WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 6, 596-613. Review Article ISSN 2277– 7105

ARTIFICIAL BLOOD SUBSTITUTES: A LIFE SAVING TOOL

Nandita Yadav[1], Ismat Fatima Rizvi[2], Mohd. Haris Siddiqui[1,2] and Alvina Farooqui*[1]

[1]Department of Bioengineering, Faculty of Engineering, Integral University Lucknow, Uttar Pradesh, India-226026.

[2]Advanced Centre for Bioengineering and Bioinformatics (ACBB), Integral Information and Research Centre (IIRC), Integral University Lucknow, Uttar Pradesh, India-226026.

Article Received on
29 March 2016,

Revised on 20 April 2016,
Accepted on 11 May 2016

DOI: 10.20959/wjpr20166-6307

***Corresponding Author**
Dr. Alvina Farooqui
Department of Bioengineering, Faculty of Engineering, Integral University Lucknow, Uttar Pradesh, India-226026.

ABSTRACT

Blood is a specialized fluid of body that delivers necessary substances such as nutrients and oxygen to the body cells and transports waste products away from those cells. Artificial blood is a product that can act as substitutes of blood and can transport oxygen (oxygen therapeutics) and carbon dioxide throughout the body. Artificial blood will also lessen the demand for human blood supplies and give immediate response without triggering a rejection in cases of massive blood loss. They do not require any blood typing, so that they can be infused immediately and is applicable for all blood types patient. It can be produced by different methods such as synthetic production, chemical isolation, or by using recombinant biochemical technology. One basic approach to make an oxygen therapeutic is using Perfluorocarbons a chemical compound which can carry and release oxygen which maintain the circulating blood volume as well as the need of the patients.

KEYWORDS: Human Blood, Artificial blood, Blood substitutes, oxygen therapeutics, Perflurocarbons

INTRODUCTION

Blood is a special fluid which carries necessary substances like nutrients and oxygen to different body cells as per requirement. It also transports wastes away from the body cells. Artificial blood is made to act like a red blood substitutes mainly for transporting oxygen and carbon dioxide throughout the body. The initial goal of oxygen carrying blood substitutes mimics blood's oxygen transport capacity. It is a new innovation in the field of blood in

which is an artificial substance that can take the place of blood and substance could replace during some surgical procedures. On the basis of type of artificial blood, it can be produced in three different ways: Synthetic production, Chemical isolation, Recombinant biochemical technology.[1]

HUMAN BLOOD

Human blood consists of two main components:

1.Plasma- Everything that blood carries like nutrient, hormones and wastes dissolved in plasma mostly water.

2. Formal element –Cells and a part of cells floats in plasma.

Main components of the human blood are

1. Red blood cells
2. White blood cells
3. Platelets
4. Fluid (plasma)

1. Red blood cells

Red cells contain 40-50% of the total blood volume. Due to oxygenated red cells blood is of red colour. It facilitate transport of oxygen from the lung to all of the living tissues of the body and carry away carbon dioxide. Haemoglobin is the protein that makes up 95% of a red cell and consists of haemachrome.[2] Haemachrome were combine with the globin and it cause folding in globin to form its final stereo structure called a αβ dimer. They form haemoglobin when two αβ dimers form a 2α2β tetramer. Normally the haemoglobin of a healthy adult consists four polypeptide chains. Each and every polypeptide chain contain of a heme group and a single oxygen-binding site, all of these four chains were arranged as a tetrahedron. The structure of haemoglobin is stable but the oxygen affinity is very weak. There will be a structural change when oxygen binds with one heme of one polypeptide chain. The structure changes at interfaces between these four polypeptide chains.[3]

2. White blood cells

They are also called leukocytes or leucocytes. These are the cells present in the immune system which are involved for protecting the body against both infectious disease and foreign organisms.[4] It consists of neutrophil, eosinophil, basophil and lymphocytes.

i) Neutrophil

They are the most abundant white blood cell, it constitutes 60-70% of the circulating leukocytes.[5] They defend against bacterial or fungal infection. They have a multi-lobed nucleus, which consists of three to five lobes connected by slender strands.[5]

ii) Eosinophil

It compose of about 2-4% of the total WBC. It rises in response to allergies, parasitic infections, disease of the spleen and central nervous system. They are very less in the blood, but much more in the mucous membranes of the respiratory, digestive, and lower urinary tracts of human body.[6]

iii) Basophil

They are responsible for allergic and give antigen response by releasing the chemical histamine which causing the dilation of blood vessels. Because of the rarest amount of the white blood cells (less than 0.5% of the total count) and share physicochemical properties with other blood cells, they are difficult to study.[7]

iv) Lymphocytes

They are more found in the lymphatic system as compare to blood. They are different from other cells by having a deeply staining nucleus that are eccentric in location, and relatively small amount of cytoplasm. Lymphocytes include:

