# PBLD 5: Current Status of Hemoglobin Free Blood Substitutes

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*"Just as the body cannot exist without blood, so the soul needs the matchless and pure strength of faith."*
*Mahatma Gandhi*
*"A pint of sweat will save a gallon of blood." George S. Patton*

Learning Objectives

1. Discuss therapies moving towards your OR

2. Review outcome data related to hemoglobin substitutes

**CASE #1:**
80 year old female with a history of dypsnea on exertion (DOE) and syncope. Workup reveals aortic stenosis (AVA=0.8cm2) and normal coronary arteries by cardiac catheterization with moderate concentric hypertrophy and an EF of 50%. Her other PMH is unremarkable. She was advised by her cardiac surgeon to seek a preoperative anesthetic consultation because she joined the Watch Tower organization last year and became a Jehovah's Witness. She refuses all blood products even in the face of death. She says that she has heard of hemoglobin substitutes that have been used in humans and wants to talk to you more about that.

*Questions:*
*What are hemoglobin substitutes? Are they from human blood or are they purely synthetic? How are they prepared? Do they all contain hemoglobin or human proteins?*

You start to talk to her about the synthetic blood products and she is very happy to hear that they are being studied. She asks you which ones are available now and what are the current indications? She asks you if they have been used off-label.

*What do you tell her? Are there any that are FDA approved? Which ones are approved outside of the US? Are there case reports whereby these products have been used off-label?*

**Case #2:**
65 year old male with a history of HTN and DM (well-controlled on medication/compliant) was found to have severe 3 vessel disease on cardiac catheterization done due to an abnormal stress test and new bundle branch block on EKG. Laboratory workup revealed hemoglobin of 7.5g/dL. He is on Metoprolol, Avandia and Lisinopril. He has an allergy to egg yolks. Upper and lower endoscopies were noted to be negative as were stool guiac. All other causes for low hemoglobin were ruled out and the patient was scheduled for coronary artery bypass grafting (CABG). He was admitted the day prior to surgery and is transfused 2 units of PRBC's. During the transfusion, he

becomes dyspneic and tachycardic. His temperature was noted to be elevated to 39.5 °C. He starts feeling dizzy and loses consciousness.

*Questions:*
*What is your differential diagnosis? What is the most likely cause of his syncope? What are other complications of blood transfusions?*
Immediate ACLS is instituted. He is resuscitated successfully. A transfusion reaction is suspected and upon further investigation, it is noted that he received incorrectly labeled blood. The medical student you are working with asks whether or not he was a candidate for blood substitutes.

*How do you respond? Do blood substitutes involve the same type and cross-matching? How long can they be shelved? Is a blood substitute contraindicated in this patient?*

## Case Discussion

Risks of blood transfusions include transfusion reactions, infections, delayed wound healing, transfusion related lung injury, and immunomodulation. Recent research focuses on the age of the stored blood and its relation to increased morbidity in the recipient. Requirements for storage and portability as well as the need for cross-typing add to the challenges faced with blood transfusions especially in the setting of out of hospital injury and/or trauma [1]. Finally, a recent study investigated the potential link between blood transfusion and cancer progression [2].

Ideally blood substitutes would overcome these barriers and have a low risk to benefit ratio. Some characteristics are listed below in the table [1,3]:

| |
|---|
| Lack of antigenity |
| Ability to interact with oxygen species |
| Absence of renal toxicity |
| Room temperature storage capability |
| Adequate half-life |
| Immediate availability |
| Ease of administration |
| Lack of vasoactivity both in pulmonary and systemic circulation |
| No oxidation and free radical formation |
| Does not form methemoglobin |
| Long term storage ability |
| Does not overload reticuloendothelial system |

