August 2007

ARTIFICIAL BLOOD

HEALTH TECHNOLOGY ASSESSMENT UNIT

MEDICAL DEVELOPMENT DIVISION

MINISTRY OF HEALTH MALAYSIA

008/07

Prepared by:

Dr Izzuna Mudla bt Mohamed Ghazali
Principal Assistant Director
Health Technology Assessment Unit
Ministry of Health Malaysia
August 2007

Dr Mohd Aminuddin bin Mohd Yusof
Principal Assistant Director
Health Technology Assessment Unit
Ministry of Health Malaysia
August 2007

Reviewed by:
Datin Dr Rugayah bt Bakri
Deputy Director
Health Technology Assessment Unit
Ministry of Health Malaysia
August 2007

ARTIFICIAL BLOOD

1. INTRODUCTION

Artificial blood or blood substitutes are solutions intended to replace transfusion of banked red blood cells. Alternatives to red blood cell transfusions are designed to overcome known limitations; short supply of donor blood, risk from contamination and clerical error and the requirement for cross-matching.[i,ii] The risks of allogenic blood transfusions are multiple and include infectious transmission, delayed postoperative wound healing, transfusion reactions, transfusion related acute lung injury, immunomodulation and potential risk of cancer recurrence.[iii]

The term artificial blood or blood substitutes is not accurate since human blood performs many important functions. The preferred and more accurate terms are volume expanders for inert products (crystalloid-based or colloid-based) and oxygen therapeutics for oxygen-carrying products.[iv] Although volume expanders are well-established in its use, oxygen therapeutics are still in clinical trials. Oxygen therapeutics can be further classified into two categories based on the transport mechanism. They are namely perfluorocarbon based and hemoglobin based.[v]

Efforts to produce artificial blood started over a century ago. Since then many types of artificial blood products have been produced. Based on the time of production, the artificial blood products can be divided into the first generation products and the second or next generation products.[vi] The first generation products include polymerized Hb, intramolecular cross-linked Hb, perfluorocarbons emulsions and lipid vesicles encapsulated Hb. Among the second generation products, Sangart's Hemospan® is currently in clinical trials.[vi]

This technology review was requested by the Deputy Director General of Health (Medical) as a response to a newspaper article on proposal to set up a facility in Malaysia for artificial blood production.[vii] This review focused on oxygen therapeutics or oxygen carriers and did not include volume expanders.

2. OBJECTIVES

To determine the safety, effectiveness and cost-effectiveness of oxygen therapeutics in substituting true blood for transfusion.

3. TECHNICAL FEATURES

Depending on the type of the artificial blood, it can be produced in different ways using synthetic production, chemical isolation or recombinant biochemical technology. The ideal product has the following characteristics[iii]:

- Safe to use and compatible within the human body i.e. different blood types should not matter when it is used. It should be free from all disease-causing agents.
- Must be able to transport oxygen throughout the body. Effective oxygen delivery is dependent on the ability to load oxygen in the pulmonary capillary bed, transport the oxygen in the circulation to the tissues, unload oxygen at tissue oxygen tensions and permit the diffusion of the off-loaded oxygen into the tissue.
- Must be shelf-stable (stored for over the year or more).
- Does not increase arterial and pulmonary blood pressure
- Sufficient half-life in the circulation
- Does not form methemoglobin, activate complement, increase white blood cell count, reacts with plasma substitutes or platelets
- Absence of renal toxicity
- Immediate availability
- Easy to administer
- Does not overload reticuloendothelial system
- Does not cause oxidation and free radical formation

The development of artificial blood can be tracked in five cycles which started in the second half of the 19th century.[viii] The first cycle occurred in the later half of the 19th century which led to the development of balanced salt solutions. The second cycle followed the recognition of colloid osmotic activity. Haemoglobin-based oxygen carriers are the third cycle and perfluorocarbon oxygen carriers are the fourth cycle. The last cycle is platelet substitutes. The technical features of perfluorocarbons and hemoglobin based oxygen carriers (HBOC) will be discussed further.

3.1 PERFLUOROCARBON / PFC EMULSIONS

These products are based on perfluorocarbons materials (biologically inert materials) that have 50 times higher solubility of oxygen compared with blood plasma.[ix] These PFC are hydrocarbon in which all of the hydrogen atoms have been replaced by flourine. PFCs have the following advantages: they do not react with oxygen or other gases, increase oxygen solubility in the plasma compartment, the dissolved oxygen is not subject to the effects of temperature, pH, 2,3-DPG amongst others, thus the oxygen dissociation curve is linear and facilitate the effortless transfer of oxygen from the red cells to the tissue.[ix] As PFC does not bind oxygen in a cooperative manner as haemoglobin, 100% oxygen is necessary for its effectiveness.[v]

PFC are relatively inexpensive to produce and can be made devoid of any biological materials.[vi] These PFCs compound are not miscible in water and hence cannot transport water soluble metabolites or waste products in the circulation which may limit their ability to sustain life over long periods.[x] For the same reason, they must be combined with emulsifiers prior to intravenous use. The reticuloendothelial system is responsible for the systemic removal of perfluorocarbons that are finally exhaled via the alveolar surfaces in the lungs which result in a short dose dependent circulatory half-life.[x]

The specific PFC usually used is perflubron. PFC particles are about 40 times smaller than the diameter of red blood cell (RBC) and enable it to traverse capillaries through which no RBC is flowing.[v]