- **B cells** make antibodies which bind to pathogens, block pathogen invasion, activate the complement system, and enhance pathogen destruction.
- **T cells**
  - **CD4+ helper T cells**: T cells contain co-receptor CD4 are known as CD4+ T cells. Helper T cells make cytokines and perform other functions which help in coordinate the immune response.
  - **CD8+ cytotoxic T cells**: T cells contain co-receptor CD8 are known as CD8+ T cells. These cells were to bind antigens presented on MHC I complex of virus-infected or tumour cells and kill them.
  - γδ T cells possess is an alternative T cell receptor that found in tissue more commonly than in blood, γδ T cells which share the characteristics of helper T cells, cytotoxic T cells, and natural killer cells.

- **Natural killer cells** are able to kill cells of the body which are do not display MHC class I molecules, or display stress markers such as MHC class I polypeptide-related sequence A (MIC-A).

3. Platelets

These are found in mammalian blood. There are100-300 thousand platelets per cubic millimetre in human blood. Platelets perform the function of stopping bleeding. When the concentration of platelets is lower than 70 thousand per cubic millimetres it will difficult for stopping bleeding. If the concentration of platelets is low than 40 thousand per cubic millimetres it resulting in spontaneous haemorrhage.[8]

4. Plasma

Plasma transports materials which are required by cells and removed those materials which are unnecessary to cells like various ions (Na+, Ca2+) , glucose, amino acids, other organic acids , cholesterols and other lipids , hormones and wastes. In plasma, two types of osmotic pressure are:

Crystal osmotic pressure

This pressure formed by inorganic salt. It is the summation of crystal osmotic pressure and colloid osmotic pressure.

Colloid osmotic pressure

Osmotic pressure formed by plasma proteins. It has most important function to retain the balance of blood and tissue fluid. In plasma colloid osmotic pressures high and low depend on content of plasma protein. When colloid osmotic pressure is too high, human body will be dehydrated.[8]

ARTIFICIAL BLOOD CELLS

Artificial blood does not perform all the function of real blood. Sometimes, it is unable to replace lost blood volume. It work on that situations where a person's red blood cells is unable to carry oxygen in their own way. To perform this function artificial blood is synthesized as “oxygen therapeutic” which is also called as oxygen therapy**.** Usually in the oxygen-carrying sense artificial blood or blood surrogates is a substance which are used to help in increase and fulfil some important functions of biological blood. The main motive is to provide an alternative for safe blood transfusion, in which transferring blood or blood-

based products from one person into another. It not contains plasma, red blood cells, white blood cells and platelets of human blood because it designs to perform only functions to transport and deliver oxygen to the body's tissues.[1]

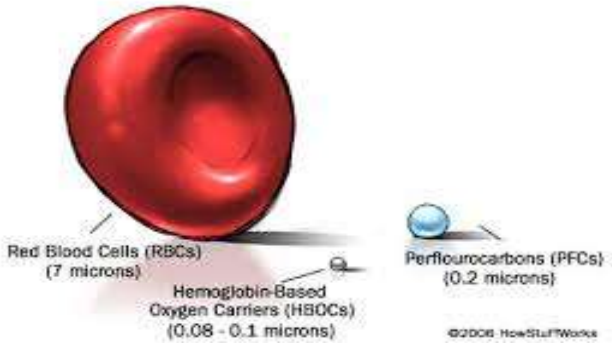

Fig: 1 Artificial blood substitutes

COMPOSITION OF ARTIFICIAL BLOOD SUBSTITUTES

Perfluoro-octyl bromide

FO-9982

Yolk lecithin

DSPE-50 H

PURPOSE TO CREATE ARTIFICIAL BLOOD

Military

They requires a large volume of blood products which can be easily stored and easily shipped to the site of casualties.

Human immunodeficiency virus

With the advent of this virus, the medical community and the public suddenly became aware of the significance of transfusion-transmitted diseases and became concerned about the safety of the national blood supply.

