

REGULAR REVIEW

Artificial Blood Substitutes

J Tappenden

Department of General Surgery, Northern General Hospital, Herries Road Sheffield S5

Introduction

Haemorrhagic shock kills. The maintenance of intravascular volume is essential and resuscitation with isotonic crystalloid or volume expanders such as gelofusin or pentastarch is fundamental in the management of shock, with the addition of packed red cells when available, to restore oxygen carrying capacity and oxygen delivery to reinstate tissue perfusion - although the timing and target blood pressure for resuscitation is still not universally agreed. Donor blood however is a limited resource for which there is increasing demand. Fourteen million units of blood were used in the US alone last year. England and Wales require 8,000 units of blood per day (1). Currently there are only 5.1 days' stock of O negative blood in the UK banks (1). 50% of RBC transfusions are used in trauma or surgery, to compensate for major blood loss (2). The increase in demand for blood is due to an increase in the ageing population, and decreased allogenic donation. Vamvakas *et al* predict an annual shortfall in the US of 4 million units of blood by 2030 (3). Artificial oxygen carriers may provide a solution to the shortfall of donor blood available for transfusion.

Artificial oxygen carrier infusions offer volume expansion and oxygen carriage. The advancement of artificial blood substitutes encompasses two different pathways of development. Synthetic blood substitutes are based on perfluorocarbons, whilst haemoglobin-based oxygen carriers use human or bovine haemoglobin as the core product. The aim of these products, both in the elective and trauma setting, is to reduce the need for allogenic blood transfusion and maintain tissue oxygenation.

The US military have been the greatest advocates of the development of artificial blood substitutes because of the vital need and benefits in the battlefield scenario. In Vietnam, 2774 casualties received almost 20,000 units of blood, most receiving 2-5 units (4). Blood is currently unavailable far forward on the battlefield for predominantly logistic reasons. Casualties may be salvageable if intervention is available far forward and earlier. Artificial oxygen carriers could provide part of the solution to this problem.

Coupled with unavailability of blood far forward on the battlefield, the wastage of blood resources during war is also of great concern. 60% of the 1.3 million units of blood dispatched during the Vietnam War and 95% of 120,000 units during the Gulf War were discarded (5). This was largely due to the units passing their expiry date. During the same years of the Vietnam War, 15-35% of blood for civilian use was wasted (6). Artificial blood substitutes have been developed with this in mind, and have been engineered to have a long shelf-life. These factors have propagated the military investment in the development of these products. The U.S. military has spent approximately \$30 million on the enterprise to date.

Correspondence to: Miss J Tappenden
Registrar in General Surgery, Department of Surgery,
Northern General Hospital, Herries Road, Sheffield S5
Email: janinetappenden@hotmail.com

Allogenic blood transfusion is associated with potential significant immunomodulatory and infective complications, as outlined in Table 1. The combination of potential adverse effects of transfusion, impending shortage of blood, high cost of packed red cells storage and transfusion, and limited availability in pre-hospital scenario and battlefield, has led to an accelerated push in the development of a clinically employable alternative.

Acute	Hypothermia Hyperkalaemia Hypocalcaemia Acute haemolytic reaction secondary to ABO incompatibility Transfusion Related Acute Lung Injury (TRALI) Endotoxic shock
Delayed	Transmission of infection (HIV, HCV, HBV, CMV, parvovirus, vCJD) Delayed haemolytic transfusion reaction Increased tumour recurrence
Massive transfusion	Coagulopathy Hypothermia Acidosis TRALI

Table 1 Complications of Red Blood Cell Transfusion

This paper presents a narrative review outlining the current state of development of artificial oxygen carriers.

The Benefits of Artificial Oxygen Carriers

Artificial oxygen carriers have been developed to obviate the shortcomings of packed red cells.

Compatibility. Artificial oxygen carriers are universally compatible with all blood types. There is no need for typing or cross-matching, preventing the risk of ABO incompatibility associated with human error which still occurs despite rigorous checking of packed red cells in 1:34,000 units transfused (2).

Contraindications. As the artificial blood substitutes are universally compatible, they can be used in patients with allo-autoantibodies such as those with sickle cell disease. **Refusal.** Artificial oxygen carriers have also been accepted by Jehovah's Witness' patients when allogenic blood transfusion has been refused. **Shelf life.** Blood has a limited shelf life of 42 days when stored at 4°C and 5 hours at room temperature. Artificial haemoglobin carriers have an extended shelf life of 1-3 years at room temperature, which allows for stock-piling for emergencies, trauma, disasters and warfare. **Disease transmission.** Allogenic blood transfusion carries the risk of disease transmission. Blood substitutes provide a disease-free source of blood, which is of great benefit to countries with a high HIV/ AIDS population, where disease-free blood is a limited resource and HIV and hepatitis transmission in blood remains widespread. South Africa

