blood substitutes may not. Red blood cells transport oxygen, white blood cells defend against disease, platelets promote clotting, and plasma proteins perform various functions. The preferred and more accurate terms are volume expanders for inert products, and oxygen therapeutics for oxygen-carrying products [1].

Examples of these two "blood substitute" categories:

Volume expanders

They are inert and merely increase blood volume. These may be crystalloid-based (Ringer's lactate, normal saline, D5W dextrose 5% in water) or colloid-based (Voluven, Haemaccel, Gelofusin).

Oxygen therapeutics

They mimic human blood's oxygen transport ability. Examples: Hemopure, Oxycyte, Oxygent, PolyHeme and Perftoran. Oxygen therapeutics is in turn broken into two categories based on transport mechanism: Perfluorocarbon based and Hemoglobin based.

When severe trauma occurs, a serious danger is that blood volume will be reduced to a point where the remaining red blood cells can no longer oxygenate body tissue, which can result in tissue damage or death. In such an emergency situation, doctors will often give patients volume expanders, like saline, to make up for lost blood volume. This helps to 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 patients blood transfusions to replace some of the lost blood. Blood transfusions are also fairly common during some surgical procedures.

Corresponding Author:- **S.Shalini** Email:- shalsels@gmail.com

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.

This is where artificial blood comes in. 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 as oxygen therapeutics [2].

HISTORY

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. Milk was one of the first of these materials. In 1854, patients were injected with milk to treat Asiatic cholera. Physicians believed that the milk helped regenerate white blood cells. In fact, enough of the patients given milk as a blood substitute seemed to improve that it was concluded to be a safe and legitimate blood replacement procedure. However, many practitioners remained skeptical so milk injections never found widespread appeal. It was soon discarded and forgotten as a blood replacement [3].

Another potential substitute was salt or saline solutions. In experiments done on frogs, scientists found that they could keep frogs alive for some time if they removed all their blood and replaced it with a saline solution. These results were a little misleading, however, because it was later determined that frogs could survive for a short time without any blood circulation at all. After much research, saline was developed as a plasma volume expander.

Other materials that were tried during the 1800s include hemoglobin and animal plasma. In 1868, researchers found that solutions containing hemoglobin isolated from red blood cells could be used as blood replacements. In 1871, they also examined the use of animal plasma and blood as a substitute for human blood. Both of these approaches were hampered by significant technological problems. First, scientists found it difficult to isolate a large volume of hemoglobin. Second, animal products contained many materials that were toxic to humans. Removing these toxins was a challenge during the nineteenth century.

A significant breakthrough in the development of artificial blood came in 1883 with the creation of Ringer's solution, a solution composed of sodium, potassium, and calcium salts. In research using part of a frog's heart, scientists found that the heart could be kept beating by applying the solution. This eventually led to findings that the reduction in blood pressure caused by a loss of blood volume could be restored by using Ringer's solution. This product evolved into a human product when lactate was added. While it is still used today as a blood-volume expander, Ringer's solution does not replace the action of red blood cells so it is not a true blood substitute [4].

Karl Landsteiner, who has been called the father of immunology, was the only child of Leopold Landsteiner, a prominent Austrian journalist and editor, and Fanny Hess Landsteiner. Landsteiner was educated at the University of Vienna, where he received his medical degree in 1891. While in medical school, Landsteiner began experimental work in chemistry, as he was greatly inspired by Ernst Ludwig, one of his professors. After receiving his medical degree, Landsteiner spent the next five years doing advanced research in organic chemistry for Emil Fischer, although medicine remained his chief interest. During 1886-1897, he combined these interests at the Institute of Hygiene at the University of Vienna where he researched immunology and serology. Immunology and serology then became Landsteiner's lifelong focus. Landsteiner was primarily interested in the lack of safety and effective-ness of blood transfusions. Prior to his work, blood transfusions were dangerous and underutilized because the donor's blood frequently clotted in the patient. Landsteiner was intrigued by the fact that when blood

from different subjects was mixed, the blood did not always clot. He believed there were intrinsic biochemical similarities and dissimilarities in blood [5].