Shortage of blood donors

Approximately 60% of the population is eligible to donate blood, but fewer than 5% are regular blood donors. It improve the shortage of blood.[9]

BENEFITS OF ARTIFICIAL BLOOD

- They are readily available and have a long shelf life.

- Allowing them to be stocked in emergency rooms and ambulances and easily shipped to areas of need.
- They can undergo filtration and pasteurization processes to virtually eliminate microbial contamination.
- No product can claim to be 100% risk-free for infectious agents.
- They do not require blood typing, these substitutes have a greatly increased level of safety.
- They can be infused immediately and for all patient blood types.
- Able to transport and release oxygen where needed.
- They do not appear to cause immunosuppression in the recipient**.**
- Compatible in the human body**.**

DRAWBACKS OF ARTIFICIAL BLOOD

- It is currently expensive.
- It may also increase chances of a heart attack.
- Body immune systems may sometimes react negatively to the foreign blood that isinserted into the body.

ARTIFICIAL BLOOD SUBSTITUTES

1) Red cell substitutes

Two major types of red cell substitutes are under development:

- Haemoglobin based
- Perfluorocarbon (PFC) based

Haemoglobin based

Red cell substitutes use haemoglobin from several different sources:

Human

Human haemoglobin were obtained from donated blood that reached at itsexpiration date and small amount of red cells collected as a by-product during plasma donation. One unitof haemoglobin solution can be produced for every 2 units of discarded blood.

Animal

Animal haemoglobin was 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 haemoglobin, has an affiliation with a local breeding farm, allowing close monitoring of the health and diet of the animals.

Recombinant

Recombinant haemoglobin was 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 haemoglobin solution can be produced from 750 L of Escherichia coli culture.[9]

TYPES OF HEMOGLOBIN BASED RED CELL SUBSITUTES

Surface modified haemoglobin:

This was created by attaching large molecules such as polyethylene glycol to surface lysine groups. This modification increases the viscosity and oncotic pressure of the solution. There are two companies which developed surface-modified haemoglobin solutions.

- Enzon
- Apex Bioscience

Both of the companies' products, however, triggered moderate vasoconstriction after infusion. Enzon is targeting its polyethylene glycol–conjugated haemoglobin product for treatment of patients with stroke and cancer in which a small size of the haemoglobin molecules are allows them to pass through constrictions and oxygenate areas which cannot be reached by red blood cells. For patients with cancer, these solution can deliver oxygen to tumor cells and increase susceptibility for radiation or chemotherapy. Apex Bioscience is developing its product for treatment of hypotension induced by septic shock.

Cross-linked haemoglobin

For producing a cross-linked haemoglobin, a small bridges of sugar molecules are attached covalent to the dimers and create a stable tetramer. The US Army had partnered with Baxter Corporation for develop a cross-linked product i.e. Hem Assist. The product development was discontinued when increased mortality was noted in phase III trials. Baxter had also partnered with Somatogen to produce Optro, which is a recombinant product produced by E. coli. These product are also no longer under development.

Polymerized haemoglobin

For polymerize hemoglobin, surface amino acid groups are linked by reagents such as glutaraldehyde.[9]

Table No.1: Haemoglobin Based substitutes

Name	Sponsor	Description	Figure
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 made up of chemically stabilized with cross-linked bovine (cow) haemoglobin in a salt solution.	
Oxyglobin	Biopure Corp	Oxyglobin is currently approved for veterinary use in US and Europe. Oxyglobin solution is the first and only oxygen therapeutic which has both US FDA and European Commission approved for veterinary use. It has been used primarily for blood transfusions and for treatment of anaemia in dogs. Currently, they can only be used in canines and not in humans.	
PolyHeme	Northfield Laboratories	PolyHemeis a unique human haemoglobin-based oxygen-carrying blood substitute which isin development for urgent, the treatment of large volume blood loss in trauma and surgical settings. It is the only blood substitute which has completed a Phase III trial.	
Hemospan	Sangart	Hemospan is currently in Phase II trials in the United States They are produced in powder form, which can then be mixed into liquid form and transfused immediately, regardless of a patient's blood type.	
Dextran Haemoglobin	Dextro-Sang Corp	Dextran-Haemoglobin is currently in vetrinary trials. It is created by the Dexto-Sang Corporation. It has create a conjugate of the polymer dextran with human haemoglobin molecules which increases its half-life inside the body, and prevents tissue damage.	