has licensed the use of the artificial Haemoglobin-based oxygen carrier Hemopure (HBOC-201) in part for this reason. **Oxygen delivery.** Transfused blood may take up to 24 hours to achieve full oxygen transfer capacity due to 2,3 DPG depletion. Artificial blood substitutes are at full oxygen capacity immediately. **Immunological effects.** It has been postulated that artificial blood substitutes do not prime the circulating neutrophils unlike transfusion with packed red cells, and therefore reduce the incidence of multi-organ failure which occurs with use of blood after prolonged storage (7,8). **Availability.** Abundant supply of blood substitutes will enable use in pre-hospital scenario, battlefield use, and in remote locations where blood may be unavailable. As no typing or cross-matching is required, the blood substitutes will be immediately available for infusion. **Cost.** The current cost of one unit of blood in the US is approximately \$250. This encompasses donor recruitment, phlebotomy, administrative, testing, hospital and storage costs. As manufacturing of artificial oxygen carriers becomes refined and with no storage costs, the cost of blood substitutes may fall potentially below that of packed red cells. **Rheology.** As the haemoglobin is in the plasma, there is increased diffuse transport of oxygen to the microcirculation. Cellular oxygen delivery from artificial oxygen carriers is three times that of red cells (9). HBOCs can reach post-stenotic and poorly perfused tissues with plasma flow where erythrocytes cannot due to their smaller size. HBOCs are 1/1000th the size of erythrocytes. Hughes *et al* demonstrated increased splanchnic perfusion, skeletal muscle oxygenation and restoration of pancreatic microcirculation in a rat model (10).

Artificial Oxygen Carrier	Product name	Current Trial
Perfluorocarbon	Fluosol-DA	Phase III Withdrawn 1994
	Oxygent	Phase III (Stopped)
Diaspirin cross-linked haemoglobin	Hemassist	Phase III (Stopped)
Human polymerised haemoglobin-based oxygen carrier	Polyheme	Phase III
Polymerised bovine haemoglobin-based oxygen carrier	Hemopure	Phase III RESUS (Restore Effective Survival in Shock) trial
Haemoglobin raffimer	Hemolink	Phase II (Stopped)
Maleimide-activated polyethylene glycol-modified haemoglobin (MP4)	Hemospan	Phase II
Human recombinant haemoglobin	rHb1.1 rHb2.0	Phase II (Stopped)
Enzyme cross-linked polyhaemoglobin	-	No large animal studies or human studies to date
Haemoglobin containing vesicles	-	Animal studies only
Allosteric modifier	RSR13	Animal studies unfavourable
Stem cells	-	Ex-vivo studies only
Dendrimers	-	Early research only

Table 2 Current Status of Development of Artificial Oxygen Carriers

The Development of Artificial Oxygen Carriers

Two distinct pathways of development have emerged in the search for a clinically employable oxygen carrier with the aforementioned characteristics: synthetic oxygen carriers (perfluorocarbons) and haemoglobin-based oxygen carriers. The development of artificial oxygen carriers began in 1933, when Mulder *et al* took cats and dogs and performed total exchange transfusion, infusing bovine haemolysates, and maintained oxygen consumption (11). In 1949 Amberson *et al* took the same haemoglobin solution and infused it into humans, hoping for the same successful outcome, but unfortunately it proved to be toxic, resulting in vasopressor effects and acute renal failure (12). It wasn't until 1978 before the next cohort of haemoglobin-based oxygen carriers were developed. These were stroma-free haemolysates, which still proved to be nephrotoxic due to haemoglobin tetramer instability (13). Single molecules of haemoglobin are nephrotoxic and lead to vasoconstriction and therefore the haemoglobin molecules were polymerised, resulting in haemoglobin stability and losing the vasopressor and nephrotoxic side effects. These products were the forerunners of the present market leaders, polymerised haemoglobin based oxygen carriers, Polyheme and Hemopure. However there have been many products developed during the search for a clinically employable blood substitute as summarised in Table 2.

Perfluorocarbon emulsions

Perfluorocarbons are synthetic oxygen carriers. They are chains of eight to ten hydrocarbon molecules where the hydrogen has been replaced by fluorine. Perfluorocarbons are biologically and chemically inert. They possess high gas dissolving properties. Perfluorocarbons are not miscible with water and therefore have to be brought into an emulsion prior to use. Perfluorocarbons have a short half-life of 12-18 hours, but are only cleared from the body weeks later, preventing multiple doses in a short time span (14). Perfluorocarbons are initially taken up by the reticulo-endothelial system forming droplets of emulsion which are then broken down and taken up by blood again bound to lipid. They are then transported to the lungs, by which route they are excreted via exhalation. This whole process takes several weeks. It is also mandatory for oxygen to be inspired to a supra-physiological level with 100% oxygen during infusion of the product, as perfluorocarbons carry oxygen proportional to the inspired oxygen. Perfluorocarbons have a linear relationship with the partial pressure of oxygen and oxygen content rather than the sigmoidal curve of human haemoglobin. They require a partial pressure of oxygen greater than 300mmHg to be effective (14). Perfluorocarbon emulsion droplets are dissolved within blood rather than being bound to haemoglobin in red blood cells and therefore are smaller (<0.2µm vs. 7-8µm diameter of RBC) allowing increased oxygenation by facilitated diffusion and passage into narrower capillaries where red blood cells are unable to pass, thus increasing local tissue oxygenation.

The first blood substitute approved by the FDA for phase III trial was a perfluorocarbon-based product called Fluosol-DA-20 produced by Green Cross Corporation, Osaka, Japan. It was approved by the FDA in 1989 for use during PTCA, with the premise of reducing myocardial hypoxia distal to the angioplasty balloon. Fluosol-DA remains the only product to have been fully approved by the FDA. Despite this, the product was withdrawn in 1994 due to its limited success, poor sales figures, complexity of use and side effects (anaphylaxis, pulmonary hyperinflation and interference with PMN function) (15). Oxygent, a second generation perfluorocarbon developed by the Alliance Pharmaceutical Corporation was trialled in patients undergoing cardiac surgery in 2001. It was

produced as a completely man-made powder, which could be stored indefinitely. Oxygent side effects noted include decreased platelet count which returns to normal by day 7, myalgia, flu-like symptoms, and headache.

