An artificial blood, electron microscope image, support, principles, benefits, and fusion methods: a review

An artificial blood, electronmicroscope image, support, principles, benefits, and fusion methods: A Review

Auns Q Al-Neami[1], Noor S Shalal[2], and Khawla H Rasheed[3]
[(1),(2)]Biomedical Engineering Department, College of Engineering, Al-Nahrain University, Baghdad, Iraq
[(3)]Ibn Sina University of Medical and Pharmaceutical sciences, Baghdad, Iraq

uns_alneami@yahoo.com;

Abstract. Over several decades, artificial blood become an important thing to develop in order to save life of many people because the main blood function in the body is the transporting oxygen to body's tissue and carries carbon dioxide to the lung, protecting the body against bacteria and viruses, dominance of bleeding, in addition to proteins and water found in it. This review paper presents the studies for systems that have been used for manufacturing artificial blood and their side effects. Also include the recent studies and articles that discussing the development in artificial blood to make it closer to normal blood and more compatible with body and have many characteristics as those of native RBCs.

Keyword: artificial blood, RBCs, polymrized hemoglobin, cross linked hemoglobin, connjugated hemoglobin, recombanent hemoglobin, nanocapsules.

1. Introduction

Studies of producing artificial blood as an RBCs substitute have been started for several decades. Several materials such as Ale, Wine, and Opium could be utilizing for blood substitute as proposed by Sir Christopher Wren in 17th century. Revealing of human blood antigens in 1901 by Karl Landsteiner was at the same time of starting of blood transfusion [1,2]. Karl Classified human blood groups into A, B, C (its name changed to O later) and after one year, AB group has been included. Because of the shortage in saving method and in proper anticoagulation, the theory of blood transfusion have a finite limit, although blood transfusion became available as approved by Ottenberg in 1913. After the first and second wars, some changes and developments have occurred on the blood transfusion process which allows it to be available [3].

It's important to make an overview about the role of blood in the body and its functions before everything. Blood is a noble fluid, constitute about five liters in an adult, it supplies the body's cells with nutrients and take up the waste outputs of them, it conforms to body's need over the circulatory system. The blood consists of several contents each of them perform a unique function, these contents include: red blood cell (RBCs), white blood cell (WBCs), platelets as shown in Fig. (1), and plasma,. Plasma has a large amount of water, in addition to proteins, sugar, hormones, and fats. It accounts for more than sixty percent of blood in the body. The RBCs represent the most common types of cells in the blood, its shape is a biconcave and doesn't have mitochondria or nucleus. Its shape helps it to pass through tight blood vessels. Its shape increases red blood cell surface area. Red blood cell is responsible in definition the blood type by an identifiers found on RBC's surface, these are known antigens [4,5]. Hemoglobin which is the most important protein contains within Red blood cell is responsible of providing body's tissue with oxygen from the lungs and take up carbon dioxide again back to the lungs by an iron. The white blood cell protecting the body against bacteria and viruses. While the platelets have a major role in preventing bleeding when wound occur [6].

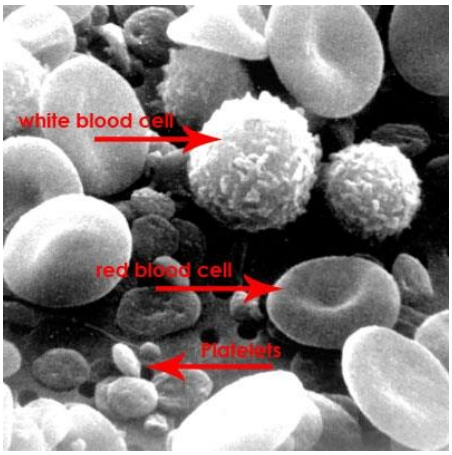

Figure 1. Human blood as scanned by an electronic microscope[7].

Due to its importance role in the body, changing in amount of blood inside the body leads to problems in the body. Decreasing blood in the body below its normal value leading to many risks attack the human body among them is anemia. There are many reasons lead to decrease its amount such as bleeding, during surgery, blood loss after childbirth, cancer, accidents and so on. Therefore, the need for compensation is essential. To compensate the shortfall, the patients need to be supply with blood transfusion for substitute the lost blood.The process of blood transfusion from donor people have several confrontations include the following:

1. There is a disadvantage related to its scrubby life in circulation system of about less than 40 days [8]. This makes it undesirable for cases in which the blood is needed to save for relatively long time such as in Military medicine. Patients with severe bleeding may not being alive if they are treated with volume expander only [7].

