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Artificial blood

Mine Ün[1], Oytun Erbaş[2]

[1]Istanbul Aydın University Medical Faculty, Istanbul, Turkey
[2]Department of Physiology, Demiroğlu Bilim University Faculty of Medicine, Istanbul, Turkey

ABSTRACT

Problems with blood collection and preservation have led to the development of blood substitutes. Artificial blood studies mainly aim towards the artificial realization of the functions and movements of the natural hemoglobin molecule found in erythrocytes. Therefore, artificial blood studies are on two main products: hemoglobin and perfluorocarbons. It has been proven that it is possible to produce recombinant hemoglobin in yeast. Currently, commercial production of recombinant hemoglobin is very expensive. After three-times reduction of costs and establishment of large production facilities, hemoglobin can be produced on a large scale. The challenges that still exist can be solved by synthetic biology and metabolic engineering. As a result, recombinant hemoglobin production can provide access to safe and affordable blood substitutions for the entire world population.

Keywords: Artificial blood, modified hemoglobin, perfluorocarbons, recombinant hemoglobin.

BLOOD

Blood is a unique connective tissue that consists of white blood cells (leukocytes), red blood cells (erythrocytes), platelets (thrombocytes), and plasma. It has various functions in the body.[1]

White blood cells are responsible for the body's defense. They detect foreign materials that enter the body and reduce their effects to a minimum. Platelets provide coagulation along with various proteins in the plasma. Red blood cells give blood its bright red color. Two drops of blood contain about one billion red blood cells. These cells are responsible for the most basic functions of blood: carrying oxygen to tissues, and removing carbon dioxide from tissues. These functions are carried out by red blood cells found in blood and the oxygen-carrying protein called hemoglobin.[1]

Artificial blood was developed to carry out only the functions of red blood cells. Real blood plays a role in many different functions, while artificial blood only transports oxygen and carbon dioxide in the body.[1]

STUDIES ON SOLUTIONS TO BLOOD DEFICIENCY

In order to prevent anemia-related deaths, studies on blood transfusions as well as finding an alternative fluid to blood have been conducted since the 15th century.[2]

Efforts to produce artificial blood as an alternative to real blood date back to the early 1600s and are still ongoing.[1] No real progress was made until 1616, when William Harvey explained how the blood circulated through the body. After 1616, countless substances such as beer, urine, milk, plant resins and sheep's blood were tested as an alternative to blood.[1-4]

As the search for alternative fluid to the blood has consistently failed, the researchers have focused on blood transfusion studies. The first successful blood transfusion was performed in

İletişim adresi: Mine Ün. İstanbul Aydın Üniversitesi Tıp Fakültesi Öğrencisi, 34295 Sefaköy, Küçükçekmece, İstanbul, Türkiye.
e-posta: unmine13@gmail.com

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1667. However, as blood groups were unknown in that time period, subsequent transfusions resulted in patient deaths and the practice was discontinued.[1,5]

Blood transfusions were unsuccessful until the year 1901. In addition, artificial blood as a substitute to real blood had still not been found. In 1901, experiments by Karl Landsteiner resulted in the discovery that humans had different blood groups. Blood groups were classified as A, B, and O. In 1902 a larger study was conducted and with the discovery of the AB group, blood groups were classified into four groups as A, B, AB, and O. As a result of these studies, donor and recipient blood groups were assessed before blood transfusion to perform transfusion between two compatible people. Thereby, blood transfusions became a safe and routine medical practice and blood bank systems were established.[1]

Blood bank systems worked so well in developed countries that, for a period, artificial blood studies decreased in these countries. Over time, blood bank systems became insufficient and with the discovery that HIV and hepatitis could be contracted with blood transfusions, the efforts to develop artificial blood were restored.[1,5-11]

LEANING TOWARDS ARTIFICIAL BLOOD

One of the important steps in the development of artificial blood was the discovery of Ringer's solution. Ringer's solution consists of sodium, potassium, and calcium salts. Scientists discovered that a frog's heart continues to beat in this solution.[1]

Ongoing studies found that Ringer's solution increased blood pressure when it lowered due to decreased blood volume. Although still used as a blood volume enhancer today, Ringer's solution cannot perform the functions of red blood cells, so it cannot be considered artificial blood.[1]