As there is potential for the reticulo-endothelial system to be overloaded by perfluorocarbons, resulting in hepatosplenomegaly and impairment of immunological function (16) the dose of the product has been limited by the manufacturers to avoid overdose. The dose is limited to the equivalent of half unit RBC. They therefore have no role as a resuscitation fluid, but only as an adjunct but which provides improved tissue oxygenation. In an elective surgical setting they have been trialled with acute normovolaemic haemodilution (ANH) of patients to avoid allogenic transfusion.

Spahn *et al* conducted a multi-centre European phase III study with 492 patients undergoing major non-cardiac surgery with the aim of reducing allogenic transfusion (17). Patients underwent ANH to a haemoglobin of 8. They were given perfluorocarbon at the time of skin incision. A second dose was given if the haemoglobin fell to less than 6.5 intra-operatively. A third dose was given post-operatively if haemoglobin fell below 5.5 or there was a physiological trigger. At the end of surgery all ANH blood was re-transfused. At 24 hours the percentage of patients avoiding transfusion was 53% in the treatment group compared to 43% in the control group ($p < 0.05$). At the day of discharge this fell to 26% in the perfluorocarbon group compared to 16% in the control group ($p < 0.05$). The adverse event rate was comparable in both groups as was the mortality (2% in control vs 4% in the treatment arm). The trial concluded that patients with a blood loss of greater than 20ml/Kg needed significantly less allogenic transfusion in the perfluorocarbon group.

However the Alliance Pharmaceutical Corporation suspended enrolment into a Phase III trial of cardiac patients as interim analysis revealed those treated with Oxygent were more likely to suffer a stroke when compared to the standard treatment group (18). All current North American human studies of perfluorocarbons have since been terminated (19).

Diaspirin cross-linked Haemoglobin

Following the problems found with the modified haemoglobin products due to the instability of the haemoglobin tetramers leading to vasopressor side effect, haemoglobin was modified to avoid this. Diaspirin cross-linked haemoglobin (DCLHb) was the first modified haemoglobin product to be developed. DCLHb is produced from outdated donor blood. The two α subunits of haemoglobin are cross-linked with 3, 4-dibromosalicyl-fumarate (diaspirin). It was developed to avoid the instability of haemoglobin's tetrameric structure. A DCLHb, Hemassist produced by Baxter, was the first Haemoglobin-based oxygen carrier to be evaluated within Phase III trials. Animal studies supported its continued development. Xu *et al* demonstrated in a haemorrhagic rat model improved wound healing and decreased splanchnic bacterial translocation in the diaspirin group when compared to red blood cell transfusion group (20). Habler *et al* found overall survival was better with DCLHb compared to albumin in a pig haemorrhagic model (21).

Initial human studies demonstrated decreased allogenic transfusion rate in cardiac and non cardiac patient trials. Schubert demonstrated 23% of patients avoided allogenic transfusion compared to none of the control group (22). Sloan *et al* conducted a multi-centre US randomised controlled single blinded efficacy trial of diaspirin cross-linked haemoglobin in 1999 with 112 patients. Patients accepted for the study had traumatic haemorrhagic shock and were unstable. They were randomised to 500mls of DCLHb or saline followed by a further 500mls. The trial was stopped prematurely after interim analysis demonstrated excess



Figure 1: A bag of Polyheme ready for use in the Phase III trauma trial

28 day mortality in the treatment group of 46% vs. 17%, thought to be mediated by scavenging of nitrous oxide, invoking vasopressor action and pulmonary hypertension (23). The 28 day multi-organ dysfunction score was also 76% higher in the DCLHb treatment arm (24). Similar outcome of excess mortality and worse outcome scores were also demonstrated by Saxena *et al* in a trial of 85 patients with acute ischaemic stroke treated with either DCLHb or saline. The European "On-Scene" multicentre study randomised patients to 1000mls of 10% DCLHb on scene or normal saline. The trial terminated early as interim analysis showed the efficacy not to be sufficient enough to compensate for concerns regarding significant side effects (25). In September 1998 production of Hemassist was terminated by Baxter due to its prevailing adverse event profile.

Polymerised Haemoglobin-based Oxygen Carriers

The polymerisation of haemoglobin was the next development following modified haemoglobins. Polymerisation was identified as solving the vasopressor problems associated with modified haemoglobins such as DCLHb. A primate study by Rosen *et al* demonstrated the maintenance of tissue oxygen consumption and haemodynamics in 6 adult baboons which had undergone total exchange transfusion with a polymerised pyridoxylated haemoglobin solution (26).

The human polymerised haemoglobin, Polyheme, began as a military venture following Vietnam. Polyheme (Figure 1), along with Hemopure (HBOC-201) are the only products that have undergone phase III FDA approved human trials and have continued commercial backing. Both products are glutaraldehyde cross-linked polymers of haemoglobin. They can be infused at room air oxygen concentration as opposed to perfluorocarbons, which require oxygen to be inspired. Further characteristics are outlined in Table 3.