Hemotech	HemoBiotech	Hemotech is currently approved for Phase I trials 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	

Perflurocarbons (PFC)-based substitutes

Perfluorocarbons are derived from a group of hydrocarbons in which the hydrogen atoms are replaced by fluorine atoms. They are chemically inert due to the strength of the carbon-fluorine bonds, used tocreate artificial blood during surgeries. Now a day's most of the Perfluorocarbons oxygen carriers are mixtures of perfluorocarbons with emulsifying agents. Emulsifying agents are substances that help stabilize two seemingly unbendable things. Perfluorocarbons oxygen carriers utilize Puronic-68, egg yolk phospholipids and triglycerides.[10]

Advantages

1. Perfluorocarbons do not react with oxygen.
2. Perfluorocarbons allow easy transportation of the oxygen to the body.
3. They allow increased solubility of oxygen in plasma.
4. It minimize the effects of factors like pH and temperature in blood circulation

Disadvantages

1. This is often caused by phagocytosis of the per fluorocarbon emulsion by the recipient organism'simmune system.
2. Often causes flu-like symptoms.
3. Unable to remain mixed as aqueous solutions –thus, they must be prepared as emulsions for use in patients.

Table No. 2. Perflourocarbon based substitutes Platelets substitutes Infusible platelet membranes

Name	Sponsor	Description	Figure
Oxygent	Alliance Pharmaceuticals	Oxygent is currently approved for Phase II Trials in US and Europe which is developed to reduce the need for donor blood during surgery. They are surrounded by a lecithin surfactant in a water-based solution.	
Oxycyte	Oxygen Biotherapeutics	Oxycyte is currently approved for Phase II-b Trials in the United States. They targeted as an oxygen therapeutic with successful small scale open label human trials for treating traumatic brain injury at Virginia Commonwealth University.	
Perftoran	Russia	Registered in Mexico as PERFTEC, distributed by KEM Laboratory (Mexico).they facilitates oxygen delivery with remaining red blood cells together at blood replacements.	

They are produced from expired human platelets which are fragmented and virally inactivated then lyophilized. They have their shelf life 2 years. Although the platelet membranes stillexpress some blood group and platelet antigens, they has to appear asresistant to immunedestruction. One company, Cypress Bioscience Incorporated, manufactures an IPM productthat is currently in phase II trials. These company is used its product for use in patients whohave become refractory to platelet transfusions because of the formation of antibodies to HLA antigen or platelet antigens. These products was successfully stopped bleeding about 60% of such patients. Overall, these products appear to be safe.

Thrombospheres

Thrombospheres (Hemosphere, Irvine, Calif) are not platelets. They are created by cross-linkedhuman albumin with human fibrinogen bound to the surface. This mechanism of action has notyet been elucidated. Experimentally, they appear to enhance platelet aggregation but do nothave ability to activate platelets themselves. A similar product, Synthocytes (AndarisGroupLtd, Nottingham, UK), has just entered for clinical trials in Europe.

Lyophilized human platelets

These products have been under development since the late 1950s. The current process involvesfixing human platelets in paraformaldehyde prior to freeze-drying in an albumin

solution. This fixation step can kills microbial organisms, and the freeze-drying which has greatly increasestheir shelf life. These product is currently in animal trials.[11]

DELIVERY OF ARTIFICIAL BLOOD SUBSTITUTE

Two types of approaches for effective delivery of blood substitutes:

- Vesicular approaches
- Non vesicular approaches

1. Vesicular approaches

Liposomes

The cellular structures of liposomes has been exploited as a carrier for drugs, genes, enhancing blood retention, targeting a site because of encapsulation by membranes which prevent the material against degradation and enhance bio-distribution. Drugs in liposome forms such as AmBisome® andDoxil®, are in clinical trials. Haemoglobinencapsulated in sterile liposomes made from chloroform, HSPC, cholesterol, negatively charged DMPG and alpha-tochopherol, showed oxygen carrying capacity of 20%, a half-life of 15 -20 hrs as measured in mice. These liposome have a phospholipid bilayer, with cholesterol molecules added for increased rigidity and mechanical stability which then encloses a stroma free haemoglobin solution and 2,3 DPG or inositol hexaphosphate as a gelatinous fluid.[12] Use of cell-free Hb solutions is associated with many deterious side effects and to overcome these, liposome-encapsulated haemoglobin (LEHb) -based artificial blood substitutes are being used since it has no blood group antigens and can therefore be stored for a longer time.[13,14] Plain liposomes undergo deformation and destruction when exposed to fluid stress so in a LEHbstudy, demonstrated that 10–20% of the encapsulated Hb in LEHbdispersions is released when dispersion is subjected to a well-defined flow field.[15]

