

## SECTION IV. NEW HORIZONS IN SYNTHETIC BLOOD SUBSTITUTES

### Hemoglobin-Based Oxygen Carriers

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**Learning Objectives:** 1) To describe the need for and use of hemoglobin-based oxygen carriers, especially in the trauma population. 2) To describe the adverse effects of allogeneic blood transfusions. 3) To describe the method of preparation of PolyHeme and its potential clinical indications. 4) To summarize prior research done on PolyHeme in trauma patients and in other populations. 5) To describe the current phase III trial investigating PolyHeme in trauma patients with hemorrhagic shock

#### Abstract

There has been increasing concern about the potential adverse effects of red blood cell (RBC) transfusion. Trauma patients are very likely to receive transfusions while in the hospital and are especially prone to these adverse effects. There has consequently been a worldwide interest in the development of a clinically useful oxygen carrier that could serve as a blood substitute, particularly in the trauma population. Hemoglobin-based oxygen carriers (HBOCs), such as PolyHeme, may serve this role and decrease or potentially eliminate the need for blood transfusion.

PolyHeme is a human hemoglobin-based temporary RBC substitute that is presently under clinical evaluation for the treatment of life-threatening blood loss when an oxygen-carrying fluid is required and RBCs are not available. The potential benefits of PolyHeme in clinical care are that it is immediately available, is in abundant supply, and has a prolonged shelf life. It is universally compatible with all blood types and therefore does not require time-consuming typing and cross-matching. It is also sterile and free from disease transmission, antigenic reactions, and immunologic effects. A multicenter, randomized, controlled phase III trial investigating PolyHeme in trauma patients with hemorrhagic shock was recently completed. Final results of this study are still pending but may herald the introduction of HBOCs to patient care.

The potential benefit of a blood substitute is probably most appreciated by trauma surgeons. Trauma patients often bleed massively even before hospital admission, and blood is usually not available in the prehospital setting. In addition, there has been increasing concern about potential adverse effects of red blood cell (RBC) transfusion.<sup>1-5</sup> Trauma patients are very likely to receive RBC transfusions while in the hospital, and about 3% will receive massive transfusion, defined as more than 10 units of RBCs or the normal amount of blood in the human body, which makes them more prone to these adverse effects.<sup>6</sup> Limitations in the donor blood supply have precipitated the search for a blood substitute. Consequently there has been a worldwide interest, especially in the trauma community, in the development of a clinically useful oxygen carrier that could serve as a blood substitute. Hemoglobin-based oxygen carriers (HBOCs) may serve this role and decrease or potentially eliminate the need for blood transfusion.

The adverse effects of RBC transfusion have been known for decades, but recently there has been increased recognition of the dysfunction in the immune system associated with transfusion.<sup>2,3,5</sup> Although the public is most aware of the infectious risks of transfusion, these risks are small compared with the noninfectious hazards of transfusion. These noninfectious risks include acute hemolytic reactions from incompatible blood transfusions, delayed serologic and hemolytic reactions, transfusion-associated acute lung injury, graft-versus-host disease, fluid overload, and febrile nonhemolytic transfusion reactions, among others.<sup>3,7</sup> The immune dysfunction associated with transfusion may result in a higher incidence of infection, acute respiratory distress syndrome, intensive care unit (ICU) length of stay, and mortality in those who have received transfusion.<sup>1,2,4</sup>

The risks involved with infusions of blood in trauma patients have been extensively investigated. Moore and colleagues<sup>8</sup> evaluated 513 consecutive trauma patients older than 16 years with an Injury Severity Score (ISS) >15 who survived more than 48 hours, and found that more than six units of RBCs transfused in the first 12 hours postinjury was an independent risk factor for the development of multiple organ failure. In a report of 63 trauma patients, Zallen et al<sup>5</sup> found that the age of transfused blood in the first 6 hours postinjury correlated with the incidence of multiple organ failure. This was thought to be due to the proinflammatory effects of stored RBCs. In 2002, Claridge and coworkers<sup>1</sup> found that the infection rate among patients who received at least one transfusion was more than 4 times greater than those receiving no transfusions. In a report on more than 5,000 patients with a moderate degree of injury (ISS <25) from blunt trauma, Croce et al<sup>2</sup> found that delayed transfusion, defined as the first transfusion being given more than 48 hours from admission, was independently associated with ventilator-associated pneumonia, acute respiratory distress syndrome, and death in trauma patients, regardless of injury severity. Finally, Malone et al<sup>4</sup> reported on 15,534 trauma patients and found that blood transfusion was a strong independent predictor of mortality, ICU admission, ICU length of stay, and hospital length of stay. Clearly, RBC transfusions are associated with adverse outcomes among injured patients. The risks of allogeneic blood are summarized in Table 1.

