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Review Article

A REVIEW ON ARTIFICIAL BLOOD: A SOURCE WE NEED

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ABSTRACT

Blood is a liquid tissue, in which abundant chemical factors and millions of different cells are dissolved. It is one of the most demanding sources in clinical and medical aspects. The issues and cost of human blood collection and storage directed this procedure toward the use of alternative blood. Thus, came an invention of artificial blood and blood substitutes. These alternative blood or blood substitute is a substance which is made to play as a substitute of erythrocytes. Thus, the main objective is to replace the normal human blood with artificial blood substitutes in the place of blood transfusion during surgeries and organ transfusion. Two major and focused blood substitutes in pharmaceutical aspects are perfluorocarbons and hemoglobin-based oxygen carriers (HBOC's). Among these HBOCs vaguely resemble normal human blood. These blood substitutes are to allow flow through the blood stream to carry the oxygen and supply it to heart and other parts of the blood. They are used to fill the lost fluid volume. They are also called as plastic blood with iron atom as the base. They are found to serve as a good oxygen carrier. The results showed by these products are discussed, and they proved that they can act as a blood substitute and also they can reach the human tissue easier than erythrocytes and can control oxygen directly. However, these artificial blood products are being processed in research laboratories for good outcome. Their important functions are oxygen carrying capacity and to replace the lost blood volume in the human body. Their special features are survivability over a wider range of temperatures, eliminating cross matching, cost efficient, pathogen free, long shelf life, minimal side effects. Thus, artificial blood products are really a good alternative source which we need for replacing normal human blood.

Keywords: Artificial blood, Blood transfusion, Plastic blood, Perfluorocarbons, Hemoglobin-based oxygen carriers, Cross matching, Shelf life.

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INTRODUCTION

The main aim of this concept is to find a source which can replace a human blood. Blood is a fluid which is pumped into the body by the pumping action of the heart and through blood vessels, they are circulated to all over the parts of the body. From the inhaled air, the arterial blood carries oxygen with the help of lungs, and the cells produce the waste product Co_2 of this whole process or metabolism is carried by the venous to the lungs to get exhaled [6]. The questioning of why to replace a human blood is to be answered as, in present, the value of blood has increased and it has become one of the most demandable sources. In practice of surgeries and other procedures like organ transfusion, one can lose their body blood volume, thus the time of need for blood arises. In our country, the receptor's level is much more increasing than donor's level. If in case of donated blood, the patients should face the following complications such as same blood group and pathogen-free blood should be screened to check whether the blood is free from infectious diseases. The normal method of blood transfusion is mostly suggested, but it cannot meet the need of demanded blood all the time [20].

Blood transfusion and iron chelation therapy have improved the quality of life and life span to an age of around 30 years, but frequent blood transfusions cause progressive iron overload, which is a major clinical complication of the treatment [16]. Iron overload can result in multiple progressive organ damages grouped together under a condition called hemosiderosis [17]. Hence, it is to be noticed that an artificial product could replace the human blood and that is called as blood substitute (or) artificial blood [6].

BLOOD TRANSFUSION

Blood transfusion is a technique where the blood is transmitted from one person to other. It is also defined as injecting the blood and blood products to one's circulation. This is one of the widely used methods in various medical and clinical fields to replace the lost components in the blood. Thus, in proceeding the artificial blood products on to the blood stream, the blood transfusion method is analyzed. Many trials were conducted with different blood samples, to find the common effect of treatment effect using meta-analysis [19,23]. To considered different average percentage of donors which would not support the motivator goodness of fit test were used [24]. Furthermore, using blood plasma, platelets, and frozen blood, a supply chain mechanism has been invented for the supply of blood products [21]. During the blood transfusion of hospitalized patients, the implementation of restrive method strategies has proved to have the capability to decrease the severe health-care infections [22]. Blood transfusion not only has improved the lifespan but also frequent transfusions will lead to a major clinical complication in the treatment [18].