	Hemopure (HBOC-201)	Polyheme	RBC
Origin of Haemoglobin	Bovine	Human	-
Haemoglobin (g/dL)	13	10	13
P50* (mmHg)	38	29	26.8
Half-life	19 hours	24 hours	31 days
Shelf-life @ 4°C	≥ 3 years	≥ 1.5 years	42 days
Shelf-life @ 21°C	≥ 2 years	≥ 6 weeks	< 6 hours

Table 3 Characteristics of Haemoglobin-Based Oxygen Carriers. *P50 = the partial pressure of oxygen at which 50% of haemoglobin is saturated

Hemopure is produced by the lysis of bovine red blood cells from a disease free US herd of cattle less than thirty months old. It is ultrapurified to remove the stroma and diafiltrated to remove potential prions. It is then polymerised with glutaraldehyde. One of its greatest advantages over Polyheme is its 3 year half-life at 1-38°C. Polyheme has to be refrigerated to maintain its shelf-life. Polyheme is produced from outdated human donor blood. A major drawback of Polyheme and Hemopure is their short half-life of less than 24 hours, necessitating repeated infusions. Favourable outcomes have been reported in several trials of Hemopure. Jahr *et al* evaluated 693 patients undergoing major orthopaedic surgery in 2001. The results demonstrated Hemopure increased the number of patients avoiding transfusion from 0% to 59% with comparable adverse events (27). They report mild self-limiting adverse events such as fever, GI disturbance jaundice and pyrexia. A further trial of 98 patients undergoing cardiac surgery demonstrated 34% compared to 0% avoided allogenic transfusion (95% confidence interval 21%-49%) (28).

Polyheme has the most trial data available in the literature supporting its ongoing development. Phase III trials have concentrated on trauma patients, unlike trials of Hemopure which have largely been based on reductions of transfusion in elective patients. Polyheme is currently being assessed in a large pivotal clinical trial in the US (29). As of April 2006, 32 Level 1 trauma centres in the U.S. have been involved in the trial, which under FDA special approval, has allowed the use of Polyheme in an emergency urban trauma setting with short transport time. Trauma patients were randomised to receive Polyheme or saline at the scene and during transportation to the hospital and then Polyheme or red blood cell transfusion on arrival at the trauma centre for up to 12 hours or up to 6 units. The primary endpoint of the study is 30 day survival. The trial recruited 720 patients and recruitment closed in 2006, with early outcome data suggesting no statistical evidence of safety concerns and full results expected to be released in June 2007 (29).

There has been much controversy surrounding the ethics of the Polyheme trauma trial which has sparked debate across America. The FDA under regulation 21 CFR 50.24 have given approval for Polyheme to be given in the emergency trauma setting without gaining informed consent from the patient if it is precluded by the extent of their injuries. This waiver of consent only applies if available treatments are unproven or unsatisfactory. Blood is an accepted standard effective treatment of haemorrhagic shock when available. The pre-hospital phase of the trial negates this ethical issue as blood is not available in the ambulance for use at the scene or during transportation, and resuscitation with crystalloids, albeit standard treatment, is unsatisfactory, therefore enabling Polyheme to be given under the waiver ruling. However, on arrival at hospital many ethicists

and others believe the continuation of treatment with Polyheme for up to 12 hours rather than blood under the CFR 50.24 waiver of consent is no longer ethical as blood is available for use and is an accepted standard treatment in the management of haemorrhagic shock, thus informed consent should be acquired if Polyheme is to be continued (30). Northfield refute all allegations questioning the ethical status of the trial. The study protocol was reviewed by 32 Institutional Review Boards and approval was granted to continue the trial. The study was also subject to four reviews of mortality and serious adverse events by the Independent Data Monitoring Committee, which allowed the trial to continue without modification (31). Community-based awareness campaigns were held to highlight the trial to the local communities and citizens were able to opt out of the trial by wearing blue hospital bands provided by Northfields Laboratories, stating, "I decline the Northfield Polyheme Trial". The trial did however reach its full enrolment in 2006 but the media and public interest has remained heightened regarding the ethics surrounding the study.

Gould *et al* have presented further Polyheme trial data supporting the use of Polyheme to sustain life in haemorrhaging patients when red cells may not be available (32). In 2002 they compared 171 trauma patients to a historic control group of 300 surgical patients who refused blood transfusion on the basis of religious beliefs. The trauma patients received varying amounts of Polyheme. 45 patients received 1-2 units, 4 patients received 3-4 units, 47 patients received 5-9 units, 34 patients received 10-20 units. Of the trauma patients, 40 had a nadir haemoglobin <3 g/dL, with a mean of 1.5 g/dL. Their total haemoglobin was maintained at a mean of 6.8g/dL with infusion of Polyheme. The 30 day mortality was 25% in the Polyheme group compared with 64% in the control group. Of 12 patients with a haemoglobin <1g/dL nine survived. A haemoglobin of less than 2 is accepted as being incompatible with life and these 9 patients would have otherwise died if blood was not available. The trial has been put forward by Gould *et al* as demonstrating the life sustaining capacity of Polyheme in instances where transfusion may not be available.