**Table 1. Risks of Allogeneic Blood Transfusions**

- Disease transmission
  - Human immunodeficiency virus
  - Human T-cell lymphotropic virus
  - Hepatitis B, C viruses
  - West Nile virus
  - Yersinia, malaria, babesia, chagas
  - ? Variant Creutzfeldt-Jacob disease
- RBC membrane compatibility
  - Hemolytic reactions
- Allergic response
  - Fever, rash, bronchospasm, anaphylaxis
- Immunodysfunction
  - Decreased allograft rejection
  - Increased tumor recurrence
  - Increased postoperative infection
  - Transfusion-related acute lung injury
  - Increased postinjury multiple organ failure
- Compromised O<sub>2</sub> delivery
  - Reduced RBC deformability
  - Decreased 2,3-diphosphoglycerate

RBC, red blood cell.

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**Table 2. Alternatives to Allogeneic Blood Transfusion**

- Lower transfusion thresholds
- Recombinant human erythropoietin
- Recombinant activated factor VIIA
- Hemoglobin-based oxygen carriers
  - Hemopure, PolyHeme\*

\*Hemopure, Biopure Corp., Cambridge, MA; PolyHeme, Northfield Laboratories, Inc., Evanston, IL.

**Table 3. Characteristics of PolyHeme\***

Index	Measurement
Volume	500 mL
Hb mass	50 g
Hb concentration	10 g/dL
P <sub>50</sub>	28-30 mm Hg
Methemoglobin concentration	<3%
Tetramer	<1%
Half-life	24 hr
Shelf life	>1 year

\*PolyHeme, Northfield Laboratories, Inc., Evanston, IL.

Hb, hemoglobin.

Reproduced with permission from Gould SA, Moore EE, Moore FA, et al. Clinical utility of human polymerized hemoglobin as a blood substitute after acute trauma and urgent surgery. *J Trauma* 1997;43:325-332.

Several strategies have been identified to limit RBC transfusions in order to avoid these transfusion-related effects (Table 2). The first is the use of lower transfusion thresholds. The Transfusion Requirements in Critical Care (TRICC) study was a prospective randomized study in which critically ill patients with euvoemia were randomized to receive a single unit of RBCs at either a transfusion trigger at a hemoglobin (Hb) level of 10 g/dL (liberal group) or a transfusion trigger of 7 g/dL (restrictive group).<sup>9</sup> The conclusion was that maintenance of a Hb level of 7 to 9 g/dL was at least as effective as and possibly superior to a more liberal transfusion strategy in this group of patients. However, caution was advised in patients with ischemic heart disease. Subsequent studies have supported these findings.<sup>10-12</sup>

Another strategy that has been used to limit RBC transfusion is the use of recombinant human erythropoietin; however, its role in critically ill patients is uncertain, and erythropoietin is not effective in the management of acute blood loss.<sup>13,14</sup> Recombinant activated factor VIIA has an evolving role in the management of acute blood loss secondary to trauma.<sup>15-18</sup> It has been shown to reverse coagulopathy and to decrease the need for both RBC transfusion and also massive transfusion in certain trauma patient populations. Finally, HBOCs may be given in the prehospital or inpatient setting and have the potential for limiting allogeneic RBC transfusions and their consequences.<sup>19,20</sup>

The greatest need for a blood substitute such as an HBOC is in patients with a large unanticipated blood loss, the most likely scenario for which is in the setting of trauma. This is especially the case if RBCs are unavailable. This situation may occur both in the civilian and military settings. At present, there are two blood substitutes that may be capable of delivering oxygen in these settings.

