Why Do We Need Blood Substitutes?
Red blood cell (RBC) membrane contains blood group antigens; typing and matching are needed before they can be transfused into patients. This results in delays in emergency situations. The storage time for donor blood using standard method requires storage at 4°C and is only good for less than 42 days. There is increasing concern that even this length of storage may result in unsafe donor blood. RBCs cannot be sterilized to remove infective agents like hepatitis viruses, HIV, and other potential emerging infective agents. There is also a shortage of donor blood, especially in major surgery, emergency, war, or disaster situations.

First Generation Blood Substitute in the Form of Oxygen Carriers
Nanobiotechnology has allowed the development of a first generation blood substitute that is a simple polyhemoglobin (PolyHb) oxygen carrier. This has been rather extensively tested in clinical trials. PolyHb does not have blood group antigens and can be used immediately on the spot without cross-matching or typing. One of these could be stored for more than 1 year at room temperature. Infective agents like HIV and other viruses and micro-organisms can be removed.

Basic Principles
Hb is a tetramer (α1β1α2β2) that breaks down into toxic dimers (α1β1 and α2β2) that cause renal toxicity and other adverse effects. Even in the form of tetramers, Hb molecules can cross the intercellular junction of blood vessels to cause adverse vasopressor effects. The first use of nanobiotechnology is to assemble and cross-link a number of Hb molecules together into soluble polyHb (Fig. 332.1). The glutaraldehyde method has been developed independently by other groups for clinical trials.
PolyHemoglobin
1964 Chang (Diacid)
1971 Chang (Glutaraldehyde)

Conjugated Hb
1964 Chang (polyamide)
1968 Wong (dextran)
1970 Abuchowski & Davis (PEGylation)
1980 Iwashita (polyethylene glycol)

Crosslinked tetrameric Hb
1968 Bunn & Jandl
1979 Walder et al (Diaspirin)

Recombinant Human Hb
1990 Hoffman et al

Present Status of PolyHb in Clinical Trials

Gould’s group has developed PolyHb using human hemoglobin from outdated donor blood. They have carried out clinical trials on 171 patients, showing that PolyHb can successfully replace extensive blood loss in trauma surgery and maintain the Hb level at the 8 to 10 g per deciliter needed for safe surgery. Transfusion of this polyHb in patients with Hb level as low as 2 g per deciliter can raise the Hb level to within the 8 to 10 g per deciliter level with the patients recovering from surgery. They have infused up to 10 L of polyHb into individual severely bleeding trauma surgery patients. They have more recently carried out clinical trials on its use in prehospital ambulance patients because no typing and cross-matching is needed and it can be used right on the spot. Patients in the control group received saline in the ambulance on an average of about 30 minutes ambulance ride. They needed donor blood transfusion after admission into the hospital. Each patient in the PolyHb group received PolyHb transfusion in the ambulance, which was continued for the first 12 hours after admission into the hospital for up to a total of 6 units of PolyHb. Unlike the control group that needed blood transfusion on admission to the hospital, the PolyHb group only needed donor blood about 14 hours after admission. However, in this clinical trial, there was a 3% incidence of myocardial infarction as compared to 0.6% in the control group. Therefore, they proposed that this might be used in critical situations where donor blood is not available. This may be critical and lifesaving in situations where donor blood is not available, such as traffic accidents, traumatic injuries in remote areas, major surgeries requiring large amount of donor blood, and cases of areas of major disasters or war. The circulation halftime of PolyHb is about 24 hours. In their earlier trauma surgery trial, they had infused up to a total of 20 units. This could mean a possible 40 hours or more before donor blood is needed. The supply of Hb from outdated donor blood is limited. A glutaraldehyde-cross-linked bovine polyHb has been developed and tested in phase III clinical trials. For example, in one study, a multicenter, multinational, randomized, single-blind, RBC-controlled Phase III clinical trials have been carried out in patients undergoing elective orthopedic surgery. A total of 688 patients were randomized 1:1 to receive either the polyHb or RBCs at the time of the first perioperative RBC transfusion decision, and 59.4% of the patients receiving polyHb required no RBC transfusion all the way to follow-up, 96.3% avoided transfusion with RBC on the first postoperative day, and up to 70.3% avoided RBC transfusion up to day 7. In South Africa, where there are higher incidences of HIV, this has already been approved for routine clinical use in patients for a number of years.