Using blood samples from his colleagues, he separated the blood's cells from its serum, and suspended the red blood cells in a saline solution. He then mixed each individual's serum with a sample grom every cell suspension. Clotting occurred in some cases, in others there was no clotting. Landsteiner determined that human beings could be separated into blood groups according to the capacity of their red cells to clot in the presence of different serums. He named his blood classification groups A, B, and O. A fourth group AB was discovered the following year. The result of this work was that patient and donor could be blood-typed beforehand, making blood transfusion a safe and routine medical practice. This discovery ultimately earned Landsteiner the 1930 Nobel Prize in physiology or medicine.

Blood transfusion research did not move forward until scientists developed a better understanding of the role of blood and the issues surrounding its function in the body. During World War I, a gum-saline solution containing galactoso-gluconic acid was used to extend plasma. If the concentration, pH, and temperature were adjusted, this material could be designed to match the viscosity of whole blood, allowing physicians to use less plasma. In the 1920s, studies suggested that this gum solution had some negative health effects. By the 1930s, the use of this material had significantly diminished. World War II reignited an interest in the research of blood and blood substitutes. Plasma donated from humans was commonly used to replace blood and to save soldiers from hemorrhagic shock. Eventually, this led to the establishment of blood banks by the American Red Cross in 1947 [6].

In 1966, experiments with mice suggested a new type of blood substitute, perfluorochemicals (PFC). These are long chain polymers similar to Teflon. It was found that mice could survive even after being immersed in PFC. This gave scientists the idea to use PFC as a blood thinner. In 1968, the idea was tested on rats. The rat's blood was completely removed and replaced with a PFC emulsion. The animals lived for a few hours and recovered fully after their blood was replaced.

However, the established blood bank system worked so well research on blood substitutes waned. It received renewed interest when the shortcomings of the blood bank system were discovered during the Vietnam conflict. This prompted some researchers to begin looking for hemoglobin solutions and other synthetic oxygen carriers. Research in this area was further fueled in 1986 when it was discovered that HIV and hepatitis could be transmitted via blood transfusions [7].

DESIGN

Ideal blood substitute

Blood substitutes or synthetic blood are currently labelled as "oxygen carriers". This is because they are unable to mimic many of the other functions of blood. They do not contain cells, antibodies, or coagulation factors. Their main function is replacing lost blood volume and oxygen carrying capacity.

The ideal blood substitute could be defined by the following terms:

- Increased availability that would rival that of donated blood, even surpass it
- Oxygen carrying capacity, equaling or surpassing that of biological blood
- Volume expansion
- Universal compatibility: elimination of cross matching
- Pathogen free: elimination of blood contained infections
- Minimal side effects
- Survivability over a wider range of storage temperatures
- Long shelf life
- Cost efficient

ADVANTAGES

Oxygen therapeutics, even if widely available, would not eliminate the use of human blood, which performs various functions besides oxygen transport. However oxygen therapeutics has major advantages over human blood in various situations, especially trauma.

Blood substitutes are useful for the following reasons:

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. Although the blood supply in the US 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.
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. 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. There is no practical way to test for prior-transmitted diseases in donated blood, such as Mad Cow and Creutzfeld-Jacob disease, and other disease could emerge as problems for the blood supply, including smallpox and SARS.
6. 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.
7. Blood substitutes can be stored for much longer 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
8. 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.

Basic Approaches

There are two basic approaches for constructing oxygen therapeutics:

- Perfluorocarbons (PFCs), chemical compounds which can carry and release oxygen. The specific PFC usually used is perfluorodecalin.
- Haemoglobin derived from humans, animals, or artificially via recombinant technology

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. 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. In theory this can benefit damaged, blood-starved tissue, which conventional red cells cannot reach. PFC solutions can carry oxygen so well that mammals and humans can survive breathing liquid PFC solution, called liquid breathing [8].

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.

The first approved HBOC was a Perfluorocarbon-based product called Fluosol-DA-20, manufactured by Green Cross of Japan. It was approved by the Food and Drug Administration (FDA) in 1989. Because of limited success, complexity of use and side effects, it was withdrawn in 1994. However, Fluosol-DA remains the only oxygen therapeutic ever fully approved by the FDA.