Hemopure (Biopure Corp., Cambridge, MA) is a polymer of bovine hemoglobin that has been used successfully in elective cardiac, aortic, and hepatic surgery.<sup>19,20</sup> It has been approved for use

in Europe and South Africa; however, clinical trials in the United States have been halted by the Food and Drug Administration pending safety concerns. PolyHeme (Northfield Laboratories Inc., Evanston, IL) is the only HBOC that has been evaluated in severely injured patients in the United States to date<sup>19</sup>; therefore, it will be the focus of the remainder of this discussion.

PolyHeme is a human hemoglobin-based temporary RBC substitute that is presently under clinical evaluation for the treatment of life-threatening blood loss when an oxygen-carrying fluid is required and RBCs are not available.<sup>21</sup> The characteristics of PolyHeme are listed in Table 3. RBCs may not be available for clinical care in many situations, including at the scene of injury, during transport to definitive care, in the operating room in the case of unplanned hemorrhage, in rural or remote settings, in situations involving multiple or mass casualties, in cases of incompatibility, and in cases of religious objection to blood. The potential benefits of PolyHeme in clinical care (Table 4) are that it is immediately available, is in abundant supply, and has a prolonged shelf life (>1 year).<sup>22,23</sup> It is universally compatible with all blood types, and therefore does not require time-consuming typing and cross-matching.<sup>21</sup> It is isotonic, iso-oncotic, sterile, and pyrogen-free.<sup>23</sup> The mass of Hb in one unit of PolyHeme (50 g) is approximately equivalent to the mass of Hb in one unit of RBCs.<sup>21</sup> It is available in approximately 500-mL quantities (Fig. 1). It is safe during rapid, massive transfusion and has the ability to support life in humans in the virtual absence of any of the patient's own RBCs.<sup>24</sup>

The side effects that have been reported with HBOCs include interference with laboratory tests that are based on colorimetric changes from dissolved plasma Hb, inaccuracy of transcutaneous oxygen saturation monitoring because of methemoglobin, mild elevations of serum amylase without evidence of pancreatitis, and transient skin rashes (Table 5).<sup>19</sup> Because PolyHeme is an acellular

<b>Table 4. Potential Clinical Benefits of Hemoglobin-Based Oxygen Carriers*</b>	
Availability	<ul style="list-style-type: none"> <li>• Abundant supply</li> <li>• Universally compatible</li> <li>• Prolonged shelf life</li> <li>• Storage at room temperature</li> </ul>
Safety	<ul style="list-style-type: none"> <li>• No disease transmissions</li> <li>• No antigenic reactions</li> <li>• No immunologic effects</li> </ul>
Efficacy	<ul style="list-style-type: none"> <li>• Enhanced oxygen delivery</li> <li>• Improved rheologic properties</li> </ul>

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**Figure 1. PolyHeme is available in approximately 500-mL quantities.** (Reproduced with permission Northfield Laboratories Inc. [www.northfieldlabs.com/polyheme.html](http://www.northfieldlabs.com/polyheme.html).)

<b>Table 5. Side Effects Reported with Hemoglobin-Based Oxygen Carriers</b>	
Interference with laboratory tests	<ul style="list-style-type: none"> <li>• Prothrombin time</li> <li>• Activated partial thromboplastin time</li> <li>• Fibrinogen</li> <li>• Creatine kinase</li> <li>• Aspartate aminotransferase</li> <li>• Alanine aminotransferase</li> <li>• Alkaline phosphatase</li> <li>• Gamma-glutamyl transpeptidase</li> <li>• Lactate dehydrogenase</li> <li>• Total bilirubin</li> <li>• Mild elevation of serum amylase without evidence of pancreatitis</li> </ul>
Inaccuracy of transcutaneous oxygen-saturation monitoring	
Transient skin rashes	

form of Hb, the total Hb is the sum of the Hb provided by PolyHeme and the Hb contained in RBCs. The hematocrit will thus be lower than would otherwise be expected for the measured Hb.