2. A dead result will take place if the patient receive a blood type not match its own blood so, it is important to be make sure that the donated blood type match the blood type of the corresponding patient [9].

3. The shortage of quantity of donated blood in the hospital, this make patient's life is threated [10].

4. The most important confrontation is that there is a possibility of transmission of viruses (such as Hepatitis A, Hepatitis B, Hepatitis C), and Bacterial infection (such as Syphilis) [9].

The above conformations mean it must be a substitute to ensure safety and constant supply of blood to use in medicine. So, in order to avoid these limitations, artificial blood has been introduced. Artificial blood can only replace the work of red blood cell. It might be better to give the name of oxygen carrier in state of artificial blood due to its unique function like that of red blood cell [10].

2.Artificial Blood

Artificial Blood is a product utilized to mimic limited function of human blood of carrying oxygen. Artificial blood was introduced to provide a substitute to donated blood from other people [11]. There are many conformations make an oxygen carrier out of being as artificial that all efforts of 2011 have failed to overcome the conformations [12]. Plasma, white blood cell, platelets have not been a part of artificial blood. It works until bone marrow recover the normal ratio of red blood cell in the body. Its function as oxygen carrier to body's tissue and carbon dioxide to expel by lungs out of the body.Progressing of artificial blood was the most important thing for researchers they were need to reach a point that artificial blood can exactly do the function of hemoglobin [3]. The label of artificial blood is not precise since it is substitute only the function of RBC. The accurate and more suitable name is Volume

expander for inactive products (crystalloid-based or colloid-based) and for oxygen- carrying products has been given the name oxygen therapeutics [13]. Oxygen therapeutics are in development and in clinical trials, depending on its transport mechanism it can be classified into two categories: perfluorocarbon based and hemoglobin based [14].

2.1 Advantages of Artificial Blood

The main benefits of the artificial blood as follow:

1. There is no possibility of infection, as compared to donated blood which has the possibility of transmission of viruses,Hepatitis B,HIV/AIDS, and organisms [15].

2. Typing and cross matching is not required so it can provide for all patients. Because it hasn't antigens that defines Blood type [7].

3. Its life time is longer than donated blood of approximately 42 days. Artificial blood can stay more than three months without need for refrigeration [15].

4.Artificial Blood might preferable for people who reject donated blood for educational or religious reasons [7].

5. Available for emergency situations, because it transmits the blood faster than donated blood [15].

6. It is not collect in body's tissue but it has full excretion.

7. Nonimmunogenicity, nonantigenicity, and noncarcinogenicity, and it have less toxic [16].

3. Design

There are two types of products that can be used as artificial blood but still in progressing these are include Perfluorocarbon (PFCs) based and Hemoglobin based [10]. Figure (1) shows the methods of hemoglobin synthesis.

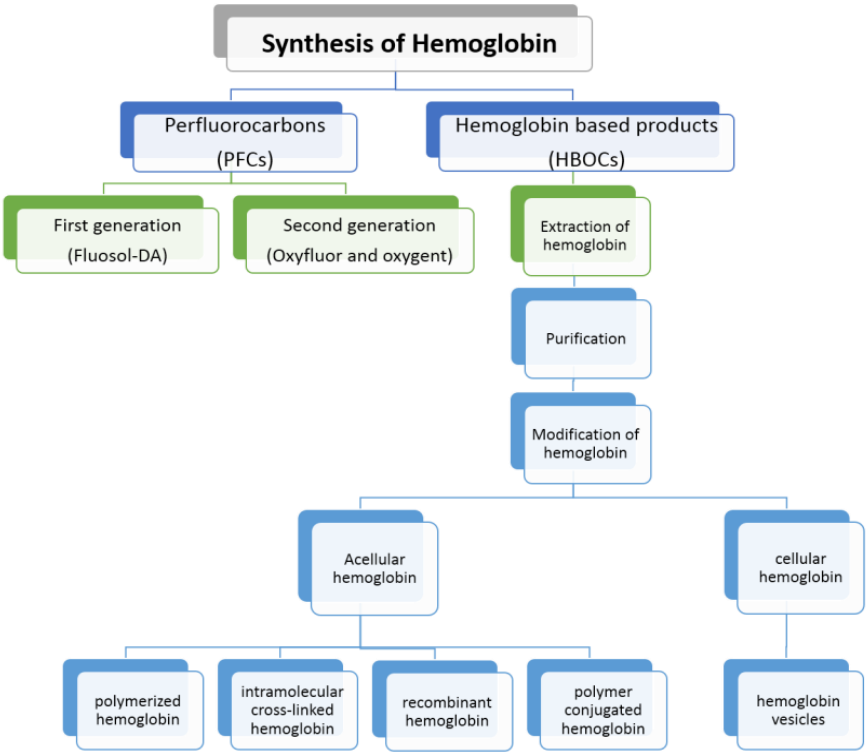

Figure 2. Methods of Hemoglobin Synthesis.