ARTIFICIAL BLOOD

Artificial blood is a substance made to function as a substitute of blood and also for the oxygen transfer throughout the body. They are also called as blood substitutes and artificial oxygen carriers [3]. They can perform the main functions such as oxygen carrying and expanding the blood volume. The main aim of artificial blood is to provide alternative way for blood transfusion. They are smaller in size around 0.08-0.2 μ [6]. They can be in microsize, macrosize, and also in nanosize this comes under cellular dimensions. An unlimited types of material bounded to a single structure called as artificial cells. Thus, these are also called as nanomedicine [15]. The shape and size of the blood substitutes were compared with red blood cells (RBCs) and the figure is depicted in Fig. 1.

Need for artificial blood

Due to shortage in blood supply, the minimum stock of blood a country needs is 1% of its total population. In India, the total blood collection

recorded is 4 million units. In the case of 10% million requirements, it can only meet the need of 40% units. The recent reports say that the blood donation has increased from 4.4 million units to 9.3 million units. During blood transfusion from one person to another, there is a chance of transmission of chronic and infectious diseases like HIV, Hep B, Hep C, etc., and other blood-borne diseases which can be prevented/ stopped by the use of artificial blood. Mistransfusion can also occur due to error. The cost for collecting the blood, followed by the screening process, then storing it with all requirements, and administration is quite high for normal blood transfusion process [7].

Ideal artificial blood

Artificial blood has increased availability that would replace the demand of donated blood. It has most promising oxygen carrying capacity [11] equaling that of biological blood. It has the ability of volume expansion of blood inside the body. It is universal compatibility (elimination of cross matching). They are pathogen free and have minimal side effects. Ability to survive [2]. The two most promising artificial blood products are perfluorocarbons (PFC's) and hemoglobin (Hb)-based oxygen carriers (HBOC's).

PFCs

To modify human or animal, Hb is one of the ways to create a blood substitute and thus helps to solve the problem of lack of blood replacement; another way is to use materials better than Hb. Thus, fluorocarbon chemicals were invented to replace the function of Hb [6]. PFC's are linear cyclic hydrocarbons of low molecular weight. In liquid fluorocarbons, hydrogen ions have been replaced by fluorine since they are neutral chemical compounds. They are sufficiently better solvents for carbon dioxide and oxygen to maintain the respiration process in animals, colorless, stable at high temperatures also, odorless, partially soluble, and entering weakly into chemical reactions.

PFC's are able to release and carry a high volume of oxygen to tissue, and the volume of physically dissolved gas which relies on the solubility coefficient of the PFC's utilized and is proportional to the gas partial pressure. This situation leads to increasing oxygen concentration in the air inspired by the patient due to several folds of increase in O_2 concentration in the emulsion of the PFC. The release of O_2 to tissues is greatly enhanced, and extraction rates and ratios are a lot higher with PFCs because of weak interaction between O_2 molecules, to compare with 25-30% of Hb PFC is able to reach 90% oxygen of its carrying [8]. The structure of PFC is depicted in Fig. 2.

Generations of PFCs

The different generations of PFCs along with its example are given in Table 1.

Perfluorocarbon emulsions

Fluorocarbon emulsion is usually well-tolerated emulsions. They often cause a fail in arterial blood pressure at the beginning of perfusion.

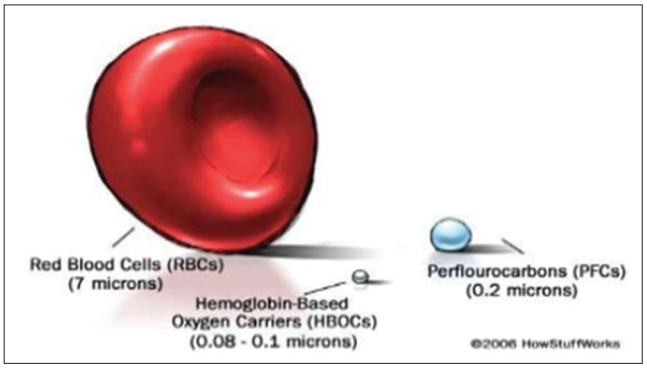

Fig. 1: The shape and size of blood substitutes (Jani *et al.*, 2012)

Some of the emulsifiers are used for this process including lipids, bovine albumin (cow) and polyols, for example, a mixture of polyoxypropylene and polyoxethylene and also Plulronic F68. By the use of mechanical energy or ultrasound, the emulsions are produce. For ensuring easy flow through the capillary vascular system, the greater importance should be given to the selection of right degree of dispersion, gas diffusion capacity for obtaining the viscosity and also the time retention of the fluorocarbon in the circulation [4]. The structure and composition of perfluorocarbon emulsion are depicted in Fig. 3.