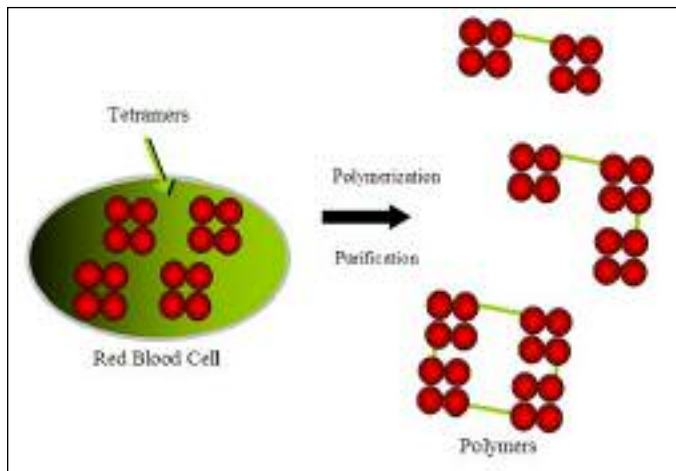
Adult Hb is a tetramer consisting of two alpha and two beta chains, each of which is bound to a heme group capable of binding to one molecule of  $O_2$ .<sup>19</sup> Removing the Hb moiety from the protective environment of the RBC membrane allows it to carry oxygen while avoiding the antigenicity inherent to the RBC membrane. However, this introduces two major problems. First, once the Hb is removed from the RBC, low levels of 2,3-diphosphoglycerate cause the oxyhemoglobin dissociation curve to shift to the left. The Hb oxygen affinity becomes too great for effective tissue oxygenation. The second problem is that Hb tetramers outside the RBC membrane rapidly dissociate into their component monomers and dimers.

Early attempts at transfusion of stroma-free Hb were complicated by the development of transient renal dysfunction and vasoconstriction.<sup>23</sup> Previous HBOCs, such as diaspirin cross-linked Hb (DCLHb), were associated with these problems.<sup>25</sup> DCLHb was tested as an adjunct to other therapies used for enhancing oxygen delivery, such as intravenous fluids, blood transfusions, and operative intervention. It was not tested as a substitute for blood. The study objective was to determine if the infusion of up to 1,000 mL DCLHb (100 g Hb) during the first 2 hours of hospitalization could reduce 28-day mortality in injured patients with evidence of persistent hypoperfusion secondary to acute blood loss. The mortality was 46% (24/52) for those in the study group, whereas it was only 17% (8/46) for those who received saline. This was believed to be due to vasoconstriction caused by the DCLHb, which occurred secondary to the scavenging of nitric oxide with additional endothelin release. This vasoconstriction then leads to a decrease in cardiac output. DCLHb is no longer in production.

At present, most of the most successful HBOCs are polymerized Hb solutions.<sup>19</sup> Polymerization appears to attenuate the vasoconstriction associated with Hb solutions.<sup>23</sup> Unmodified tetramer and its component monomers and dimers will extravasate through the vascular endothelium and bind nitric oxide, resulting in vasoconstriction.<sup>23</sup> Similarly, renal dysfunction probably occurs because of a combination of monomer and dimer filtration through the kidney with secondary mechanical tubular damage.<sup>23</sup> Other adverse effects seen with unmodified human tetrameric Hb include fever, coagulation defects, liver dysfunction, abdominal symptoms, and other nondescript complaints.<sup>23</sup> Unmodified human tetrameric Hb has clearly been found to be unsafe for human use.

The goal in preparing a safe modified Hb solution is to prevent both infiltration into the kidney and extravasation beyond the vascular endothelium. The approach used in creating PolyHeme was to create a large iso-oncotic molecule through Hb polymerization with removal of all unmodified tetramer.<sup>24</sup> PolyHeme is prepared from units of expired RBCs that are subjected to four inactivation steps.<sup>21,22</sup> The first step is the extraction and filtration of Hb from RBCs. After this, native tetrameric Hb is polymerized using glutaraldehyde to attenuate vasoconstriction. Pyridoxal phosphate is then used to destabilize deoxyhemoglobin and obtain a  $P_{50}$  that is elevated compared with normal. Finally, essentially all residual unreacted tetramer is removed, and the product is incorporated into an electrolyte solution (Fig. 2).