3.1Perfluorocarbons (PFC)

Scientists started their studies of artificial blood with use of perfluorocarbons substances so, these substances were considering as the first artificial blood generation [17]. Perfluorocarbons are colorless, clear liquid, nontoxic, low boiling temperature, chemical inert substance. Its chemical structure consists mainly of fluorine and carbon atoms. It is unsolvable in water so it is hydrophobic also it doesn't solvable alcohol [18,19]. Figure (2) shows the Perfluorocarbon.

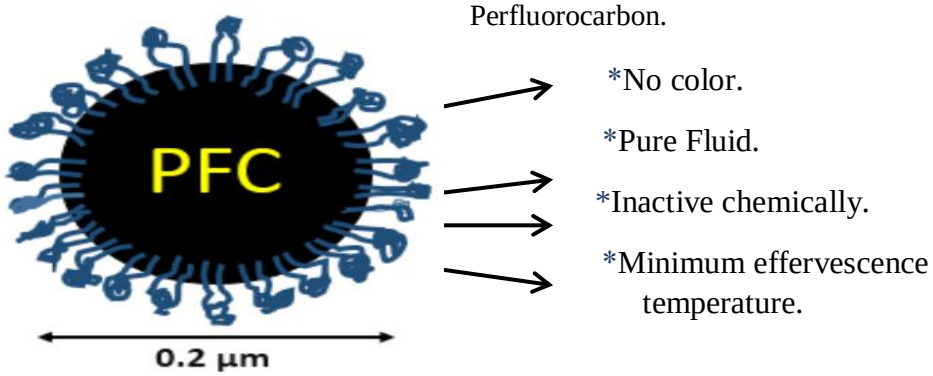

Figure 3. Perfluorocarbon.

In 1966, Clark explained the perfluoro carbon oxygen carrying ability for the first time; PFCs contain cyclic or straight hydrocarbon chains [20]. The better one of carrying oxygen is the straight shape than cyclic shape [16]. General chemical formula of PFC is C_nF_{2n+2}[21].

In order to be used as artificial blood there are two challenges that must be overcome. These challenges represent the following: firstly, it has the property of insoluble in water so that it must be concerned with emulsifiers- fatty compound known as lipids that have the capacity to hang small particles of perfluoro chemicals in the blood [10]. Secondly, cavities are composed between PFCs molecules [22]. The capability of perfluoro carbons to carry oxygen is in linear relationship to partial pressure of oxygen in balance with emulsions. Thus, hemoglobin can connect more oxygen than those resolve in perfluoro carbon at a known value of partial pressure [23]. As a result, large amount of perfluoro carbons is needed [10]. Generation of relatively stable perfluoro carbon emulsion in addition to its small size (median diameter of less than 0.2 µm) have been available with the development of technology [8].

Perfluorocarbons have a number of benefits include:

1. It can transmit oxygen without interact with it also it isn't interact with other gases [24];

2. It can thawed oxygen 20 times more than that of thaw of water and 2 times more than plasma, its thawed for oxygen is at a concentration ranging between 40%-50%;

3. Solubility of carbon dioxide is about 130–160 mL in perfluoro carbon which is more than the ability of water to dissolve it [25];

4. Its tiny particles as compared with red blood cell of a diameter 0.025 as that of RBC so it can pass blood vessels of small size that RBC can't pass through it [26].

5. PFCs have a property of being withstood against heat and temperature of about 300 C° and more without any alteration in it. This property makes it resist against heat sterilization [27].

6. It can be made without any contact with a biological substance; it is also low in cost [28].

There are many people who deny blood transfusion based on human or animals source. So, perfluorocarbons offer another advantage that can be preferable for those people. It has been

show the role of these products during surgeries for patients suffer from traumatic or hemorrhagic shocks even in war casualties by Chen et al [25]. PFC decreases the need for allogeneic RBC transfusions in non-cardiac surgery [29].

3.1.1First Generation of Perfluorocarbons. The first acceptable product based PFC substance was developed in Japan and first tested was done in November 1979in the United States. This product is known as "Fluosol-DA"[30], emulsifying agents of egg yolk phospholipid and pluronic-68 utilized for this product [31]. FDA assented to it in 1989 [32]. Fluosol-DAis an emulsion of perfluoro decaline (PFD) and perfluorotri- propylamine (FTPA) [33].The chemical structure and specification of Fluosol-DA are presented in Figure (3) and table (1) respectively.