In 1966, researchers conducted experiments on mice, which showed that mice survived even after immersion in perfluorocarbons (PFC).[1] The blood of rats was entirely removed and replaced with a PFC emulsion. The animals continued to survive after a few hours. As a result, researchers proposed PFC for artificial blood.[1] During this time, some researchers searched for hemoglobin solutions, and other synthetic oxygen carriers. One group of researchers discovered they could use solutions containing hemoglobin isolated from red blood cells instead of blood. Thus, two products that could be used instead of blood emerged: PFC and hemoglobin solutions.[12]

PERFLUOROCARBONS

Perfluorocarbons are hydrocarbon-like stationary compounds to which fluorine atoms are attached instead of hydrogen atoms.[13] They can dissolve about 50 times more oxygen than blood plasma. They are relatively inexpensive to produce and can be deprived of any biological material. This eliminates the possibility of contraction of infectious diseases spread by blood transfusion. There are two important obstacles from a technological standpoint that must be overcome before PFC can be used as an artificial blood: First, they are insoluble in water, so they are given in combination with emulsions. Second, they are capable of carrying much less oxygen than hemoglobin-based products. This indicates that more PFC should be used instead of hemoglobin-based products. Perfluorocarbons have been approved for use by the Federal Drug Administration (FDA), but have not been commercially successful because the amount required to provide a benefit is too high.[1] Hemoglobin studies have been concentrated on and have achieved the expected success as artificial blood through hemoglobin studies.[10,11,14]

HEMOGLOBIN

Hemoglobin is a very important protein in the body as it transports oxygen to organs and tissues.[6] Every hemoglobin molecule contains four heme groups with iron at its center, and four polypeptide chains (two alpha and two beta chains). This oxygen-heme bond changes the shape of the hemoglobin molecule, which allows hemoglobin to gradually attract even more oxygen molecules (Figure 1).[15,16]

HEMOGLOBIN SOLUTIONS

Researchers discovered that solutions containing hemoglobin isolated from red blood

cells could be used as a substitute to blood. Artificial blood studies on rodents experimented with hemoglobin solutions prepared with hemolyzed red blood cells and found that this solution could carry oxygen to tissues, but had a toxic effect on the liver and caused hypertension. In addition, blood transfusion studies conducted with hemoglobin solution also showed toxic effects on the kidneys.[1,3,4,10,11,14,17-19]

Since the toxic effect of natural hemoglobin was demonstrated, studies have focused on developing modified hemoglobin, and these studies have increased by the day.[1]

While hemoglobin solutions were tested initially, later studies emphasized purification of hemoglobin molecules and modifications.[20]

MODIFIED HEMOGLOBIN

With the demonstration of hepatic and renal toxicity in studies conducted with natural hemoglobin, studies focused on development of modified hemoglobin and capsulation of hemoglobin.[4,19]

Today, clinical testing is ongoing for development of modified hemoglobin variants including polyhemoglobin, conjugated hemoglobin, cross-linked tetrameric hemoglobin, recombinant hemoglobin, and hemoglobin vesicles. The most important of these is recombinant hemoglobin (Figure 2).[4,19-21]

With developments in synthetic biology and metabolic engineering, recombinant hemoglobin was produced in transgenic organisms.[4,20,22]

PRODUCTION OF RECOMBINANT HEMOGLOBIN

The first organism to be selected for recombinant hemoglobin production, with the ability to produce human hemoglobin, was *Escherichia coli (E. coli)*. It was able to synthesize a single beta globin in the first attempt.[23] In later studies, *E. coli* was able to synthesize alpha and beta chains, and produce recombinant hemoglobin by adding endogenous heme.[24] Despite the successful production of recombinant hemoglobin by bacteria, it was later discovered that the vital functions of hemoglobin produced by bacteria were altered by their hosts. This is due to certain differences in protein synthesis of bacteria.[25]

Later, the yeast Saccharomyces cerevisiae was used for production. Recombinant hemoglobin production was 87% more successful compared to previous attempts.[26]

After successful production of recombinant hemoglobin, it was mixed with water and other