PolyHeme has been extensively studied during the past 10 years (Table 6). In 1997, Gould et al<sup>22</sup> published a prospective, nonrandomized study of 39 patients who received up to six units of PolyHeme during their initial resuscitation from acute blood loss after trauma and urgent surgery. Although RBC hemoglobin fell as low as 2.9 g/dL, the total hemoglobin was maintained at acceptable levels. Also, 59% of patients avoided allogeneic transfusions during the first 24 hours after blood loss. There was no change in



**Figure 2. Preparation of polymerized, tetramer-free hemoglobin from human red blood cells. (Reproduced with permission Northfield Laboratories Inc. [www.northfieldlabs.com/polyheme.html](http://www.northfieldlabs.com/polyheme.html).)**

temperature, mean arterial pressure, heart rate, or creatinine clearance. It was concluded that PolyHeme effectively delivers oxygen and maintains total Hb in lieu of RBCs after acute blood loss, thereby reducing the need for allogeneic transfusions. These results were confirmed in a prospective, randomized trial of 44 patients the following year.<sup>23</sup>

In another prospective, randomized study, patients requiring urgent transfusion were given up to six units of PolyHeme or stored RBCs during their initial resuscitation.<sup>26</sup> Indications for transfusion included hypotension refractory to crystalloid infusion, physiologically apparent anemia (i.e., refractory tachycardia, oxygen extraction ratio >30%), or profound anemia (Hb <7 g/dL). Seven patients received RBCs and six received PolyHeme. There were no significant differences in systemic arterial pressure, pulmonary arterial pressure, cardiac index, pulmonary capillary wedge pressure, or systemic or pulmonary vascular resistance. The authors concluded that PolyHeme lacks the vasoconstrictive effects associated with other Hb-based blood substitutes.

To assess the mortality difference in patients who received PolyHeme and those who did not, Gould et al<sup>24</sup> compared the 30-day mortality in 171 patients who were given up to 20 units of PolyHeme and 300 historical patients who refused blood on religious grounds. Forty patients had a nadir Hb ≤3. Total Hb, however, was maintained at a mean of 6.8 ± 1.2 g/dL, because of plasma Hb provided by PolyHeme. The 30-day mortality in this subset of patients was 25.0% (10/40) in the PolyHeme group compared with 64.5% (20/31) in historical controls. Twenty-nine patients had a nadir Hb ≤2. Their 30-day mortality was 27.6% with PolyHeme compared with 100% (7/7) in control patients. The authors concluded that PolyHeme increased survival at life-threatening Hb levels by maintaining total Hb in the absence of RBC transfusions.

There has been concern that lipid and cytokine mediators present in RBCs may augment the postinjury inflammatory response that predisposes to multiple organ failure. This raises the question of whether PolyHeme (in lieu of stored RBCs) will attenuate the proinflammatory effects associated with allogeneic RBC transfusions and thus reduce the risk of multiple organ failure. In 2003, Johnson et al<sup>27</sup> reported a prospective study that compared the rates of multiple organ dysfunction in injured patients who received PolyHeme and those who received RBC transfusions. Patients included had an ISS >15 and were expected to receive six or more units of RBCs in the first 12 hours after injury. Seven patients received RBCs and 18 received PolyHeme. Both groups had significant increases in the proinflammatory cytokines interleukin (IL)-6 and IL-8 as well as the counterregulatory cytokine IL-10, but the relative increase was substantially less with PolyHeme.

One study investigating PolyHeme given to patients undergoing elective abdominal aortic aneurysm repair demonstrated possible adverse outcomes and has led to a great deal of controversy regarding its use.<sup>28,29</sup> This was a phase III trial in which 152 patients were randomized to receive PolyHeme (81 patients) or RBCs (71 patients) during acute normovolemic hemodilution (ANH), in which the patient's own blood is removed and stored prior to the surgery, replaced with colloid, and returned to the patient after the operation. The goal of ANH is to minimize the loss of the patient's own RBCs during surgery and potentially reduce the use of allogeneic RBCs. The study was designed to assess whether the use of PolyHeme

**Table 6. Summary of Recent PolyHeme Studies\***

Study	No. of Patients	Indication for PolyHeme	Result
Gould et al, <sup>22</sup> 1997	39	Received up to 6 units of PolyHeme during initial resuscitation in lieu of RBCs	PolyHeme maintains total Hb in lieu of RBCs after acute blood loss
Gould et al, <sup>23</sup> 1998	44	Randomized to receive RBCs or up to 6 units of PolyHeme as initial resuscitation	Amount of RBCs given on day 1 were significantly less for the PolyHeme group
Johnson et al, <sup>26</sup> 1998	13	Randomized to receive RBCs or up to 6 units of PolyHeme as initial resuscitation	PolyHeme lacks the vasoconstrictive effects associated with other Hb-based blood substitutes
Gould et al, <sup>24</sup> 2002	171	Compared mortality in patients who were given up to 20 units of PolyHeme with historical patients who refused blood	PolyHeme increased survival at life-threatening Hb levels by maintaining total Hb without RBC transfusions
Johnson et al, <sup>27</sup> 2003	25	Compared rates of multiple organ failure in injured patients receiving PolyHeme with those receiving RBCs	Relative increase in proinflammatory cytokines as well as a counterregulatory cytokine was less with PolyHeme