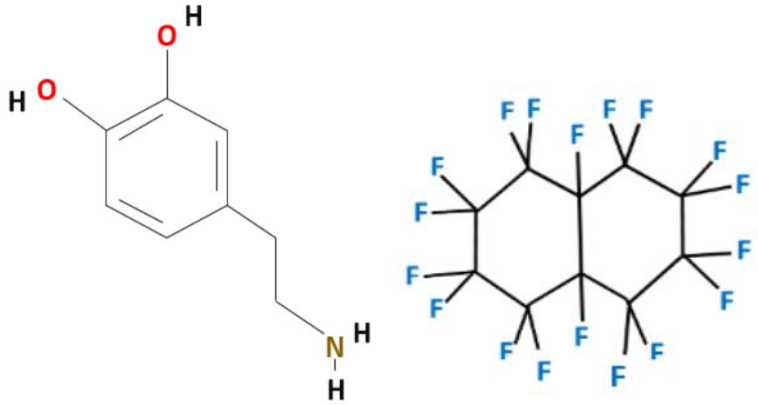

Figure 4. Fluosol-DA Chemical Structure.

Table 1 Fluosol-DA Specification[34].

Molecular Formula	**$C_{18}H_{11}F_{18}NO_2$**
Chemical Names	Fluosol-DA, hydroxyethyl starch, perfluorodecalin.
Molecular Weight	615.3 g/mol

The perfluorotri- propylamine (FTPA) has been provided with large stability, while PFC is the major content of it and is the oxygen carrier. Life time of them has different from each other. PFC has 3 to 6 hours while FTPA persisted in the body for several months [24]. People must breathe pure oxygen by mask or in hyperbaric chamber in order to allow it carries sufficient oxygen quantity [35].

In 37C° red blood cell can carry more oxygen than Fluosol-DA which is able to carry only about 7.2%. This product has several side effects lead it to reject and its manufacturing is stop in 1994 these side effects include: full activation in Fluosol-DA by factor of the emulsion lead to reaction of pulmonary this effect can be avoiding by steroid injection, Storage of the emulsion is hard (rewarming and congealed saving) [36,37].

3.1.2 Second Generation of Perfluoro carbons. Fluosol had set the way for more investigation of PFCs. Reiss and Le Blanc et al. describe the eligible features of the second generation of PFCs [38] include the following:1)Ability of Resolving of oxygen is high; 2) Have short survival in tissue and has faster secretion; 3) Have small side effects; 4) Raising pureness; 5) Big existence and high massive manufacturing.

Progressing of second-generation emulsions of perfluoro carbon by west has concentrated on perfluoro octyl bromide (C8F17Br), perfluoro decyl bromide (C10F21Br) and perfluoro dichlorooctane (C8F16Cl2) which are linear perfluoro carbons [19]. Alliance Pharmaceutical Corporation improved a steady and concentrated (60% w/v) emulsion of perfluoro carbon. Oxygent, has a mean size of 0.16-0.18 μm in diameter consisting of 2% w/v perfluoro decyl bromide, and 58% w/v perfluorooctyl bromide. By utilizing triglyceride, perfluoro dichloro octane, phospholipid of egg yolk, hemagen/perfluoro carbon improved perfluorocarbon emulsion. Oxyfluor has a mean size of (0.22-0.25) μm in diameter.

Oxyfluor and oxygent are under safe testing, although they represented the foundation for clinical testing by authorities, most of these studies aren't declared by scientific proprietary. These products are associated with several side effects include: flu marks attributed to working of phagocytic cells of retiendothelial system, thus a tiny particle (sized between 0.1-0.2 μm) are not discovered by retiendothelial as that of large particles larger than 0.2 μm, they also give raise to moderate temperature [19,39,22]. Also, difficulties in introduce the functional dose of Oxyfluor management, in addition, oxegent management lead to raising peril of stroke [40]. Nevertheless, it has been shown that any reaction between PFCs product and contents of blood didn't happen excluding a little alteration in clotting factors [41]. Moderate thrombocytopenia occurs under the effect of PFCs (causes shortage in platelets of about ten percent to fifteen percent) [42].