## HBOC's – Hemoglobin Based Oxygen Carriers

The circulation of blood was first described by William Harvey in 1628 and soon thereafter, Christopher Wren experimented with wine as a potential blood substitute. In

the late 19th Century, a combination of blood and milk was employed without success during an outbreak of cholera. Sellards and Minot in 1916 were the first to experiment with lysed human blood cells. They took varying amounts of lysed, washed red cells and infused them into 33 patients. All experienced hemoglobinuria and 3 reported different degrees of flushing, nausea, fever, chills, headache and difficulty sleeping. In 1934, Amberson purified the first bovine hemoglobin and administered it to animals and in 1949, he administered it to anemic patients. The first generation of Hemoglobin-Based Oxygen Carriers (HBOC's) were born [1,3].
Initially stroma free hemoglobin (SFH) was experimented with. This is from washed, lysed human red blood cells (RBC). SFH simply refers to hemoglobin free of red cell membranes. Use of SFH produced varying degrees of successes and significant failures. [1,3].

The next generation of hemoglobin based oxygen carriers were developed in the 1950's after the discovery of two main findings. One, the chemical structure of hemoglobin was revealed and the ability to chemically modify hemoglobin was discovered. These two findings led to polymerized hemoglobin The second generation of HBOC's is also made from RBC's from outdated human blood as well. The SFH solution is then put through a process that attempts to create a tetramer-free solution that contains only multiple sized polymers [3].

In 1997, Biopure was approved by the FDA for canine anemia and by the EU in 1998. In 2001, the South African Drug Council approved the first HBOC for human use – Hemopure. Over the course of the next decade, attempts were made to explain the etiology for the physiological effects of the HBOC's (e.g., vasoconstriction, hypertension and bradycardia). Other reported side effects include activation of the immune system, abdominal pain & discomfort, renal toxicity and increase in cardiac events, progression of stroke and multi-organ failure. Some of these findings led to the discontinuation of some products as listed below in the table [1,2].

With respect to their vasoactivity, it is now believed that the vasoconstriction was due to the scavenging of NO. Other possibilities include disturbances in autoregulation. The autoregulatory theory of vascular control states that the most significant determinant of tone in the vascular bed is ambient oxygen tension. High oxygen tension provided in an overabundance by cell-free hemoglobin then results in local vasoconstriction. Therefore, another form of cross-linking was utilized to increase the numbers of uncrosslinked hemoglobin without generating polymers [2].

The current research is increasingly focused on oxygen carriers that cater to the microcirculation by manipulating the oxygen affinity and size of the carrier molecule. Specific HBOC's are listed below as well as their current status. There are currently no FDA approved HBOC's available.

**HemoLink:**
Hemolink is a second generation HBOC and was studied in patients undergoing coronary artery bypass grafting in both Phase 2 and Phase 3 Clinical trials. It was compared to

10% Pentastarch and 6% Hetastarch and initially was found to be well tolerated. The most common side effect was perioperative hypertension, as well as gastrointestinal discomfort. No renal toxicity was appreciated in both Phase 2 and Phase 3 clinical trials. In the Phase 3 trial, the Hemolink group received fewer transfusions overall and a lower volume of allogeneic RBC's and non-RBC allogeneic blood products than the control groups. However, due to unexpected cardiac toxicities and adverse events, HemoLink was discontinued [3-5].

**HemeAssist:**
HemeAssist is an example of a third generation HBOC. It was extensively studied in patients undergoing cardiac, orthopedic, and vascular surgery as well as severe hemorrhagic shock and acute ischemic stroke [6-12]. In cardiac surgical patients, HemeAssist did result in a decrease in the number of blood transfusions compared to the control group. Perioperative hypertension was noted as well as jaundice, and gastrointestinal discomfort [10]. Saxena et al., concluded that at 3 months, HemeAssist was an independent predictor of unfavorable outcome in patients with acute ischemic stroke in the anterior circulation [9]. Furthermore, in a study involving ICU patients 24 hours after abdominal aortic aneurysm repair, administration of HemeAssist resulted in increased afterload which the authors concluded may pose serious problems with left ventricular function [12]. Other side effects noted were jaundice, pancreatitis and urinary side effects [5]. HemeAssist was discontinued due to safety concerns.