There are case reports in the literature where Polyheme has been used in specific cases with excellent results. The Lancet published a case report from the US of use in a patient with severe sickle cell anaemia, who underwent a total hip replacement for avascular necrosis. Post operatively the patient developed sepsis secondary to urinary tract infection. They received 5 units of blood initially, but the transfusion reaction stopped due to delayed haemolytic transfusion reaction secondary to alloimmunisation. Their haemoglobin dropped to 2.8 g/dL. The patient received 5 units of Polyheme, erythropoietin and iron supplementation. The patient was discharged on day 16 with a haemoglobin of 7.8 further supporting the use of Polyheme when red cell transfusion cannot be used (33). Biopure have given Hemopure for use in 33 patients in the US on compassionate grounds (34). A further US case report supporting the use of Polyheme was in a Jehovah's Witness trauma patient with splenic injury and pulmonary contusions, whose haemoglobin was kept above 6 with Polyheme to bridge to gap before bone marrow production could compensate. The patient was discharged on day 19 (35). Interestingly, despite Polyheme's strong supporting trial data, Hemopure is the only product which has been licensed for use. It has been available for commercial use in South Africa since April 10th 2001 in patients with acute peri-operative anaemia eliminating the need for blood transfusion. It has been licensed as an "oxygen bridge", with a maximum dose of 7 units.

In 2006 Levien reported the South African clinical experience to date since the product was registered for usage (36). Levien reports the clinical outcome of 336 patients who received

Hemopure between April 2001 and February 2005 registered to the ongoing clinical surveillance programme of the product. Patients had to reach a transfusion trigger, where ordinarily they would receive two units of allogenic blood. An initial dose of 30g of Hemopure was infused over one to three hours. Further doses were given dependent of the clinical stability of the patient. A mean of 2.252 units of Hemopure in an individual patient were infused. Results demonstrate that allogenic transfusion was avoided in 88% of the first 200 patients receiving Hemopure. The product has been shown to be well tolerated, with adverse events being rare. The surveillance cohort included 23 deaths. These were reported by the leading clinician to be related to the underlying disease process and none were felt to be directly related to the product itself. In 5% of patients a rise in systolic blood pressure greater than 30mmHg was reported. This was managed with an infusion of calcium channel antagonist, nitrate or beta-blocker, and never reported to be a clinical problem. Three patients died of acute severe anaemia when they became unstable 24-36 hours following infusion of Hemopure, as the treating physician failed to realise the product effect had worn off as the half-life of 16-24 hours had been passed. Further product education has resolved this issue. Hemopure has been increasingly used to increase oxygen delivery and treat acute tissue ischaemia, with clinicians describing improved wound outcome. However, further trials are needed to establish the efficacy of the product as an oxygen therapeutic for acute reversible tissue ischaemia.

Despite Biopure's acquisition of a license for the use of Hemopure in South Africa in 2001, the product only obtained its registration enabling commercial sales in 2005, and the first commercial sale was only made in January 2006. According to corporate documentation, sales of this product have been very limited (34). The Hemopure for the ongoing clinical surveillance programme in South Africa was provided without charge by Biopure. Biopure have applied for FDA approval for a pre-hospital trial of Hemopure in trauma patients in the US. Biopure have received \$4 million of funding from the government, with the input of the US Navy for the aptly named RESUS trial. However, the FDA asked for further animal studies to be conducted before any further human trials with Hemopure due to concerns regarding its safety profile. These studies have been completed and the outcome of the FDA's decision regarding the approval for the RESUS trial is awaited in early 2007.

A further product from Biopure called Oxyglobin has been licensed by the FDA for veterinary usage in the US and Europe. It has been used to treat anaemia in dogs. It is the only product on the market for usage in animals. They have sold 177,000 units of Oxyglobin for use in dogs. Supply by Biopure is low, as the company have concentrated its efforts on its main product Hemopure. Interestingly, a Spanish cyclist, Jesus Manzano, admitted to using Oxyglobin illegally before a Spanish time trial and in the 2003 Tour de France. He crashed out of the Tour de France after experiencing nausea prior to the accident.

Human Recombinant Haemoglobin

Hoffman *et al* described human recombinant haemoglobin expressed in *e.coli* in 1990. Looker *et al* modified recombinant haemoglobin as artificial oxygen carrier in 1992, producing rHb1.1 (37). The natural $\alpha\beta_2$ Haemoglobin tetramer is stabilised by fusing the two α chains, eliminating the renal toxicity associated with the tetramer structure. rHb1.1 is the second artificial blood substitute produced by Baxter. It is structurally an analogue of their first attempt, DCLHb. It has an intravascular retention time of two hours. Animal studies have proved favourable with Malhotra *et al* demonstrating survival benefit over colloid in a pig shock model (38).

However, like its predecessor DCLHb, it has shown a similar adverse event profile, with nitrous oxide (NO) scavenging leading to significant pulmonary hypertension. The second generation recombinant haemoglobin, rHb2.0, has demonstrated less NO scavenging than rHb1.1 but still at a significant level. Production has been halted by Baxter in 2003 despite favourable biological effects as the side effect profile is similar to DCLHb and appears insurmountable at present.

Haemoglobin Raffimer

The haemoglobin-based oxygen carrier Hemolink has been developed from oxidised raffinose cross linked polymerised human haemoglobin. The haemoglobin only undergoes partial polymerisation with 40% remaining as tetramers. It has a one year shelf-life. Hemosol, the Canadian-based company manufacturing the product has received sizeable funding from the Canadian Defence Department. In a Phase I trial reported by Carmichael *et al*, Hemolink was shown to be beneficial but induce vasoconstriction especially in hypertensive subjects (39). A Phase II trial involving 60 patients undergoing CABG demonstrated a reduction in allogenic transfusion but jaundice and hypertension were observed as side effects (40). In a further Phase III trial, the transfusion rate in 299 patients having coronary artery bypass surgery was decreased to 56% compared to 76% in the control group. A similar adverse event profile was noted between the two treatment groups; these events included hypertension, jaundice and increased pancreatic enzymes (41). Enrolment into a further Phase III trial was suspended when interim analysis demonstrated excess cardiac ischaemia and adverse events in the unrelated HLK 213 Phase II cardiac surgery trial in 2003. Production of Hemolink has since been terminated. Hemosol, the proprietors of Hemolink filed for bankruptcy in November 2005.