Use of UV irradiation and redox inhibitors cause polymerization of unsaturated phospholipids incorporated in the vesicle membrane and provides mechanical strength to the vesicle.[16-17]

Instead of introducing covalent linkages between the hydrophobic tails, stabilization of the liposomes can also be carried out by coating the liposomes with help of polymers.[18-22]

Akama et. al increased the mechanical strength of the membrane of Hb encapsulated vesicles, by introducing actin matrix in the inner aqueous core of the vesicles and these are used as a potential artificial blood substitute[23].The sub micron size of the liposomes makes it by pass the RES system and inhibits recognition of the opsonins.[24] Because of the smaller size as compared to RBC s also enables them to pass through blockages, clots in cases of stroke and heart attack.[25] Efficacy and quality of liposomes can be further increased bymodifying them with polyethylene glycol (PEG), which makes the liposomes even more stable, increase the half-life, induce water soluble property, lower immunogenicity and antigenicity along with site specific targeting.[25-26] HbV, a cellular oxygen carrier has high concentration of haemoglobin encapsulated in phospholipid bilayers membrane with PEG and is advantageous as the oxygen affinity can be easily manipulated by changing the concentration of allosteric effector such as pryidoxal 5'-phosphate.[27] PEG coating and tailored size of 250nm enhances time of blood circulation as compared to other vesicles (t1/2 for cell free Hb and PEGylatedHb in rats of 1.5 and 10.9 hr, respectively.[28] Encapsulation of Hb helps to suppress renal excretion but eventual capturing by phagocytes in the mononuclear phagocyte system(MPS) does take place in case of these HbV.[29] These unique features of increased circulation time, as reported in rats, mouse and haemorrhagic rat model, of the vesicles make them comparable to RBCS.[30-35] Instead of conjugation of multiple copies of PEG-5k which imparts an enhanced molecular volume to the vesicle, PEG chains gives a low density atom in PEG shell relative to protein core and the chain increases the hydrodynamic drag on the molecule and slows the sedimentation rate. The same influence is seen with PEG-Hb whenused as a blood substitute.[36]

Dendrimers

Dendritic polymer having a well defined nanoscopic size of 1 nm, compromises of fluorocarbon and hydrophilic moieties. Compatibility with plasma is due to discrete well-defined globular shapes, flexibility, chemical stability low cytotoxicity; and

hydrophilicity of exterior makes it a major step forward in the field of blood substitutes e.g Poly amidoamino dendrimers (PAMAM).[37]

Polymersomes

Vindico Nano biotechnology Inc. (Vindico) is developing a hemoglobin-based cellular oxygen therapeutic (blood substitute), NanoHeme, based on its proprietary nanoparticle-based delivery platform known as polymersome. Polymersomes are synthetic polymer vesicles that are formed in nanometric dimensions which can efficiently encapsulate oxygen-carrying proteins such as hemoglobin (Hb). The lead Nano Heme formulation comprises of a diblock copolymer comprising hydrophilic polyethylene oxide (PEO) and hydrophobic polycaprolactone (PCL). It demonstrates all the characteristics of ideal oxygen therapeutic, such as tunable oxygen binding capacity, uniform and small size, viscosity and oncotic pressure characteristics similar to human blood as well a s ease of mass production and storage. Encapsulation of Hb inside polymersome core protects surrounding tissues and blood components from direct contact with Hb and it also allows for the use of less expensive animal Hb.[38]

Polymeric shell

Encapsulation of hemoglobin in polymeric shells, which are suspension of fine particles of artificial blood substitutes, avoids drawbacks such as nephrotoxicity, activation of complement C3a associated with liposomes as carriers for delivery of artificial bloodsubstitutes. In addition to its ability to carry poorly soluble agents, hemoglobin encapsulated in polymeric shells has high binding capacity for oxygen. Key difference between protein microspheres and polymeric shells is that in former case they don't have proteins shells rather proteins are dispersed throughout volume of microspheres. Polymeric shells are completely degraded by proteolytic enzymes as the polymer present is protein, which result in minimal side effects as compared to current formulation. Coating of polymeric shells with PEG result in increase circulation time maintain high levels of artificial blood substitutes.[39] Number of biocompaptible materials such as natural and synthetic proteins,ploypeptide or oligopeptide etc. having sufficient sulfhydryl or disulfide group for crosslinking are used successfully formulating polymeric shells. Recently new proteins such as albumin and haemoglobin are exploited for formation of polymeric shells. By encapsulating haemoglobin with in core of fluorocarbons it possible to deliver large amount of oxygen forshort periods as in case tissue ischemia and tumor therapy.