**Hemospan:**
Hemospan is a fourth generation HBOC that is based on the autoregulatory theory which states that vascular tone is the result of the amount of oxygen supplied. Cell-free oxygen is thought to provide oxygen in overabundance and therefore research focused on titrating the amount of oxygen provided by the hemoglobin substitute and this resulted in the development of Hemospan. It underwent Phase II testing in a multi-center double-blinded study in orthopedic patients [13]. It was found that Hemospan was well-tolerated and no significant difference was found between the two groups with respect to serious adverse events. Of note, patients treated with Hemospan did experience mild elevations in hepatic enzymes and more bradycardic events [13]. Hemospan is currently being manufactured commercially and a recent study evaluating escalating doses of Hemospan in ASA I and II patients undergoing spinal anesthesia for orthopedic surgery revealed that doses up to 1000mL were well tolerated. No significant adverse events were noted [14].

**PolyHeme**:
PolyHeme is second generation HBOC [15]. It has been studied in the setting of acute trauma and emergent surgery where it was found to be safe as treatment for acute blood loss. 44 trauma patients were randomized to receive either red blood cells or PolyHeme and the results revealed that up to 6 units of PolyHeme could be administered without adverse events. Vasoconstriction, renal dysfunction and fever were not present in any study subject during the three days of follow-up [16]. It is currently undergoing Phase 3 Clinical Trials in the setting of pre-hospital trauma.

Several case reports are available regarding use of PolyHeme off label. These include a case study of administration of PolyHeme to a Jehovah's Witness involved in a road accident with a hemoglobin of 3.2 g/dL. She received 5 units of PolyHeme and suffered no acute distress. She was discharged with a hemoglobin of 9.8 g/dL from the hospital on Day 19 [17].

**Oxyvita:**
Oxyvita or zero-linked hemoglobin is a fourth generation HBOC. It is called zero linked because coupling between molecules does not use cross linking agents. It is currently undergoing preclinical trials [3].

## PFC's - Perfluorocarbons

Perfluorocarbons (PFC's) are low molecular weight, hydrophobic synthetic molecules that can dissolve large quantities of gas [1]. They are hydrocarbons in which the hydrogen atoms on the carbon chain have been substituted by fluoride. This results in chemical inertness and lack of metabolism. They do not have any oxygen binding capacity but act as simple solvents and are available in emulsions. The oxygen transport is based on the physical solubility and the amount of oxygen is determined by the partial pressure. They can reach small microvasculature. In the 1960's, Chang et al demonstrated that molecules from a combination of silicone rubber and hemolysate could carry and release oxygen. In 1967, Slovitier and Kamimoto discovered that rat brain function was maintained for several hours with finely emulsified fluorocarbons and in 1968, Geyer et al. revealed that an emulsified fluorocarbon solution could replace almost all of the rats' blood with good survival and recovery [3]. Like HBOC's, there are multiple generations. First generations include Fluosol DA-20 which was developed by Naito and Yokoyama in 1976 [1]. Second generations include Oxygent and Oxyfluor and the third generations are early in development. The second generations were made with egg yolk and lecithin and have greater stability and enhanced oxygen carrying capacity [1]. Current research not only focuses on the ability for these emulsions to dissolve oxygen, but also on their ability to reduce air bubble size during cardiac surgery. There are currently no FDA approved PFC's available [1].

**Fluosol DA20:**
This was developed in 1976, underwent clinical trials and was approved by the FDA for oxygen delivery during percutaneous angioplasty. It was subsequently discontinued and is no longer manufactured [1,18].

**Alliance Trials:**
After undergoing preclinical trials, PFC's were studied in the orthopedic patient population and found to reverse physiologic transfusion triggers [19]. In Phase 3 clinical trials, PFC administration was combined with acute normovolemic hemodilution (ANH) in patients undergoing noncardiac surgery [20]. No significant difference was found between the two groups with respect to serious adverse effects, although more patients in the PFC group experienced digestive system side effects (ex. postoperative ileus) and

cardiovascular side effects (hypertension). This increase in blood pressure was seen postoperatively and attributed most likely to the increase associated with re-administering blood that was initially removed for ANH (e.g., transient hypervolemia). Overall, the study revealed that during high blood-loss noncardiac surgery (>20mL/kg), using PFC and ANH can reduce the transfusion requirements [20].