Maleimide-Activated Polyethylene Glycol-Modified Haemoglobin (MP4)

MP4 has been developed by Winslow and the Sangart Corporation from outdated donor blood. The haemoglobin molecules undergo surface conjugation with maleimide-activated polyethylene glycol. It has been designed with a rather counterintuitive profile. MP4 has a low haemoglobin concentration at 4.2g/dL, high oxygen affinity with a p50 of 6mmHg, and high viscosity of 2.5cP. It has been developed with a high oxygen affinity based on the premise that the oxygen will be preferentially released to hypoxic tissue, avoiding tissue hyperoxia of normal tissue, which is thought to lead to arteriolar vasoconstriction and impaired microvascular circulation (42). It has been shown to have an intravascular retention of 24 hours in rats (43). Functional capillary density is increased indicating improved microvascular perfusion (44). A hamster haemorrhagic shock model demonstrated MP4 restored tissue oxygenation significantly better than hydroxyethyl starch. However, re-transfusion with autologous blood proved better than MP4 (44).

Enzyme cross linked polyhaemoglobin

In this product, haemoglobin is cross-linked with enzymes such as catalase superoxide dismutase (18). They have been shown to be beneficial when using haemoglobin-based oxygen carriers to treat organ ischaemia. A rat model demonstrated decreased oxygen free radicals production on reperfusion. There have been no large animal studies or studies in humans thus far (18).

Haemoglobin containing vesicles

This product is still in its pre-clinical phase, with only animal trials to date. The product has been designed to avoid the adverse events of free haemoglobin by encapsulating it in liposomes. Haemoglobin is purified and encapsulated in a lipid

mixture with phospholipids, cholesterol and α -tocopherol. The liposome vesicles measure 0.1-0.3 μ m in diameter. They have been shown to have a longer intravascular half life than other HBOCs and perfluorocarbons (45), less vasopressor effect (46), and less reperfusion injury by encapsulating other enzymes (47).

A study of haemoglobin containing vesicles placed in human albumin solution infused into hamsters which have undergone 80% blood volume exchange demonstrated microcirculatory blood flow and tissue oxygenation to be maintained (48). A further study demonstrated the haemoglobin vesicles are better in Dextran 70 than human serum albumin (49). This product still needs to be defined and large scale production may prove difficult and costly (18).

Allosteric modifier (RSR13)

RSR13 modifies haemoglobin oxygen affinity by mimicking 2,3-DPG and shifting oxygen dissociation curve to the right promoting tissue oxygenation by enhanced off-loading of oxygen to the tissues (50). RSR13 was initially developed to enhance tumour oxygenation to bring about radio-sensitisation (51). It was thought by beneficial by enhancing oxygen off-loading and thus avoiding allogenic red blood cell transfusion. However in a rat model, where the rodents were progressively haemodiluted, tolerance of anaemia was not improved despite the anticipated shift in oxygen affinity (52). This will not therefore be beneficial to reduce transfusion in humans.

Stem Cell Haemoglobin

Giarratana *et al* developed a large *ex-vivo* production of mature human RBC using haemopoietic stem cells (53). They have the same haemoglobin content, morphology, and life span as normal red blood cells. Stem cells are expected to be prohibitively expensive per unit due to the complex production involved.

Dendrimers

The Dendritech Corporation are developing nano-polymers known as dendrimers to be used as blood substitutes (54). Vögtle first described dendrimers in 1978. Dendrimers are polymers in which the atoms are arranged in branches and sub-branches with a central backbone of carbon atoms. They are entirely artificial. Dendrimers have been used in drug delivery systems and in non-medical practice such as inkjet ink and toners. They are currently being developed for gene therapy, contrast agents for MRI, and dendrimer-antibody conjugates in immunoassay for MI by Dendritech (54). The use of dendrimers as blood substitutes is still in the very early stages of research. The dendrimers solution will consist of the dendrimer polymer in an aqueous carrier, which is able to transport oxygen dissolved in the solution. Dendritech believe they can produce a very cost effective product as they can be produced in bulk quantities with pre-established manufacturing techniques and abundant supply of raw materials, unlike the HBOCs, which rely on out dated donor blood or specially reared disease-free bovine haemoglobin for their raw product (55). In 2005 Dendritech was awarded a two year \$275,000 US Army grant towards the further development of dendrimers as blood substitutes, which has aided their continued development (55).

Conclusions

Many artificial oxygen carriers have failed to demonstrate efficacy and safety and have thus either been withdrawn from the market or failed to get there in the first place. Other products are still in the early stages of development and may offer promise with future clinical development. The most promising products in the domain at present appear to be the haemoglobin-based oxygen carriers of Polyheme and

Hemopure. The safety and efficacy of both these products has been demonstrated by clinical trials, and Hemopure has been licensed for commercial use in South Africa. At present, there are no randomised controlled trials comparing the different products directly. Furthermore, there is no evidence on long-term follow up after prescription of the products, effects of long-term use, or repeated use. Currently the Hemopure dose is limited by the manufactures to 7 units and coupled with its short half-life, making its application for abolishing allogenic red blood cells transfusion entirely limited, but extremely important as an off-the-shelf, ready to use, universally compatible, life sustaining product when blood is not available. The outcome of the large Polyheme pre-hospital trauma trial and the planned Hemopure RESUS trial may well prove to be key studies in the manufacture of a widely available, clinically employable artificial oxygen carrier.