In the 1990s, because of the risk of undetected blood bank contamination from AIDS, hepatitis C and other emergent diseases such as Creutzfeldt-Jakob disease, there was additional motivation to pursue oxygen therapeutics. Significant progress was achieved and haemoglobin-based oxygen therapeutic called Hemopure was approved for Phase III trial (in elective orthopedic surgery) in the U.S., and more widely approved for human use in South Africa.

ADVANCED TECHNOLOGY

Pharmaceutical companies developed a few varieties of artificial blood in the 1980s and1990s, but many abandoned their research after heart attacks, strokes and deaths in human trials. Some early formulas also caused capillaries to collapse and blood pressure to skyrocket. However, additional research has led to several specific blood substitutes in two classes hemoglobin-based oxygen carriers (HBOCs) and Perfluorocarbons (PFCs). Some of these substitutes are nearing the end of their testing phase and may be available to hospitals soon. Others are already in use. For example, an HBOC called Hemopure is currently used in hospitals in South Africa, where the spread of HIV has threatened the blood supply. A PFC-based oxygen carrier called Oxygent is in the late stages of human trials in Europe and North America.

The two types have dramatically different chemical structures, but they both work primarily through passive diffusion. Passive diffusion takes advantage of gasses' tendency to move from areas of greater concentration to areas of lesser concentration until it reaches a state of equilibrium. In the human body, oxygen moves from the lungs (high concentration) to the blood (low concentration). Then, once the blood reaches the capillaries, the oxygen moves from the blood (high concentration) to the tissues (low concentration) [9].

HEMOGLOBIN-BASED PRODUCTS

Hemoglobin

The structure of Hb was determined in 1959 by Max Perutz for which he was awarded a Nobel Prize. Human Hb is a 64 kDa tetrameric protein comprised of two alpha subunits and two ß-globin subunits that fold into compact quaternary structure ($a_2ß_2$).

Each α and ß subunit contain an iron-heme group that binds to oxygen molecule allowing for transport. A fully saturated Hb molecule carries a maximum of four oxygen molecules. Environmental conditions such as pO_2, pH, temperature, and pCO_2 cause Hb to undergo conformational change from a high oxygen affinity state to a lower oxygen affinity state. Such a transition is also facilitated by the binding of an allosteric effector, 2, 3-diphosphoglycerate (DPG), causing a decrease in Hb oxygen affinity and facilitating oxygen offloading. As oxygen is being unloaded CO_2 binds to the globin chain, resulting in carbamino-Hb, which is then transported to the lungs. However, only about 20% of the CO_2 is transported in the blood. The rest of the CO_2 is transported in the form of bicarbonates. Local conditions in the lungs including higher pO_2, higher pH, and lower temperature, cause Hb to shift back to the higher oxygen affinity state and dissociate with DPG. Such a transition favors CO_2 release, which is then exhaled. Although blood transfusion is effective, it is not without risks. Allogenic blood transfusion may cause fatal hemolytic reactions, transmit blood-borne infectious agents, and compromise overall immune function. It is therefore highly desirable to have an artificial oxygen carrying fluid that is readily available, free of infectious agents, and can be used independent of the recipient blood type. The idea of using purified Hb as possible universal substitute for red blood cells has been around for almost a century due to Hb's unique oxygen binding property and the lack of blood type antigen. In 1916, Hb was used in human subjects in an attempt to treat anemia.

However, such early attempt to use Hb-saline solution within the clinical setting failed due to renal toxicities. It was later determined that early Hb contained erythrocyte membrane stroma lipids that were contaminated with endotoxins, causing severe nephrotoxicity in patients. Consequently, Hb solutions had to be prepared “free” of stromal lipids and endotoxin in order to prevent nephrotoxicities. Two other problems shortly emerged: stroma free Hb had too high an oxygen affinity and too short of an intravascular half life in order to be therapeutically useful. Hb had too high of an oxygen affinity because 2,3-DPG normally present in erythrocytic Hb was lost during the purification process. Such a high oxygen affinity did not result in optimal oxygen off-loading in the tissues. In addition tetrameric Hb (a 2 ß 2) dissociated into aß dimmers that were filtered by the kidneys and excreted in the urine. Consequently, the HBOCs being clinically tested today have been chemically or genetically “engineered” to produce desirable oxygen offloading characteristics and an extended circulation half time in order to become a therapeutically useful agent. Some of the key approaches of hemoglobin oxygen carriers as red blood cell substitutes are illustrated above. Once stroma-free Hb is prepared from human or bovine red blood cells they must be chemically stabilized in order to become therapeutically useful [10].