PFC's were also studied in patients undergoing cardiac surgery. 36 adult patients were randomized to either ANH alone or in combination with escalating doses of PFC. This study revealed an increase in cerebral emboli in the treatment groups and a subsequent Phase 3 trial in cardiac surgical patients with PFC was terminated early due to an imbalance in the incidence of stroke in the treatment group [18]. No PFC is currently approved by the FDA.

## Complications of HBOC's and PFC's

Table constructing using information from various sources [1,18,19,20].

| Adverse Effect | HBOC | PFC |
|---|---|---|
| Vasoconstriciton | HBOC's increase the pulmonary and systemic blood pressures. Current etiologies include NO scavenging, activation of endothelin and/or adrengergic receptors as well as the presence of cell free hemoglobin. | |
| Immune System | Impeding macrocytic function as well as activation of complement, kinin and coagulation cascade. NO scavenging may increase platelet aggregation. Free radical formation as a result of stroma-free hemoglobin, heme and iron. Formation of antibodies by recipient to hemoglobin. | Activation of complement and phagocytes. Flu-like symptoms experienced by patients – facial flushing, backache initially followed by fever and chills. An enhanced inflammatory response as a result of excessive cytokine release. |
| CNS | Hemoglobin as a neurotoxic agent – especially important in trauma where blood-brain barrier is not intact. | Increase in cerebral blood flow and/or emboli. |
| GI | Abdominal pain, discomfort, nausea and vomiting. Likely due to a NO scavenging mechanism resulting in smooth muscle spasm. | |
| Renal | Renal failure with first generation HBOC's but not with concurrent | |

| | | |
|---|---|---|
| | generations. | |
| Iatrogenic | Inability to determine accurate hemoglobin measurements. Validity of mixed venous and PO2 and other laboratory parameters. | |
| Iron deposition | May result in a state of iron overload | |
| Methemoglobinemia | Oxidation during storage and exposure to air may result in formation of methemoglobinemia. | |
| Shelf Life | Can be stored in some instances up to one year | Limited - A concern with first generation PFC's but not with subsequent generations. |

The following is a table that lists the various generations of HBOC's and their current status [3]:

| Generation | Technology | Product | Company | Effects | Status |
|---|---|---|---|---|---|
| "First" HBOC | Red cell lysis | SFH | Academic<br>Warner Lambert | Renal failure GI symptoms | Discontinued |
| "Second" HBOC | Polymerized Hb | PolyHeme | Northfield Laboratories | ? | Phase III trauma |
| | | HemoLink | Hemosol | Cardiac events<br>No renal toxicity<br>GI symptoms | Discontinued |
| | | HemoPure | Biopure | Vasoconstriction | ? Indication |
| "Third" HBOC | Intramolecular | αα-Hb | US Army | Vasoconstriction | Discontinued |

| Generation | Technology | Product | Company | Effects | Status |
|---|---|---|---|---|---|
| | crosslink | | | | |
| | | HemAssist | Baxter | Death (stroke, trauma) Increases in pancreatic enzyme and Pancreatitis/ MI/MOF | Discontinued |
| | | rHb1.1 | Somatogen | Vasoconstriction | Discontinued |
| "Fourth" HBOC | PEG conjugation | PHP | Ajinomoto/Apex | Generally safe | Sepsis trials |
| | PEG conjugation | PEG-hemoglobin | Enzon | Generally safe | Discontinued |
| | PEG conjugation | Hemospan | Sangart | Generally safe | Phase III elective surgery |
| | Zero-link | ZL-HbBv | Oxyvita | ? | Preclinical |

Abbreviations: SFH, stroma-free hemoglobin; PHP, pyridoxal phosphate hemoglobin polyethyleneglycol; ZL-HbBv, bovine hemoglobin polymerized with Zero-Link technology (see text); Hb, hemoglobin; PEG, polyethylene glycol; GI, gastrointestinal;MOF, multi-organ failure;MI, myocardial ischemia

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