References

1. <http://www.blood.co.uk/pages/stocklevel.html>. 2007.
2. Stanworth S, Cockburn H, Boralessa H, Contreras M. Which groups of patients are transfused? A study of red cell usage in London and south east England. *Vox Sang* 2002;**83**:352-7.
3. Vamvakas EC. Epidemiology of red cell utilisation. *Transfus Med Rev* 1996;**10**:44-61.
4. Mendelson J. The use of whole blood and blood volume expanders in the US military facilities in Vietnam 1966-71. *J Trauma* 1975;**15**:1-13.
5. Bowersox JC, Hess JR. Trauma and military applications of blood substitutes. *Artificial Cells Blood Substitutes Immobil Biotech* 1994;**22**:145-57.
6. Hess JR. Blood use in war and disaster: the US experience. *Scand J Trauma Emerg Med* 2005;**13**:74-81.
7. Johnson J, Moore EE, Offner P. Resuscitation with blood substitute abrogates pathological post injury neutrophil cytotoxic function. *J Trauma* 2001;**50**:449-55.
8. Johnson J, Moore E, Gonzales R, Fedel N, Partrick D, Silliman C. *J Trauma* 2003;**54**:133-9.
9. Hughes G, Fracone S, Antal E, *et al*. Haematological effects of a novel haemoglobin based carrier in normal male and female subjects. *J Lab Clin Med* 1995;**126**:444-51.
10. Hughes, Antal, Locker, Francom, Adams. *Crit Care Med* 1996;**24**:756-64.
11. Mulder *et al*. Oxygen consumption with haemoglobin ringer. *J Cell Comp Physiol* 1934;**5**:383-97.
12. Amberson *et al*. Clinical experience with haemoglobin-saline solutions. *J Appl Physiol* 1949;**1**:469-89.
13. Savitsky. A clinical safety trial of stroma free haemoglobins. *Clin Pharmacol Ther* 1978;**73**:80.
14. Tremper K. Perflurochemical: "Red Blood Cell Substitutes." The continued search for an indication. *Anaesthesiology* 2002;**97**:1333-4.
15. Gould SA, Rosen AL, Sehgal LR *et al*. Fluosol-DA as a red cell substitute in acute anaemia. *N Engl J Med* 1986;**314**:1653-6.
16. Faithfull NS. Fluorocarbons. Current status and future application. *Anaesthesia* 1987;**42**:234-242.
17. Spahn D, Waschke K, Standl T *et al*. Use of perflubron emulsion to decrease allogenic blood transfusion in high-blood-loss non-cardiac surgery. *Anaesthesiology* 2002;**97**:1338-45.
18. Spahn D KR. Artificial O₂ Carriers: Status in 2005. *Current Pharmaceutical Design* 2005;**11**:4099-114.
19. Mackenzie C BC. Artificial oxygen carriers for trauma: myth or reality? *Hosp Med* 2004;**65**:582-8.
20. Xu L, Rollwagen FM, Li Y *et al*. Cellular responses to surgical trauma, haemorrhage, and resuscitation with diaspirin cross-linked hemoglobin in rats. *J Trauma* 1997;**42**:32-41.
21. Habler O, Kleen M, Pape A, Meisner F, Kemming G, Messmer K. Diaspirin cross-linked hemoglobin reduces mortality of severe haemorrhagic shock in pigs with critical coronary stenosis. *Crit Care Med* 2000;**28**:1889-98.
22. Schubert A, Przybelski RJ, Eidt JF *et al*. Diaspirin cross-linked hemoglobin reduces blood transfusion in non-cardiac surgery: a multi-centre, randomised, double-blinded trial. *Anaesth Analg* 2003;**97**:332.
23. Sloan E, Koenigsberg M, Gens D *et al*. Diaspirin cross-linked hemoglobin (DCLHb) in the treatment of severe traumatic hemorrhagic shock. A randomised controlled efficacy trial. *JAMA* 1999;**282**:1857-64.
24. Saxena R WACHeal. Controlled safety study of haemoglobin-based oxygen carrier, DCLHb in acute ischaemic stroke. *Stroke* 1999;**30**:993-6.
25. Kerner T, Alhers O, Veit S, Riou B, Sanders . DCLHb for trauma patients with severe haemorrhagic shock: The European "On-Scene" Multicentre Study. *Intensive Care Med* 2003;**29**:378-85.
26. Rosen AL, Gould SA, Sehgal LR *et al*. Effect of haemoglobin solution on compensation to anaemia in erythrocyte primate. *J Appl Physiol* 1990;**68**:938-43.