Composition of PFC-based blood substitute

The PFC-based blood substitutes can be prepared by the following materials, and the materials are listed in Table 2.

Advantages of PFCs

The advantage of PFC's is they do not interact with oxygen and they also allow easy transportation of the oxygen to the human body. The supply of oxygen in plasma level has increased, and the level in plasma has increased. The physical parameters such as pH and temperature are minimized in blood circulation which makes the PFC's the better alternative for the blood [5].

Benefits of PFCs

The benefits of PFC's than biological blood are it can be stored for more than 1 year in a normal room temperature and the initial checking of blood grouping is not neccesary. This blood can even penetrate into small capillaries with ease allowing hem to bypass arterial blockages and deliver the oxygen to the most needed areas. This ease of blood flow is possible because of their size when compared with RBCs. It also has its effects on chemotherapy of radiation in tumor treatment for cancer patients [5].

Disadvantages of PFCs

PFC's must be prepared as emulsions since they cannot remain in aqueous phase for a longer period of time. To ensure oxygenation of tissues, the patients must breathe at a linear rate because PFCs absorb oxygen passively. High requirement of fraction of inspired oxygen is needed [2]. Platelets count in the blood will be reduced and it causes flu-like symptoms. Adverse effects of PFC's may also cause if there is an intake of continuous doses. It will also lead to impaired neutrophil function and cause allergic reactions [8].

HBOCs

Hb an obvious candidate for blood substitute that has a number of desirable features. The carrier has the ability to carry oxygen and

Table 1: Generations of PFCs

S. No	Generation	Examples
1	1st generation	Fluosol-DA™
2	2nd generation	Oxyfluor™ Oxygent™
3	3rd generation	Perftoran™ PHERO$_2$™

PFCs: Perfluorocarbons

Table 2: Composition of PFCs

Materials	Amount (%)
Distilled water	57.8
DSPE-50 H	0.12
Perfluoro-octyl bromide	28
FO-9982	12
Yolk lecithin	2.4

PFCs: Perfluorocarbons

is countless of non-antigen complexes of erythrocyte membrane. According to molecular stability improvements techniques, there are four groups of Hb cells: Surface-modified Hb - the molecule-linked Hb - polymerized Hb - the Hb liposomal capsule. HBOC's has half-life of 18-24 hrs, which are sufficient for use in acute care. Moreover, they can be maintained at a temperature of 4°C for a period of 1-2 years. HBOC has less oxygen affinity than normal human blood [1]. The structure and linking of HBOC's are depicted in Fig. 4.

Conventionally, it is recommended that for <600 ml of blood, plasma expanders should be used. For 600-1200 ml, RBCs products are essential, and blood plasma derivatives or products such as low platelets or whole blood transfusion are critical. Blood substitutes should be used as a suggestion, and complementary transfer of autologous or homologous should be used in combination with the protein.

Hb is considered as the tetramer and is linked with 2-α and 2-β polypeptide chains with iron heme group at the center of the molecule. Hb's, as having an excellent source of blood substitute, thus it allows heme bonds to have high oxygen affinity [10].

Stabilization of stroma-free Hb

The initial step toward clinical study with free Hb resulted in nephrotoxicity. Nephrotoxicity is a toxic symptom occurred in kidney, and it is poisonous. The Hb used was found to have bacterial endotoxins as well as erythrocyte membrane stromal lipids.