\* PolyHeme, Northfield Laboratories, Inc., Evanston, IL.  
RBC, red blood cell; Hb, hemoglobin.

would allow an increase in the volume of autologous blood collected during ANH, and therefore avoid the use of donated blood. Serious cardiovascular consequences occurred more commonly in the PolyHeme group. Ten of 81 (12%) patients given PolyHeme had myocardial infarction within 7 days after operation and two of these patients died. None of the 71 patients on standard therapy had myocardial infarction. The patients in this study were older, with more cardiovascular risk factors than the usual trauma patient. It cannot be determined if the reason for the higher cardiovascular morbidity was due to the more extensive ANH, the reinfusion of more blood in the PolyHeme group, or if it was related to the PolyHeme itself.

Blood is usually not available in the prehospital setting. There is little published data on the mortality of bleeding patients who do not have access to blood in the field. In one study, 112 patients received RBC transfusion during helicopter evacuation for a systolic blood pressure <80 mm Hg or absence of a palpable radial pulse.<sup>30</sup> The average transport time was 27 minutes. Overall, there were 51 deaths (46%). Excluding the 35 patients who required cardiopulmonary resuscitation at the scene or in transit, 18 of 77 (23%) patients died. Achieving mortality less than this by giving an HBOC in the prehospital setting would be comparable if not superior to giving RBCs in the field, and certainly an improvement over the expected outcome in the situation in which no blood is available.

To address this issue, a multicenter, randomized, controlled phase III trial investigating PolyHeme in trauma patients with hemorrhagic shock was completed on July 31, 2006.<sup>21</sup> In this study, patients were randomized to receive either PolyHeme (up to a total of six units) beginning in the field and continuing to the first 12 hours after injury or crystalloid in the prehospital phase and blood in the hospital phase. Eligible patients were at least 18 years old. Obviously pregnant women, those with severe brain injuries, patients requiring cardiopulmonary resuscitation or with obviously nonsurvivable injuries, and patients known to refuse transfusions or resuscitation were excluded from the study. Thirty-two level 1 trauma centers throughout the United States participated in this trial under a Food and Drug Administration special category (21 Code of Federal Regulations 50.24) that allowed waiver of informed consent. More than 700 patients were enrolled. The hypothesis of this study was that the use of PolyHeme as the initial resuscitative fluid in severely injured patients in hemorrhagic shock, beginning in the prehospital setting and continuing through a 12-hour postinjury hospital setting, will reduce mortality in comparison to control therapy. The primary end point was survival at 30 days.

The preliminary results of this study were announced by Northfield Laboratories in December 2006.<sup>21</sup> Of the 722 patients in the study, 712 were randomized and received treatment (modified intent-to-treat population). Because of the complexity of the protocol, there were 126 protocol violations, leaving a total of 586 patients (279 PolyHeme, 307 control) in the per-protocol population. In the modified intent-to-treat population, there were 46 deaths (13.2%) in the PolyHeme group and 35 deaths (9.6%) in the control group. In the per-protocol population, 30-day mortality was 10.8% in the PolyHeme group and 9.1% in the control. Day one mortality was equal (6.8%) in both groups. The preliminary safety analysis did not reveal any significant differences between the two patient groups. Final study data are expected to be released by Northfield Laboratories in the near future.

HBOCs, such as PolyHeme, have the potential to become clinically useful oxygen carriers that would serve as a blood substitute, especially in the trauma population. Although many studies have been performed, no HBOC is available at present for clinical use. Final results of the PolyHeme study, if favorable, may

herald the introduction of HBOCs to patient care. PolyHeme might then be used in situations in which blood is not available or not accepted (i.e., in Jehovah's Witnesses). It might achieve wider use even outside these situations if it is found to be safer than allogeneic blood transfusions. Further study is needed on the use of PolyHeme and other HBOCs to assure their safety and efficacy in trauma and other patient populations.

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