3.1.3 Third Generation of Perfluoro carbons. Oxycyte is pastured by Synthetic Blood International in Costa Mesa, California as a third generation curative oxygen carrier perfluoro carbon. It is introduced to provide an oxygen to body's tissue and take carbon dioxide to lungs which in turn take away outside the body. As compared to hemoglobin, Oxycyte is capable of carrying Oxygen five times than that of hemoglobin. It has been accepted for phase II, perhaps, oxycyte is useful for wound care, skill cell crisis, and heart attack. However, this product requires several research to ensure that it can be used as an artificial blood. Other Perfluoro carbons products are approved for human use include Perftoran, while other are still under development such as PHER-O2 [17]. Table (2) shows the main products of perfluoro carbons as blood substitiute.

Table 2. Perfluorocarbon Products as Blood Substitute.

PFC product	***Generation*** [43]	***Approval***	***Clinical trials***
Fluosol-DA-20	1st Generation	Approved by FDA in 1989 [32].	Stop in 1994, several problems associated with it [32].
Oxygent	2nd Generation	Doesn't Accepted. Due to its side effect [17].	Phase III clinical trials, due to its because of its stability, altitude secretion rate [38].
Oxycyte	3th Generation	No acceptance in countries, it is under evaluation [17].	Phase III clinical trials [17].
Perftoran	3th Generation	Approved in Russia in 1996, Mexico in 2005 [17].	For human use, in Russia [17].
PHER-O_2	3th Generation	No acceptance [17].	Pre-clinical trials[17].

Since the perfluoro carbon associated with several side effects, researchers interested with perfluoro carbon-based capsules [44, 45]. Mostly when wall material and biological emulsifier is biopolymer albumin. Albumin-derived perfluoro carbon-based capsules (PBCs) provide an advantages in preserved alive an isolated heart under ischemic conditions [46,47]. These capsules need more investigated in order to be used in human clinical trials. In a study that pointed to make individuals capsules moves in an environment that imitates physiological conditions by describing the holographic optical tweezer, also a statistical evaluation of the bonding based on different capsules sizes and capsules assemblage behavior were firstly investigated utilizing optical tweezer. Results shows that individual perfluoro carbon-based capsules studying is adequate using optical tweezer, in addition the last one supply information about the interaction of these capsules to be used as artificial oxygen carriers in the future by developing a flexible, faster, and simple method, also this method shows that capsules assemblage increase with increase the diameter of the capsules. This make us expect that the nano particles don't interact even less than the micro particles used in the experiments. However, to quantify the bonding forces, more studies and further experiment and more detailed statements are needed[48].

3.2 Hemoglobin Based Products

In an adult hemoglobin is consist of two beta chain and two alpha polypeptide chain each of them is connected to an iron containing heme-group which is binds one oxygen molecule. Any small change in partial pressure of Oxygen will affect the amount of Oxygen bound or released by hemoglobin because of the Oxygen-heme group binding change the shape of hemoglobin which in turn raise the affinity of Oxygen molecule by hemoglobin [3]. In mammals, the protein makes up about 96% of the red blood cells' dry content (by weight), and around 35% of the total content (including water)[49] . The name haemoglobin-based oxygen carriers (HBOCs) had given to Hemoglobin Based Products [50]. The main source of Hb for hemoglobin based RBCs substitute is from expired RBCs bags, other sources of Hb are from animals(bovine), recombinant Hb and cord blood RBCs.

Several features that Hb based animals have been introduced over Hb based human among them include the following:1)Exhibit high opposition against degeneration of heme; 2) unlimited access; 3) As an allosteric effector present in plasma, Hb based animals replaces 2,3-diphosphoglycerate (2,3-DPG) by chloride ion [51]. The 2,3-DPG is found in erythrocyte which is a particular intermediate of glycolysis, under condition of normal oxygen tension it is rapidly exhausted. In peripheral tissues when hypoxia occur, 2,3-DPG starts to gather to a level that make 2,3-DPG binds to hemoglobin decreasing its affinity for Oxygen resulting in a right-ward shift of the Oxygen-Hemoglobin promoting unloading of oxygen by hemoglobin and this effect promoting oxygen transport to tissues suffer from long term hypoxia [52]. The HBOCs have been in development as it is an alternative to RBCs [53].

The first use of hemoglobin solution was done by Von Stark in 1898 when he used it to treat patients suffering from chronic anemia [54]. Several efforts have been done in order to develop mutated hemoglobin when it turns out that native hemoglobin cause toxic to humans [3,55,56,58]. Using of native hemoglobin was started in 1937 for experimental transfusion in animals when A hemoglobin solution prepared by Amberson that produced by lysing red blood cell [59]. Although native hemoglobin provided oxygen, it causes hypertension. In addition, it was highly toxic to kidney. Native hemoglobin extracted from red blood cell stroma was less renal toxicity in animals [60]. These studies and other articles of Rabiner et al in [61], and savitsky et al in [62] reached to conclude that the existence of erythrocyte membrane's fractions and lipids had made the free hemoglobin solutions toxic and then causes hemolysis [63]. As a result, the use of native hemoglobin was stopped. Table (3) shows HBOC products as blood substitute.