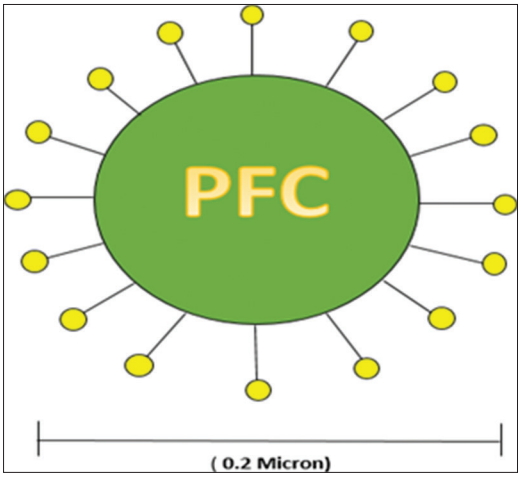

Fig. 2: The structure of perfluorocarbon (Mohan Krishna *et al.*, 2011)

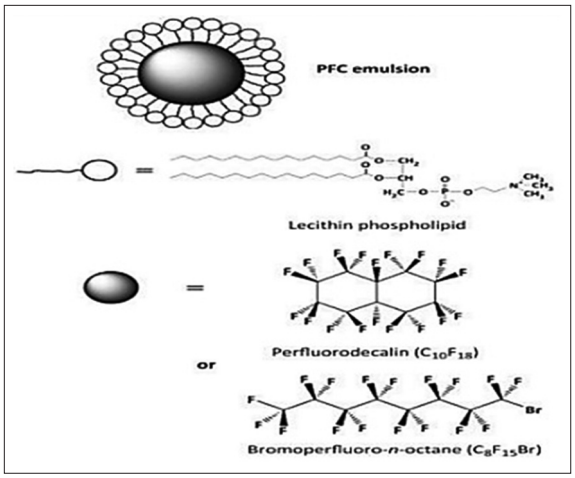

Fig. 3: Structure of perfluorocarbon emulsion (Tao and Ghoroghchian., 2014)

To get rid of these problems, stoma-free Hbs were developed. Stroma-free Hb has too short intravascular half-life because of tetrameric Hb (2-α and 2-β) dissociated into (alpha beta) dimmers. These were excreted in the urine after the filtration process of the kidney. During the purification process, the 2,3-diphosphoglycerate (DPG) is lost thus this stroma-free Hb has too high oxygen affinity. Hence, there is a need of stabilization of the Hb [7]. The structure of exact stabilization of Hb is depicted in Fig. 5.

In the above, Fig. 5 shows that there is a tetrameric stabilization taking place by intramolecular cross linking between the 2-α (or) 2-β. After the conjugation process with the help of polyethylene glycol, the Hb's molecular weight can be effectively increased. The polyfunctional cross-linking agents may produce polymerized Hb. Moreover, the liposomes can also be formed by encapsulating the Hb [7].

Intramolecular cross linking

The main aim of intramolecular modification is to cross-link the 2-α or 2-β subunits. And also to stabilize the association of the 2-α or 2-β dimers because the alpha or beta dimers are relatively stable [13]. The intramolecular cross linking of Hb is shown in Fig. 6.

3,5-dibromosalicylfumarate and nor-2-formylpyridoxal 5-phosphate are used as a support matric for cross-linking process. The oxygen affinity of Hb is reduced during cross linking, and also it prevents tetramer dissociation. Too high oxygen affinity of stroma-free Hb