Non vesicular approaches

Solution

Since hemoglobin solutions have capability to transport oxygen and because of their oncotic activity they are therefore tested to be used as artificial blood substitutes and plasma expander respectively. Reversible oxygenation property of these solutions makes them choice for rapid initial treatment of hypovolemia and tissue hypoxia.[40] Tetramers free hemoglobin solution having advantage of having half lives nearly 12-24 hrs and avoiding vasoconstriction, renal toxicity, hemoglobinuria or the other problems associated with the intravenous administration of synthetic and semi synthetic oxygen carriers and blood substitutes, can be used in patients suffering from hypovolmic shock during surgery and trauma.[41] Stroma free hemoglobin solution has been found to overcome the problems of toxicity, shelf life and stability by many folds.[42] Aqueous solutions have additional benefits such as presence of polysaccharide oncotic agent which makes them ideal to be used as synthetic plasma expanders.

Emulsions

Novel emulsions having novel surfactants such as Alkyl phosphorylcholine or alkylglycerophosphoryl choline are exploited as oxygen transport agents and artificial cell or red blood substitutes. Fluorocarbon emulsions is only class has been investigated as artificial blood substitutes over the years.[43] Fluorocarbon liquid is dispersed as physiologically acceptable emulsion as they cause vascular obstruction and death when injected intravenously.[44, 45, 46] For last one decade there is continuous research around different part of world toward developing new emulsifying agents that provide emulsion having great stability and broader utility in many industries including medical and non-medical fields.

Miscellaneous Approaches

Recombinant Technology for delivery of blood substitutes

Recombinant human gelatin like protein having isoelectric point near to 8 is an ideal method to control the clearance of artificial blood substitutes. Slow gelling due to hydroxyproplinecontent results in of the composition which allows the use of high molecular weight protein which helps to maintain suitable colloidal osmotic pressure. These can also prevent risk of anaphylactic shock which exists with most of commercially available formulations.[50]

Crosslinking technique for delivery of blood substitutes

An ideal blood substitute should have acceptable erythrocyte sedimentation rate (ESR) and excretion rate (EXC) values e.g. Dextran -Haemoglobin (Hb-Dx) conjugates having a molecular weight between 50kd and 500kd. Other linkers can be used such as acyl phosphate ester benzene pentacarbooxylate and PEG Various modified Cross-linked haemoglobin can be classified into three main categories that areintermolecular cross bridged Hb tetramer or inter and intermolecular cross bridged Hb polymer and Hb surface decorated with inert polymer such as PEG. All three classes are classified as modified haemoglobin which function primarily by preventing glomerular filtration of a cellular Hb and hence eliminate nephrotoxicity problem which is mainly associated with unmodified haemoglobin.[52] Diaspirin cross-linked hemoglobin (DBBF-Hb) is a useful model to search possible correlations between structural functional alterations and toxicity of hemoglobin-based blood substitutes, as it has been extensively evaluated in vitro and in animal models.

CONCLUSION

Artificial blood can be regarded as a blood substitute for providing increase in fluid volume and oxygen carrying capacity of vessels. By providing a blood substitute we can maintain the circulating blood volume as well as need of patient. The blood substitute carry and transport oxygen to tissue and can support life temporarily until patients either can regenerate their own red cells or can be transfused with banked blood. Synthetic chemical compounds called perfluorocarbons are currently being studied as a substitute for red blood cells. Currently artificial blood technology is limited to short-term blood replacement applications. Thus, research on artificial red blood cells and artificial platelets in Japan are progressing chiefly with the Grants-in-Aid for Health-Labour Science Research. These all efforts not only contribute to future medical care in Japan, but also lead to a considerable international contribution for many countries where safe blood supplies are falling short. For now, the long-term development is expected to be promoted in the private sector with a view to benefit humanity, though profitability will have to be secured.In the future, it is expected that new materials to carry oxygen in the body will be made available as blood substitutes.

ACKNOWLEDGEMENT

We acknowledge Integral University Lucknow India for providing facilities and supports.

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