Table 3. HBOC products as Blood Substitute.

HBOC	**Synthesis**	**Approval**	**Clinical trials**
Hemopure	Cross-lined bovine hemoglobin [17].	approved in South Africa [17].	Complete [17].
Hemospan (MP4)	It has been developed as carbon monoxide (CO) carrier (CO-MP4) [64].	No acceptance [17].	Phase II study [17].
Sanguinate	bovine Hb, it is joined on the surface lysines with PEG remnants, and it is joined to CO [65].	No acceptance.	Studies of Phase Ib studies have been plenary in sickle cell's patients [66].
Hemotech	Made from blood of cow [17].	Require more research; there is no approval [17].	Phase I trial [17].
HRC 101	Joined with hydroxyethyl oxidized starch, its content of Hb-hydroxyethyl starch is very small. The rest of it encompassing high-molecular-weight Hb-hydroxyethyl starch consolidates [67].	It shows that this product minimize sickle cell accompanied with death [67].	Experienced on mice [67].
Diaspirin cross-linked Hb (DCLHb) or HemAssist.	Human hemoglobin[68].	Institutional approval [69].	In phase lll clinical trial [70].

3.2.1 Purification of Hemoglobin. It must be taken into account that blood consists of various content (white blood cell, platelets, plasma, Red blood cell) so, red blood cell must be isolated before Hb purification [68].Several methods have been introduced as a purification process of hemoglobin [51,69-71].

Chromatographic separationis one of the most common used method [72-75] which is a biophysical technique for qualitative and quantitative analysis involving separation of mixture components that the mixture resolve into in a fluid called “mobile phase”, then it passed into another structure which have a material called “stationary phase” then the mixture will separate based on differential partitioning between mobile and stationary phases. The stationary phase can be liquid phase coated on the surface of solid phase; the mobile phase is gaseous or liquid phase. There are several chromatographic techniques based on interaction type and molecular characteristics such as ion exchange, surface adsorption, etc. There are other techniques in which chromatography based on thin layer, including column, paper

chromatography, etc. Most commonly used method for protein purification is column chromatography [76,77].

There is a problem associated with chromatography methods in which the purification process is affected by hemolysis conditions [73, 75]. Other chromatographic techniques were used for purification process such as affinity chromatography [73] and Aqueous-phase extraction [70]. Another comparatively easy and fast method has been previously searched for purification of Hb by micro- and ultra-filtration. In order to minimize carrying of viruses by hemoglobin, a filtration step is involved in this method. This step is involved in fractionation chemical modification steps in order to isolate undesirable contents and unmodified Hb from modified Hb [78]. Multi-stage filtration has been used in order to avoid closing of pores membrane by cell wreckage which is a problem associated with filtration step these cell wreckages remove gradually with progressive of filtration, but the large part of cell wreckage removes with the first part of multi-filtration process [79-81].

3.2.2 Modified Hemoglobin. As there are side effects associated with free hemoglobin solution, many researchers have been modified hemoglobin solution to be safe for use as blood substitute by eliminating risks associated with free hemoglobin such as renal toxicity, high alliance for oxygen, and erythrocyte membrane residues which causes toxicity [62]. Also toxicity of Hb result from that the Hb begin to aggregate because of being outside red blood cell so that FDA refused any HBOCs to be used in united states, also other countries had not accepted this product [82].In addition, there are several problems related to the hemoglobin stability [10].Modification of Hb can be occur in several ways depending on the type of modification process of the purified Hb, chemical, physical, or genetic including: polymerized hemoglobin, intra molecular cross-linked hemoglobin, recombinant hemoglobin and hemoglobin vesicles, polymer conjugated hemoglobin. In which conjugated, cross-linked, and polymerized hemoglobin are fall into A cellular hemoglobin while hemoglobin vesicles fall into cellular hemoglobin [83, 84].

3.2.2.1. Intra molecular Cross-Linked Hemoglobin:
In order to prevent renal filtration of hemoglobin, and to minimize affinity Hb for oxygen, Hb was cross linked as shown in Figure (4). Two types of linkers are most commonly used: Diaspirin (3,5-dibromosalicyl fumarate; DBBF), and nor-2-formylpyridoxal 5-phosphate (NFPLP). In which □-units and, □-units of Hb are cross linked or to prevent disconnection of the two alpha/beta (α-ß) dimers. Thus, keep the integrity of hemoglobin, it also might increase life time of hemoglobin tetramer (for about 12 hours), opposite to an altered one which exerted from kidney after 6 hours [23,84-87]. In 1992, Baxter have done a number of trails, excluding of ceases of heart rate and raising of blood pressure, his studies met with great success [88].