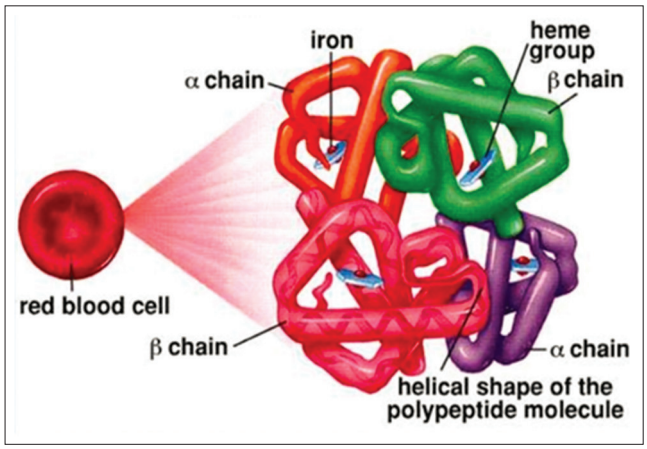

Fig. 4: Structure of hemoglobin-based molecules

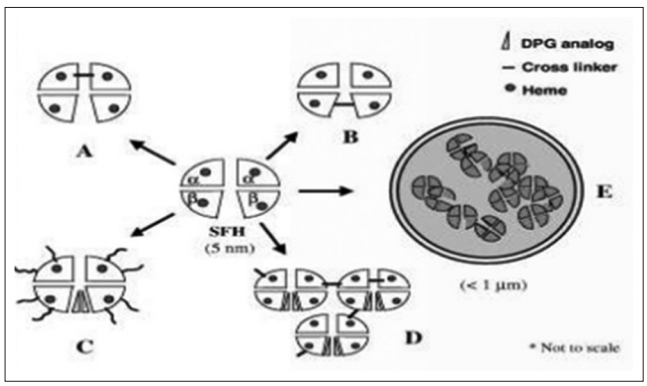

Fig. 5: Stabilization of free hemoglobin. (Mohankrishna *et al.*, 2011)

can be fixed by the addition of DPG analogs such as pyridoxal-5'-phosphate. To deliver more oxygen to the tissue, the more DPG is need in the cell. The less DPG, the less oxygen delivered to tissues. Pridoxylated stroma-free Hb has nearly normal oxygen affinity, for example, Hemopure™ [3].

Polymerized Hb

Hb oligomers are formed in the polymerization of Hb. Thus, the size of the molecules is increased by intermolecular cross linking. By the use of dialdehydes, such as glutaraldehyde and glycolaldehyde, multiple Hb proteins are linked together in this process [3].

The polymerization of Hb and its structure is depicted in Fig. 7.

Conjugated Hb

The process of conjugating Hb is also called as binding of Hb. To increase its overall size, the Hb is conjugated into a biocompatible polymer like as polysaccharid. To increase the molecule's size, multiple polyethylene glycol chains were added to Hb protein, in specific case of pegylation. The conjugated tetramers from free tetramers are shown in Fig. 8. Once pegylated, the molecule size increases from 3 nm to 15 nm. To protect the molecule from renal excretion, conjugation of Hb with PEG takes place. The intravascular circulation time of HBOC can be increased by conjugating the Hb with macromolecules [3,14], for example, Hemospan™.

Hb vesicles (hemoglobin encapsulated vesicles)

The encapsulation process takes place when the Hb is based on the idea of recreating the original and natural properties of RBC in the absence of blood group antigens. The structure of Hb vesicle is depicted in Fig. 9. The hemosome is often referred to as encapsulated Hb. The encapsulation process involves encapsulating the Hb within lipid vesicles in the solution of phospholipids. The better diffusion of O_2 and Co_2 can be done by lipid membrane [3].

Fig. 6: Structure of intramolecular cross linking (Mohankrishna *et al.*, 2011)

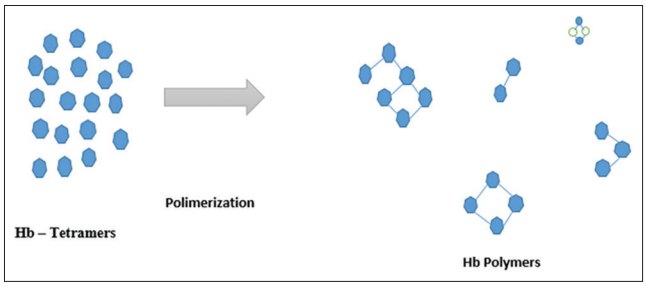

Fig. 7: Structure of polymerized hemoglobin. (Betts and Whittet, 1962)

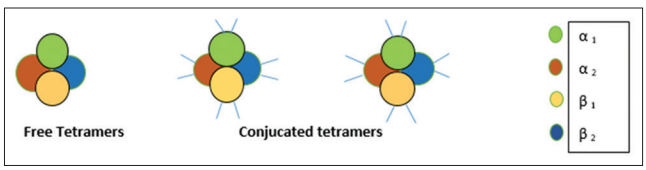

Fig. 8: Structure of conjugated hemoglobin (Mohankrishna *et al.*, 2011 and Shalini., 2012)