- (A, B) Tetrameric stabilization is accomplished by intermolecular crosslinking between the two αor ß subunits using a site-specific crosslinker.
- (C) The effective molecular weight of Hb can be increased by conjugating it to polyethylene glycol.
- (D) Polymerized Hb of molecular weights greater than the native Hb tetramer of 64 kDa may be produced through polyfunctional crosslinking agents.
- (E) Hb can also be encapsulated into liposomes in order to recreate the natural properties of red blood cells.

Intramolecular cross-linking

Preventing the Hb tetramer's dissociation is a major concern in order to suppress renal filtration. Because the alpha/beta (a-ß) dimers are relatively stable, the goal of intramolecular modification is to cross-link the two alpha (a-a) or beta (ß-ß) subunits and stabilize the association of the two alpha/beta (a-ß) dimers. The cross-linking not only prevents tetramer dissociation, but also reduces the affinity of Hb for O 2 .The most popular cross-linkers currently used are DBBF and nor-2-formylpyridoxal 5-phosphate (NFPLP).

Polymerization

Polymerization of Hb through intermolecular cross-linking increases the size of molecules through the formation of Hb oligomers. In the process multiple Hb proteins are linked together through the use of dialdehydes, such as glutaraldehyde and glycoaldeyde.

The increase of the size of the oligomers is significant because the molecular weight of the molecule exceeds 500 kDa, compared to a 64.5 kDa unpolymerized Hb tetramers. The increase in size prevents the rapid excretion of the molecule, prolonging the Hb plasma half-life. Unpolymerized Hb tetramers have, however, the unfortunate result of generating excessive viscosity, oncotic pressure, and O 2 affinity. Consequently, it is crucial to obtain high polymerization yields in the manufacturing process. Otherwise, the unpolymerized tetramers must be separated as not to create adverse reactions in the patients. In conclusion, intravascular retention times of HBOCs can be increased by intermolecular crosslinking of stabilized Hbs using crosslinker with poly-functional groups.[10]

Conjugation

Conjugation of Hb is the covalent binding of Hb to a biocompatible polymer, such as polysaccharide, in order to increase its overall size. Such a process achieves similar improvements than those made using polymerization. In a specific case of pegylation, multiple polyethylene glycol (PEG) chains are added to the Hb

protein as a means to increasing the molecule's size. It radius, for example, increases from 3 nm to 15 nm once pegylated with 6 PEG chains. Human Hb conjugation with PEG appears to protect the molecule from renal excretion. Conjugating Hb with a macromolecule extends the intravascular circulation time of a HBOC.

Encapsulation

The encapsulation of Hb is based on the idea of recreating the natural properties of RBC without the presence of blood group antigens. Encapsulated Hb is often referred to as "hemosome". The process involves the encapsulation of Hb within lipid vesicles using a solution of phospholipids. The encapsulation allows engineers to specify membrane properties of the vesicle. The negatively charged lipids, for example, have demonstrated to limit the aggregation between hemosomes. The alteration of the bilayer membrane may allow for the better diffusion of O_2 and CO_2.

Recombinant Hemoglobin

Genetic engineering is an alternative to chemically modifying Hb. With advances in recombinant DNA technologies, specially modified Hb may be produce from microorganisms, like *E. coli* or yeast. Prestabilized recombinant human Hb, for example, was produce in E. coli using an expression vector that contained tow mutant globin genes; one had a low oxygen affinity and the other tandemly fused a-globins. Modifications have been made to increase the tetramer's stability and decrease its affinity for O2. Future genetic manipulations may also be able to solve other problems such as the oxidation of Hb into metHb, reaction rate with NO, and short circulation half-life [11].