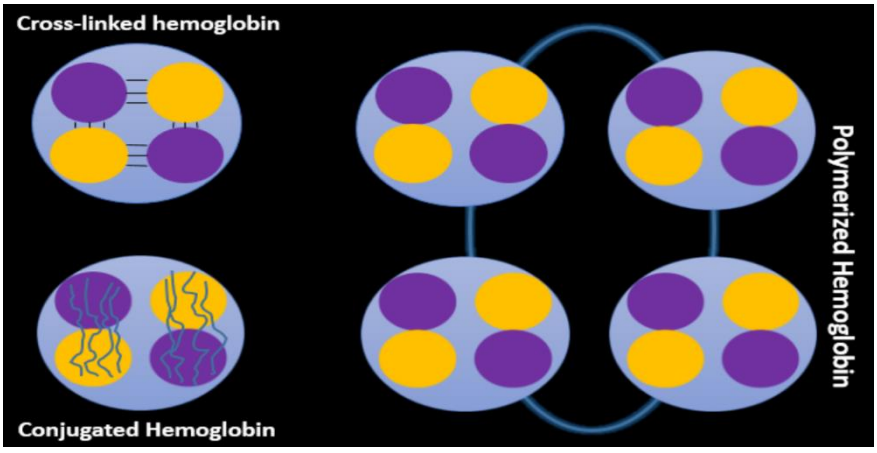

Figure 5. Three major types of acellular HBOCs.

3.2.2.2. Hemoglobin Vesicles:

In 1957, Chang was creating the first capsulation of the RBCs contents including Enzymes, Hb enclosed by artificial red blood cell and artificial membrane [89], Enzymes which being inside these artificial red blood cell include carbonic anhydrase [58] outcome short remaining in circulation system [90]. PEG– lipid vesicles as approved by Philips' group that it raising the circulation time[91]. In 1977, Djordjevich and Miller [92] able to put up hemoglobin vesicles by utilizing the method of phospholipid vesicles employed it in encapsulation. The resulting hemoglobin vesicles was out of cholesterol, fatty acid, phospholipid etc. [88]. This method has developed to mimic red blood cell, the benefits of Hb vesicles are: using of purified Hb and lipids, no modification occur [84].

Polylactide and polyglycolide are bio decompose into water and CO_2. So Polylactide has been utilized for Hb and enzymes encapsulation in micron dimension as reported by Chang [96].Also newly encapsulation made in nano range diameter of (0.08-0.18) μm [95-97]. It is possible to made Hb nanocapsule have methemoglobin shorthand system as long as glucose and other tiny molecules having tendency to mix with water [96, 97].

In order to raising the life time of these capsules inside the body, lately several types of polylactide–polyethylene glycol copolymers (PEG–PLA) have been made, for nanocapsule membrane, one has been used. Infusion of one third blood volume in rats shows no vasoactivities. These PEG–PLA nanocapsules containing hemoglobin didn't cause any change in Enzymes, Biochemistry, or histology [99-100].

3.2.2.3. Polymerized Hemoglobin:

Chang had been cross-linked the hemoglobin molecule utilizing Bi-functional agents obtaining polyhemoglobin as in Figure (4) [89, 90,101]. Chang used in his research two cross linking regent, glutaraldehyde [97], and sebacyl chloride [89,90]. The Gould's group at Northfield have been separately improved glutaraldehyde-cross-link polyhemoglobin (polyHb) firstly for animal experimentation then for clinical trial [101,102-105]. They were resulted with a production in developed stages using it for clinical trial with high dose, this production was pyridoxalated glutaraldehyde cross-linked human polyHb [102,103].

Other attempt was done using glutaraldehyde cross-linked bovine polyhemoglobin by the group from Biopure [104, 105]. Several clinical trials have been done using bovine polyhemoglobin which include less than five percent of 5% cross-linked Hb. South Africa allowed using bovine polyH for clinical trials [109].It is associated with number of side effects [70].