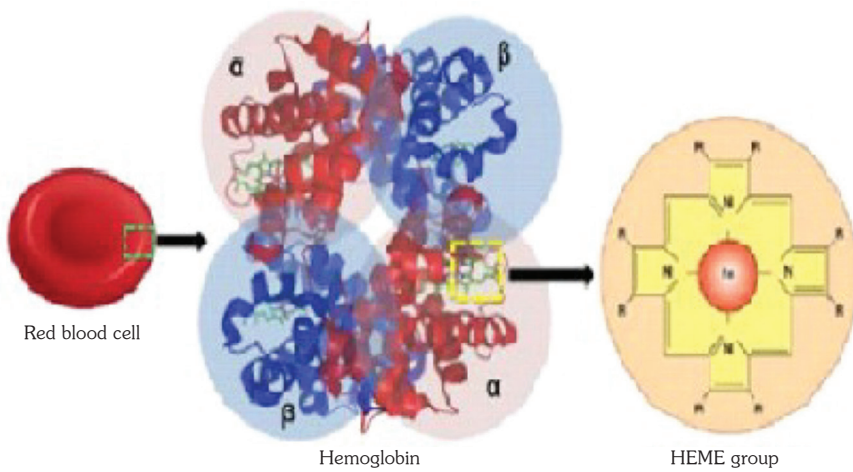

Figure 1. Hemoglobin in red blood cells consist of four heme groups and four polypeptide chains (two alpha and two beta chains) with an iron atom in the center. Each heme group in the structure of hemoglobin binds to an oxygen molecule.[15,16]

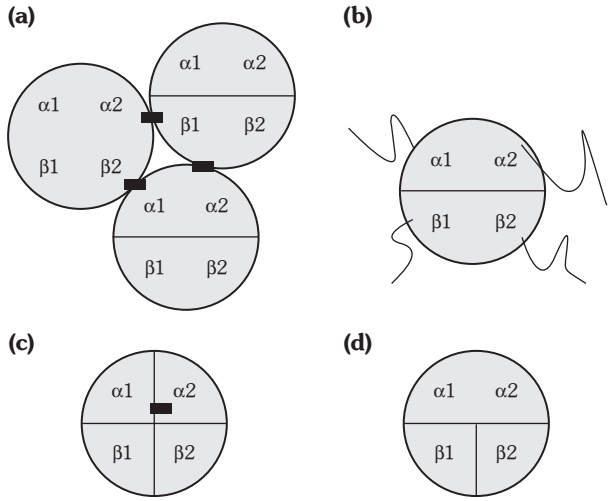

Figure 2. Different types of modified hemoglobin; **(a)** Polyhemoglobin, **(b)** Conjugated hemoglobin, **(c)** Intramolecular cross-linked tetrameric hemoglobin, **(d)** recombinant human hemoglobin.[19-21]

electrolytes. Quality of all compounds was regularly ensured throughout all processes.[1]

Following the successful first recombinant human hemoglobin, clinical studies of blood substitution with recombinant hemoglobin were initiated. Side effects such as fever, chills, and headache were recorded. In studies that used a maximum dose of 25.5 grams observed mild gastrointestinal symptoms and increased blood pressure.[13] Subsequently, studies continued on the elimination and modification of the negative effects of this molecule against nitric oxide.[13,19,20] Development of a purification process eliminated endotoxin components, reducing side effects.[13] Regardless, the method is still not safe enough. Regulatory agencies in the US and the European Union have not yet approved any hemoglobin-based oxygen carriers.[12,14,27,28]

THE IDEAL ARTIFICIAL BLOOD

First, the artificial blood must be compatible with the body. It should be applicable to all individuals regardless of blood group. It should be able to carry oxygen to all parts of the body and release it when necessary. It should be processable to eliminate all agents with potential for disease including viruses and microorganisms. It should have long shelf life and affordable production cost.[1]

COST OF HEMOGLOBIN PRODUCTION

A study conducted in the United States indicated that production of recombinant human hemoglobin was currently possible at $11/gr. However, including production costs and equipment investments, cost increased to $200/gr. Considering that one liter of human blood contains 150 grams of hemoglobin, the cost is high. Therefore, host recombinant hemoglobin production is not yet economically viable. In order to make this possible either production costs must be lowered to a third of the cost or hemoglobin expression efficiency increased three times. Synthetic biology should contribute to produce affordable recombinant hemoglobin.[14,29-31]

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