Recombinant Hb

From the microorganism, like *Escherichia coli* specially modified Hb may be produced, with the help of advance ADNA technology. Using an expression vector containing two mutant human globin genes, recombinant human Hb was produced in *E. coli*. One was fused alpha globins, and the other was a low oxygen affinity mutant. These products recombinant Hb are advanced to clinical trials, but it was completely stopped due to vasoconstriction and other harmful effects [3]. Some of the examples are depicted in Figs. 10 and 11.

The characteristics comparision between RBCs and oxygen therapeutics is given in Table 3.

The current status about the development process of HBOCs is given in Table 4.

Example of HBOCs polyheme

It is formed by extracting Hb from RBCs, and the Hb is mixed into an electrolyte solution after being associated into tetramer. It is compatible with all blood types, and it has a shelf life of approximately 12 months.

Advantages of HBOCs

They are available in larger quantities and can be sterilized by pasteurization process. It can be stored for long durations. Without typing or cross matching, it can be administered rapidly. They have many military applications also [7,25].

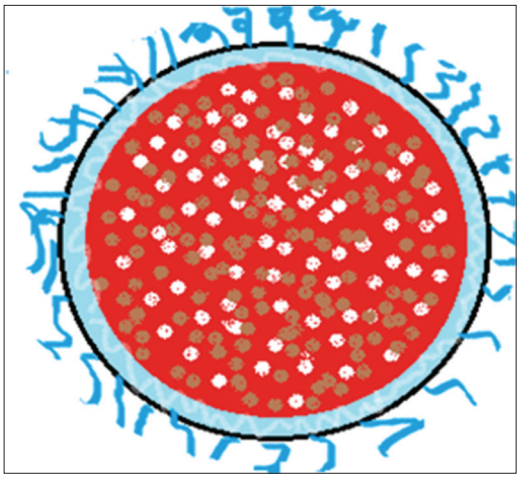

Fig. 9: Structure of hemoglobin vesicles (Shalini *et al.*, 2012)

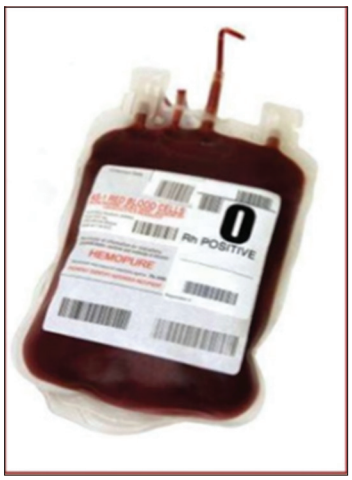

Fig. 10: Hemopure

Table 3: Comparision of biopure's oxygen therapeutics and RBC's

Characteristics	Biopure's oxygen therapeutics	RBC's
Storage	Room temperature (20°C–30°C)	Refrigerated
Shelf life	36 months	42 days
Preparation	Ready to use	Testing, typing, and cross matching
Compatibility	Universal	Type specific
Effectiveness	Immediate oxygen delivery	Depend on length of storage
Purity	Processed to remove infectious agents	Tested and screened for infectious agents
Raw material	Bovine hemoglobin	Human blood
Cost	600–800	125–425

RBC's: Red blood cells

Table 4: Current development status of HBOC's

Product type	Product name	Developer	Source and/or technology	Status
Cross-linked Hb	HemAssist™	Baxter (USA)	αα cross-linked human Hb	Discontinued
	Optro ($_T$Hb)™	Som atogen (USA)	Recombinant Hb	Discontinued
Polymerized Hb	PolyHeme™	Northfield lab (USA)	Glutaraldehyde, pyridoxal human Hb	Discontinued
	Hemopure™	Biopure (USA)	Glutaraldehyde bovine Hb	Approved
Conjugated Hb	Hemospan™	Sangart (USA)	Maleimide PEG - human Hb	Discontinued
	PEG-Hb™	Enzon (USA)	PEG conjugated bovine Hb	Discontinued
	PHP™	Apex (USA)	Polyoxyethylene - conjugated human Hb	Discontinued