It was revealed that glutaraldehyde cross-linking hemoglobin polymerization reaction produce various molecular sizes products, in addition to hardly controlling the reaction [107]. Poly hemoglobin was also produced by Hsia utilizing 2,3-DPG-sac converter derived from di-aldehyde which gotten using O-raffinose [108,109]. The Hb molecule increase in its size after polymerization from 64.5 kDa (unpolymrized Hb) to large than 500 kDa this has a benefit of being un-exerted by kidney thus prolonging the life time of Hemoglobin plasma. Unpolyermized result in several risk of raising viscosity, O_2 affinity, elevating blood pressure [86]. Hemosol improved hemolink product which polymerize human Hb fractionally [3]. Several products of polymerized hemoglobin such as Hemopure , Oxyglobin , PolyHb-Fibrinogen show a number issues, the main of them are rising the risk of cardiovascular problems, and risk of transmission of diseases [68,70]

3.2.2.4.Recombinant Hemoglobin:

Researchers produce transgenic organisms able to make massive amount of human Hb, this was the starter of experiments of recombinant hemoglobin in 1980s [110]. Nagai *et al.* in the beginning of 1980s proved thatfrom bodies in Escherichia coli, unfolded β-subunits from human Hb can be expressed and separated. In the existence of native, minimize α-subunits create effective, tetrameric Hb, the denatured globin could be re-tucked and rebuilding with

hemin [111-113]. The rHbβG83C was produced using aimed translation (β83 Gly → Cys) that the Gly had Compensated in β-chain's location 83 with Cys. amino acid. It had been approved that in fresh frozen plasma its molecular size is fixed. This production targeted several benefits of developing Vaso activity of Hb and stopping dimer transformation and related problems [115]. In another study, involving introduce another product which is rHb(βN108Q), translation of N → Q was happen in β-chain's location108. This translation which shown that it carries several advantages of minimizing of oxygen affinity, lowering auto-oxidation of the product. The rHb(βN108Q) has been manufactured utilizing E. Coli expression system [116].

3.2.2.5. Conjugated hemoglobin:
In this type of modification, surface of hemoglobin molecule is connected with an inert polymer as shown in Figure (4). PEG-conjugated hemoglobin is Hemospan, which is under clinical routine, PEG may be the best used polymer for Hemoglobin conjugation because of its toxicity is minimal, reduction of immunogenicity or anti genicity in body. Also there are other types polymers have been used for hemoglobin conjugation such as: benzene tetra carboxylate dextran, hydroxyethyl starch (called HRC 101),and albumin [117-121].Another PEG-conjugated hemoglobin which turns out that it didn't causes vasoconstriction, in addition to its efficiency for transporting oxygen to tissues suffering from lake of oxygen this product is MP4[122].

3.3 Stem Cells as a Source of RBCs Substitute
Giarratana et al. made a study in which they used hematopoietic stem cells in characterizing mature human blood cells outcomes. The results were showed that they have Hb content and morphology like that of native RBC [123]. Various sources of stem cells have been used for this goal among them are from cord, bone marrow, and induced pluripotent stem cells (iPSCs) [124]. In clinical application, the last one might appear as a source for RBCs manufacturing. Different manufacturing circumstances of providing an efficient agriculture circumstances for hematopoietic stem cells originate from cord and following co-culture of human fetal liver stromal cells with erythroid progenitors have been modified. This involve progressing of repining operation of erythroid, in addition to produce massive number of cells [125]. In this domain, there are several issues associated with it such as expensive manufacturing and mass manufacturing. Due to development in technology, there might be a production of large number of RBCs with characteristics more likely as native RBCs [124].

4. Conclusion
Due to lack of donor blood in hospital and many problems associated with it. The need became urgent to make a RBCs substitute. So, many studies have interested in this field over several decades, some systems were depending on synthesis artificial blood based on perfluoro carbons that showed several side effect. However, recently there are various changes have been done of perfluoro carbons that make it used as RBCs substitute but still under development.

Other systems are based on extraction hemoglobin from different sources and passed it into several development of purification, modification to be used as RBCs substitute, some of the modification methods such as produced products that had several side although effects. The cross-linked hemoglobin method provides a product in phase III trial although; it is associated with several problems. In the same way, the hemoglobin polymerization method has a number of products in phase I, II trials and accepted in various countries but they have side effects. The other methods also have their own benefits and side effects. Researchers have been developed a method overtaken the problems joined the above methods depends on nano encapsulation of hemoglobin. According to several studies and trials on animals, the last method was exceeded the issues related to the above methods. Stem cell was becoming a way for RBCs substitute, different sources can be used for extraction of stem cell, but this way

associated with troubles of high cost in addition to mass production. However, in all methods the resulting artificial blood carries the function of red blood cell only. Researchers hope to make in the future an artificial blood substitute all functions and components as that of human blood.

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