2) PFC Blood

Unlike HBOCs, PFCs are usually white and are entirely synthetic. They're a lot like hydrocarbons -- chemicals made entirely of hydrogen and carbon -- but they contain fluorine instead of carbon. PFCs are chemically inert, but they are extremely good at carrying dissolved gasses. They can carry between 20 and 30 percent more gas than water or blood plasma, and if more gas is present, they can carry more of it. For this reason, doctors primarily use PFCs in conjunction with supplemental oxygen. However, extra oxygen can cause the release of free radicals in a person's body. Researchers are studying whether PFCs can work without the additional oxygen.

PFCs are oily and slippery, so they have to be emulsified, or suspended in a liquid, to be used in the blood. Usually, PFCs are mixed with other substances frequently used in intravenous drugs, such as lecithin or albumin. These emulsifiers eventually break down as they circulate from the blood. The liver and kidneys remove them from the blood, and the lungs exhale the PFCs the way they would carbon dioxide. Sometimes people experience flu-like symptoms as their bodies digest and exhale the PFCs.

PFCs, like HBOCs, are extremely small and can fit into spaces that are inaccessible to RBCs. For this reason, some hospitals have studied whether PFCs can treat traumatic brain injury (TBI) by delivering oxygen through swollen brain tissue. Pharmaceutical companies are testing PFCs and HBOCs for use in specific medical situations, but they have similar potential uses, including:

- Restoring oxygen delivery after loss of blood from trauma, especially in emergency and battlefield situations
- Preventing the need for blood transfusions during surgery
- Maintaining oxygen flow to cancerous tissue, which may make chemotherapy more effective
- Treating anemia, which causes a reduction in red blood cells
- Allowing oxygen delivery to swollen tissues or areas of the body affected by sickle-cell anemia [12].

SYNTHETIC HBOC PRODUCTION

Raw Materials

Depending on the type of artificial blood that is made, various raw materials are used. Hemoglobin-based products can use either isolated hemoglobin or synthetically produced hemoglobin. To produce hemoglobin synthetically, manufacturers use compounds known as amino acids. These are chemicals that plants and animals use to create the proteins that are essential for life. There are 20 naturally occurring amino acids that may be used to produce hemoglobin. All of the amino acid molecules share certain chemical characteristics. They are made up of an amino group, a carboxyl group, and a side chain. The nature of the side chain differentiates the various amino acids. Hemoglobin synthesis also requires a specific type of bacteria and all of the materials needed to incubate it. This includes warm water, molasses, glucose, acetic acid, alcohols, urea, and liquid ammonia.

For other types of hemoglobin-based artificial blood products, the hemoglobin is isolated from human blood. It is typically obtained from donated blood that has expired before it is used. Other sources of hemoglobin come from spent animal blood. This hemoglobin is slightly different from human hemoglobin and must be modified before being used.

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. PFC products involve a polymerization

reaction. A method for the production of a synthetic hemoglobin-based product is outlined below.

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 further segregated and purified using fractional distillation. This standard Once fermented, the hemoglobin is purified and then mixed with water and other electrolytes to create useable artificial blood [13].

column separation method 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.

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 batches as large as 2,640 gal (10,000 L).

BENEFITS AND CHALLENGES OF HBOCs

Benefits:

The general benefits of HBOCs over transfused red blood cells are i.No prior planning, ii.Faster & better O 2 distribution, iii. Ready to use, iv. No waste, v. No equipment, vi. Long shelf life, vii. No refrigeration, viii. Universally compatible, ix. Immediately offloads oxygen, x. Can be use by Jehovah's Witnesses.

The challenges associated with the development of HBOC can be categorized into the following:

- Availability – Ironically while one of the primary reasons to develop oxygen carriers is to have a readily available solution to ease the projected future shortages in blood supply, some approaches to the development of HBOCs face a similar supply challenge. It was estimated that over 70,000 kg of Hb would be needed to replace 20% of RBC transfusion in the United States. This presents a significant challenge to human HBOC products. While production of human Hb by recombinant DNA could be a possible solution, it remains unclear whether the technology could produce such massive quantities of Hb for future demand. A study has estimated that a population of 100,000 transgenic pigs would be enquired to stably produce up to 50% of human Hb.
- Short half-life – Outside a red blood cell, Hb dissociates into 32 kDa dimmers and are freely filtered by the glomerulus resulting in severe renal toxicity. Current HBOC products have chemically or genetically cross-linked Hb chains resulting in 128 kDa or larger molecules that are not readily filtered by the glomerulus, thus possessing a greatly increased half-life.
- Increased oxygen affinity – Hb in the plasma has a much higher affinity for O 2 than it does within the context of a red blood cell. The increased affinity for O2 is due to lack of 2,3-diphosphoglycerate (DPG) in the plasma. Consequently the HbO2 dissociation curve shifts to the left, making such a high-affinity Hb not an ideal oxygen delivery substance. However, chemically cross-linking the Hb structure has the net effect of decreasing O2 affinity and optimizing intracellular oxygen delivery.
- Vasoactive properties – One of the major challenges facing the development of HBOCs is their effects on vasoactive properties. The theories regarding the mechanism of action of the vasoconstrictive effect: Nitric oxide scavenging by Hb, Excess O 2 delivery to the peripheral tissues, Direct effect on peripheral nerves, and the oxidative properties of HB.
- Soluble Hb, unlike Hb in RBCs, interacts with NO to form metHb and NO-Hb. NO by definition is a potent endothelial vasorelaxant that inhibits the conversion of proendothelium to the vasoconstrictor endothelium. In the prevailing theory on vasoactive properties, the decrease in NO concentration due to its reaction with Hb is responsible for vasoconstriction. Alternative theories suggest that too much O 2 is delivered causing an autoregulatory vasoconstrictor reflex. Yet another theory argues that oxidation of soluble Hb can result in heme loss, free radical formation, loss of reactive iron, and

oxidation of lipids. Such reaction and products result in endothelial stress causing vasoconstriction [14].

PROMISING TECHNIQUES

1). Stem cells

Recently, the scientific community has begun to explore the possibility of using stem cells as a means of producing an alternate source of transfusable blood. A study performed by Giarratana et al. describes a large-scale ex-vivo production of mature human blood cells using hematopoietic stem cells, and may represent the first significant steps in this direction. Moreover, the blood cells produced in culture possess the same haemoglobin content and morphology as do native red blood cells. The authors of the study also contend that the red blood cells they produced have a near-normal lifespan, when compared to native red blood cells—an important characteristic of which current haemoglobin-based blood substitutes are found to be deficient.

The major obstacle with this method of producing red blood cells is cost. Now, the complex three-step method of producing the cells would make a unit of these red blood cells too expensive. However, the study is the first of its kind to demonstrate the possibility of producing red blood cells which closely resemble native red blood cells on a large scale.

2). Dendrimers

Researchers at the Dendritech Corporation have begun research, aided by a 2 year, $750,000 grant from the US Army, into the use of dendrimers as substitute oxygen carriers. The precise nature of the research cannot be disclosed, as the company's patent application has not yet been approved. Researchers hope that dendrimer technology will be the first truly cost-efficient blood substitute.

3) Biodegradable micelles

To enhance circulation times, recombinant or polymerized haemoglobin can be encapsulated within micellar-forming amphiphilic block copolymers. These systems are typically between 30-100 nm in diameter. The hydrophobic core of the polymer micelle is able to solubilize the similarly hydrophobic haemoglobin protein, while the water soluble corona (which is usually polyethylene glycol) provides a steric barrier to protein absorption, and provides protection from clearance by the reticuloendothelial system (RES).

4) Placental umbilical cord blood

Cord blood collected aseptically from the placenta after the birth of a healthy baby can be used safely as a blood substitute. It has a higher haemoglobin content and growth factors than normal blood from an adult, which has the potential to benefit patients in varying diseases [10,12].