Tao and Ghoroghchain, 2014. HB: Hemoglobin, PEG: Polyethylene glycol, HBOC's: Hemoglobin-based oxygen carriers

Fig. 11: Oxyglobin

Benefits of HBOCs

It is faster and good at O_2 distribution. It has long shelf life. There is no refrigeration needed. It is universally compatible to all receptors. It is one of the ready to use substances. After injection, it immediately offloads oxygen [3].

Disadvantages of Hb-based oxygen carriers

They have not found to be safe in humans. It has reduced circulation half-life. It mainly releases the free radicals into the body. It disturbs certain physiological structures, especially the gastrointestinal tract [7].

Adverse effects of Hb-based oxygen carriers

It causes renal effects, neurotoxicity, platelet aggregation, antigenicity, pancreatic, and liver enzyme elevation. They also result in gastrointestinal side effects, vasoactivity/hypertension, nephrotoxicity, coagulopathy, and immune suppression [7].

Promising techniques

Stem cells

Stem cells are capable of propagating and differentiating to multiple lineages which give rise to a range of many specialized cells [26]. Recent scientific community has started to experiment the chance of producing an alternative source of transfusable blood using stem cells. Using hematopoietic stem cells, Giarratana *et al.* have performed a study to describe a production of mature human blood cells in large scale (*ex vivo*). This represents the first directed step in this innovation. The content and morphology of the normal blood cells are moreover the same as in the blood cells produced in culture. The founders of this study also proved that these produced RBCs nearly have normal shelf life or lifespan when compared with normal RBC.

The major difficulty in this procedure is the cost of production of RBCs. Then, again the introduction of complex three-step method in the production of cells would have make the blood cells unit too expensive. However, this is the first study to demonstrate the possibility in large-scale production of closely related RBC which almost resemble normal RBCs [2].

Biodegradable micelles

Formation of amphiphilic block copolymers by encapsulation of recombinant or polymerized Hb into the micellar is used to enhance the circulation. The diameter of this system is usually between 30 and 100 nm. The similar hydrophobic Hb protein can be able to solubilized by the hydrophobic core of the micelle polymer, whereas the steric barrier for protein absorption is provided by water-soluble corona (which is usually polyethylene glycol), and the reticuloendothelial system (RES) provides protection from clearance [2].

Advantages of artificial blood

There is no fear of infection. It can be kept at normal room temperature and can be stored for the period of more than 1 year. Patients in trauma and other severe situations require rapid treatment. The armed services in medical care would get benefit from the artificial blood. The immediate and sudden supply of full capacity oxygen transport can be allowed by artificial blood. It is an alternative source for the patients who refuse blood transmission for cultural or religious reasons. Currently, the transfused blood is much more cost effective. This may change if the manufacturing becomes refined the cost of the artificial blood may fall [5].

FUTURE PERSPECTIVES

In future, the extension in research should create an artificial blood which with more and more minimal differences from biological blood

and adverse effects. Since some blood substitutes still suffer from certain kind of limitations. Most HBOCs can no more last than 20-30 hrs. Thus, these are called as short-term blood transfusion. Thus, in future, longer lasting products which can run over a body for a longer time should be produced, and this development will have a wider range of application. Currently, some of the companies are working on it, these are curious questions that have to be answered for the companies who really interested to spend on research and production.There are many challenges in this aspect, but going through it will surely give this world a pride of science and technology. It will lead to a source to help and save our human kind.

CONCLUSIONS

Blood supply demand was increasing as compared to blood donation in all over the world. Thus, artificial blood will be ultimately useful to meet this demand. They can fulfill the receptor's blood volume until they receive it from the donor or the option of blood transfusion is not acceptable. Thus, new oxygen carriers are now invented to act and serve as normal blood. Both inversement and intelligence are needed to develop the process. If the research and development process of artificial blood or blood substitutes has increased in their range sure, the world will get an unbeatable source to save human.

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