27. Jahr JS, Stewart LM, MacKenzie C, Bourke D, Williams JP. Pivotal phase III study: Safety of polymerised bovine haemoglobin as compared to rbc in patients undergoing orthopaedic surgery. *Anaesthesiology* 2002;**96**:A243.
28. Levy J, Goodnough L, Greilich P *et al*. Polymerised bovine hemoglobin solution as a replacement for allogenic red blood cell transfusion after cardiac surgery: Results of a randomised, double-blind trial. *J Thorac Cardiovas Surg* 2002;**124**:35-42.
29. <http://www.northfieldlabs/polyheme>. 2006.
30. Kipnis K, King NMP, Nelson RM. Considering Northfields Laboratories' Polyheme Trial. *Am J Bioethics* 2006;**6**:1-4.
31. <http://phx.corporate-ir.net/phoenix.zhtml?c=91374&p=irolnewsArticle&ID=824752&highlight=>. 2007.
32. Gould SA, Moore EE, Hoyt DB. The life-sustaining capacity of human polymerised haemoglobin when red cells may not be available. *J Am Coll Surg* 2002;**195**:445-55.
33. Raff J, Dobson C, Tsai H. Transfusion of polymerised human hemoglobin in a patient with severe sickle cell anaemia. *Lancet* 2002;**360**:464-5.
34. <http://www.biopure.com>. 2006.
35. Cothren C *et al*. Blood substitute and erythropoietin therapy in a severely injured Jehovah's Witness. *N Engl J Med* 2002;**346**:1097-8.
36. Levien LJ. South Africa: clinical experience with Hemopure. *ISBT Science Series* 2006;**1**:167-73.
37. Looker D, Abbott-Brown D, Cozart P *et al*. A human recombinant hemoglobin designed for use as a blood substitute. *Nature* 1992;**356**:258-60.
38. Malhotra AK, Kelly ME, Miller PR, Hartman JC, Fabian TC, Proctor KG. Resus with a novel HBOC in Swine model of uncontrolled peri-operative haemorrhage. *J Trauma* 2003;**54**:913-24.
39. Lou Carmichael F, Ali A, Campbell J *et al*. A phase I study of oxidized raffinose cross-linked human hemoglobin. *Crit Care Med* 2000;**28**:2283-92.
40. Cheng DC, Mazer CD, Martineau R *et al*. A phase II dose-response study of hemoglobin raffimer (Hemolink) in elective coronary artery bypass surgery. *J Thorac Cardiovasc Surg* 2004;**127**:79-86.
41. Greenberg A, Kim H. Use of an oxygen therapeutic as an adjunct to intraoperative autologous donation to reduce transfusion requirements in patients undergoing coronary artery bypass graft surgery. *J Am Coll Surg* 2004;**198**:373-83.
42. Rao SV, Jollis JG, Harrington RA *et al*. Relationship of blood transfusion and clinical outcomes in patients with acute coronary syndromes. *JAMA* 2004;**292**:1555-62.
43. Vandergriff KD, Malavalli A, Wooldridge J, Lohman J, Winslow RM. MP4, a new nonvasoactive PEG-IIB conjugate. *Transfusion* 2003;**43**:509-16.
44. Wettstein R, Tsai AG, Erni D, Winslow R, Intaglietta M. Resuscitation with polyethylene glycol-modified human hemoglobin improves microcirculatory blood flow and tissue oxygenation after hemorrhagic shock in awake hamsters. *Crit Care Med* 2003;**31**:1824-30.
45. Phillips WT, Klipper RW, Awasthi VD *et al*. Polyethylene glycol-modified liposome-encapsulated haemoglobin: a long circulating red cell substitute. *J Pharmacol Exp Ther* 1999;**288**:665-70.
46. Rabinovici R, Rudolph As, Vernick J, Feuerstein G. Lyophilised liposome encapsulated haemoglobin: evaluation of haemodynamic, biochemical and haematological response. *Crit Care Med* 1994;**22**:480-5.
47. Chang TM. Future generations of red blood cell substitutes. *J Int Med* 2003;**253**:527-35.
48. Sakai H, Tsai AG, Rohlfis RJ *et al*. Microvascular responses to haemodilution with Hb vesicles as red blood cell substitutes: influence of O₂ affinity. *Am J Physiol* 1999;**276**:H553-H562.
49. Erni D, Wettstein R, Schramm S *et al*. Normovolaemic hemodilution with Hb vesicle solution attenuates hypoxia in ischaemic hamster flap tissue. *Am J Physiol Heart Circ Physiol* 2003;**284**:H1702-H1709.
50. Pagel PS, Hettrick DA, Montgomery MW, Kersten JR, Steffen RP, Warltier DC. RSR13, a synthetic modifier of haemoglobin-oxygen affinity, enhances recovery of stunned myocardium in anaesthetised dogs. *J Pharmacol Exp Ther* 1998;**285**:8.
51. Shaw E, Scott C, Suh J *et al*. RSR13 plus cranial radiation therapy in patients with brain metastases: comparison with the Radiation Therapy Oncology Group Recursive Partitioning Analysis Brain Metastases Database. *J Clin Oncol* 2003;**21**:2364-71.
52. Eichelbronner O, D'Almeida M, Sielenkamper A, Sibbald WJ, Chin-Yee IH. Increasing P(50) does not improve DO₂ CRIT or systemic VO₂ in severe anaemia. *Am J Physiol Heart Circ Physiol* 2002;**283**:H92-H101.
53. Giarratana MC, Kobari L, Lapillonne H *et al*. Ex vivo generation of fully mature human blood cells from hematopoietic stem cells. *Nat Biotechnol* 2005;**23**:69-74.
54. <http://www.dendritech.com/applications.html>. 2007.
55. <http://www.dendritech.com/news5.html>. 2007.