Other Types of First Generation Blood Substitutes

In addition to the previous two PolyHb, other first generation modified hemoglobins have also been prepared and studied (see Fig. 332.1). The size of single hemoglobin molecules allows them to cross the intercellular junctions of the endothelial lining of the vascular wall to enter into the interstitial space. There, Hb binds and removes nitric oxide needed for maintaining the normal tone of smooth muscles. This results in the constriction of blood vessels and other smooth muscles, especially those of the esophagus and the gastrointestinal (GI) tract. Our recent studies in rats show ischemic electrocardiography (ECG) changes in the form of ST elevation when rats receive infusions with a high percentage of single hemoglobin. Single hemoglobin molecules can be linked to a soluble polymer like polyethylene glycol (PEG) (see Fig. 332.1). These resulted in a high degree of water hydration, thus increasing the molecular size and preventing it from crossing the intercellular junction to cause vasoconstriction. This has been developed for use in clinical trials. PolyHb can supply oxygen, but it does not have RBC enzymes to remove damaging oxygen radicals in conditions of ischemia–reperfusion injury. Even antioxidant enzymes normally present in RBCs are not enough to prevent this problem if severe. Examples of this include reperfusion after sustained and severe ischemia as in hemorrhagic shock, myocardial infarction, stroke, donor organs, and other conditions. For example, an arterial obstruction due to clots or other causes can result in a stroke or myocardial infarction. Being a solution, PolyHb can more easily perfuse partially obstructed vessels. However, if there is a prolonged lack of oxygen, reperfusion with an oxygen-rich fluid like PolyHb may give rise to damaging oxygen radicals. D’Agnillo and Chang assembled and cross-linked hemoglobin, superoxide dismutase (SOD), and catalase (CAT) into a soluble nanobiotechnology complex of PolyHb-SOD-CAT. Powanda and Chang studied this in a rat model of combined sustained hemorrhagic shock and cerebral ischemia. Reperfusion using PolyHb-SOD-CAT did not result in any change in the blood–brain barrier, nor in any change in water contents of the brain. On the other hand, the use of PolyHb causes the breakdown of the blood–brain barrier and an increase in brain edema. Hsia’s group has extended this approach to develop a polynitroxylated hemoglobin with SOD activity.

New Generations of Blood Substitutes

In massive bleeding, replacement with donor blood or PolyHb may require supplements with platelets. Wong and Chang reported the use of the principle of nanobiotechnology to cross-link hemoglobin with fibrinogen to form a soluble PolyHb–fibrinogen complex that is an oxygen carrier with platelet-like activity. Unlike PolyHb, PolyHb–fibrinogen can replace up to 98% of the blood volume in rats with no significant increase in clotting time.

A Complete Nanodimension Artificial RBC

Chang had reported the preparation of complete RBCs with synthetic membranes as early as 1957. However, the problem has been the very short circulation time. As early as 1972, we prepared larger artificial cells with lipid membrane by supporting the lipid in the form of lipid protein membrane and lipid polymer membrane. Djordjevich and Miller, in 1980, prepared submicron 0.2 micron diameter artificial RBCs using lipid membrane vesicles to encapsulate Hb. This increased the circulation time significantly, although the circulation time was still rather short. In 1999, Philips et al.
markedly improved the circulation time by incorporating polyethylene-glycol (PEG) into the lipid membrane artificial RBC. The submicron hemoglobin lipid vesicle hemoglobin approach is being extensively developed by a group in Japan toward a clinical trial. Lipid vesicles would be useful for conditions that do not require large volume of blood substitutes. Because the smaller the diameter the larger would be the surface to volume relationship, these 200 nm lipid vesicles have a total surface area, and therefore, a lipid that is about 10 times that of 7 micron RBCs. Large amounts of lipids can cause the saturation of the reticuloenathelial system. Therefore, Yu and Chang use biodegradable polymer membranes to form complete nano dimension artificial RBCs as a third generation blood substitute. These nano artificial RBCs of 80 to 150 nm contain all the RBC enzymes. Using a polyethylene-glycol-polylactide copolymer membrane, we are able to increase the circulation time of these nano artificial RBCs to double that of PolyHb. Further studies in rats by Liu and Chang showed that one infusion with one-third the total blood volume did not result in any adverse effects or changes in the histology or blood biochemistries when followed on days 1, 7, and 21 after infusion.

## General Discussions

It is important for blood substitutes to be as safe as donor blood. There is increasing concern regarding the effect of stored donor blood on safety, including ischemic coronary events. Thus, it is important to analyze the risk/benefit factors of first generation blood substitutes, especially in critical life and death situations where donor blood is not available or unsafe. Also, first generation blood substitutes are only oxygen carriers and therefore should only be used in situations where only oxygen transport is needed. In the meantime, new generations of blood substitutes are being developed for special clinical conditions.

### REFERENCES


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**Thoracoscopic Sympathectomy for Hyperhidrosis**

**DANIEL L. MILLER**

Hyperhidrosis is defined as a pathologic condition of excessive sweating in amounts greater than physiologically needed for thermoregulation. It may develop secondary to a variety of medical conditions or it may be primary (idiopathic), with symptoms localized to the palms, axillae, and/or feet. Also, excessive facial sweating may occur with blushing. The incidence of hyperhidrosis depends on the culture, climate, and the subjective definition. Idiopathic primary hyperhidrosis affects 1% to 3% of the population—both genders equally—and is found predominantly in adolescents or young adults. Characteristically, palmar symptoms start in early childhood, axillary
Author's Query for Chapter 332—**Progress in the Field of Blood Substitutes**

**AQ1:** If this is another reference entry, please number as appropriate. The rest of the reference numbers will then have to be renumbered here and in text.

**AQ2:** Is there a specific chapter or page being referenced here? Please provide

**AQ3:** Do you mean “car accident” here?

**AQ4:** As meant?

**AQ5:** Please clarify “Roa”