FUTURE SCOPE

Artificial blood can be used in the following cases: Transfusion transmitted diseases like Hepatitis B virus, Hepatitis C, Hepatitis G virus, Human Immunodeficiency Virus (HIV), Human T-lymphocytotrophic Virus (HTLV-1), Cryoglobulinemia, TTV - Transfusion Transmitted Virus, Cytomegalovirus (CMV), Creutzfeldt-Jakob Disease (CJD), Kaposi's sarcoma (KS) and human herpes virus-8 (HHV-8), Leishmaniasis, Lyme Disease, Malaria, Chagas Disease, Babesiosis, Toxoplasmosis and Bacterial Contamination of Blood products**.**

ARTIFICIAL BLOOD CONTRAVERSY

At first glance, artificial blood seems like a good thing. It has a longer shelf life than human blood. Since the manufacturing process can include sterilization, it doesn't carry the risk for disease transmission. Doctors can administer it to patients of any blood type. In addition, many people who cannot accept blood transfusions for religious reasons can accept artificial blood, particularly PFCs, which are not derived from blood.

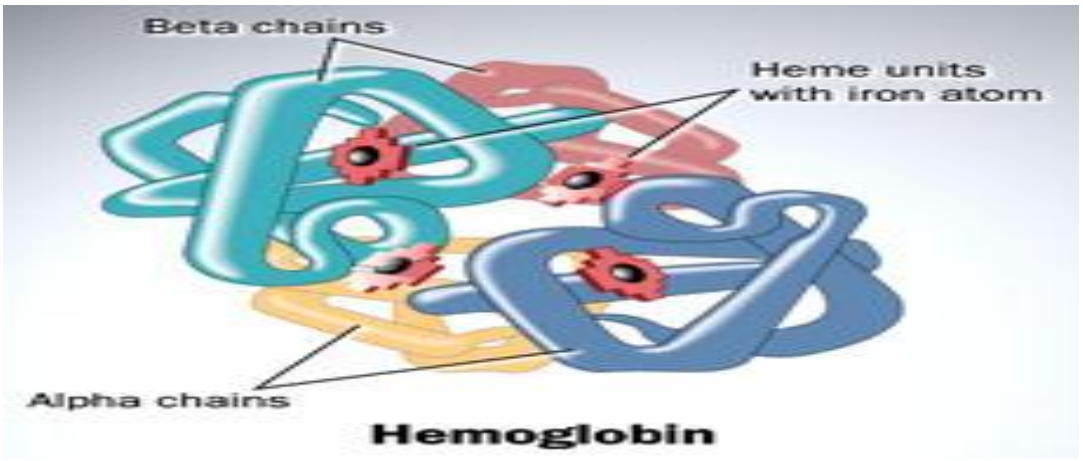
However, artificial blood has been at the center of several controversies. Doctors abandoned the use of HemAssist, the first HBOC tested on humans in the United States, after patients who received the HBOC died more often than those who received donated blood. Sometimes, pharmaceutical companies have had trouble proving that their oxygen carriers are effective. Part of this is because artificial blood is different from real blood, so it can be difficult to develop accurate methods for comparison. In other cases, such as when artificial blood is used to deliver oxygen through swollen brain tissue, the results can be hard to quantify.

Another source of controversy has involved artificial blood studies. From 2004 to 2006, Northfield Laboratories began testing an HBOC called PolyHeme on trauma patients. The study took place at more than 20 hospitals around the United States. Since many trauma patients are unconscious and can't give consent for medical procedures, the Food and Drug Administration (FDA) approved the test as a no-consent study**.** In other words, doctors could give patients PolyHeme instead of real blood without asking first. Northfield Laboratories held meetings to educate people in the communities where the study took place. The company also gave people the opportunity to wear a bracelet informing emergency personnel that they preferred not to participate. However, critics claimed that Northfield Laboratories had not done enough to educate the public and accused the company of violating medical ethics.

Blood substitutes may be used as performance-enhancing drugs, much like human blood can when used in blood doping. An October 2002 article in "Wired" reported that some bicyclists were using Oxyglobin, a veterinary HBOC, to increase the amount of oxygen in

their blood. In spite of the controversy, artificial blood may be in widespread use within the next several years. The next generations of blood substitutes will also probably become more sophisticated. In the future, HBOCs and PFCs may look a lot more like red blood cells, and they may carry some of the enzymes and antioxidants that real blood carries [13].

Fig 1. Hemoglobin



CONCLUSION

Most of the initial attempts at synthesizing blood substitutes failed because of significant adverse effects. However, continued study 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. 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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