3.1.1 First generation perfluorocarbons:

The first (and only) approved PFC by the FDA called Fluosol-DA-20 was manufactured in Japan.[3,4] This used Pluronic F-68, as an emulsifying agent and was able to maintain a balance between the oxygen carrying capacity and tissue retention. It comprised two PFCs, perfluorodecalin (PFD) and perfluorotripropylamine (FTPA). PFD was the primary component and oxygen carrier whereas FTPA was to provide the much needed stability. Each of the two components had different half lives, with PFD being only 3 to 6 hours due to its rapid clearance. FTPA on the other hand, persisted in the tissue.[ix]

3.1.2 Second generation perfluorocarbons:

The desirable characteristics in the second generation PFC included large oxygen-dissolving capacity, faster excretion and less tissue retention, lack of significant side effects, increasing purity and large scale production and availability.[ix] Three PFCs of choice were PFD, perfluorooctyl bromide (PFOB) and bis (perfluorobutyl) ethylene.
The most promising of the PFC emulsions was Oxygent, an emulsion of Perflubron develop by Alliance Pharmaceutical Corporation. Variations on the PFC emulsion formula have been developed in Russia (Perftoran®) and by a US company named Synthethic Blood International (Costa Mesa, CA). Currently, Perftoran is in clinical use in Russia and has recently been approved in Mexico.[vi]

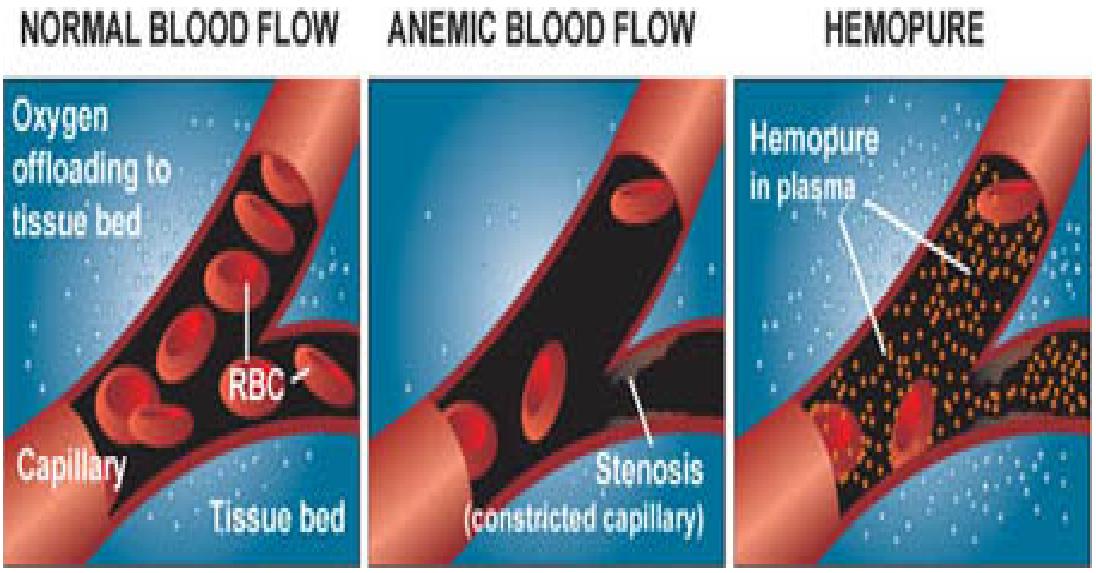
3.2 HAEMOGLOBIN-BASED OXYGEN CARRIERS (HBOC)

Hemoglobin-based oxygen carriers can be divided into two broad categories; purified hemoglobin solutions and modified hemoglobin. Modified hemoglobin can be further categorized as polymerized hemoglobin, intramolecular cross-linked hemoglobin, recombinant hemoglobin and hemoglobin vesicles.[ix]

The US Army had studied haemoglobin solutions in World War II but these were rejected due to the hypertension-induced and short time of its presence in the blood veseel.[4] The toxicities were thought due to stroma, nonhaemoglobin elements of erythrocytes. Later, unmodified cell-free haemoglobin appeared to cause renal toxicity in humans. This led to haemoglobin modification which is now the basis for the development of the present generation of potential haemoglobin-based RBC substitutes. It has five goals: (1) to decrease oxygen affinity, (2) to prolong intravascular retention, (3) to decrease colloid osmotic activity, (4) to prevent renal toxicity and (5) to develop defined products for research.

These haemoglobin products are different from the whole blood in that they are not contained in a membrane (Figure 1).[2] This eliminates the problem of blood typing. However, raw haemoglobin cannot be used because it would break down into toxic compounds.[2.3] Another problem is the stability of haemoglobin in a solution. Short half-life alone would restrict the utility of haemoglobin solutions to emergency situations and a disadvantage for surgical patients whose metabolic requirements are greatest during recovery post-surgery.[4]

Figure 1. Illustration of Hemopure, a polymerized HB in blood vessels.



The evolution of hemoglobin-based oxygen carriers is shown in Table 1.

Table 1. Evolution of Hemoglobin-Based Oxygen Carriers[1]

I. HEMOLYSATES
Bovine hemolysates into dogs/cats: preservation of neurologic function, maintenance of oxygen consumption (1933 Amberson et al, Science)
Human hemolysates into patients: transport O_2, observed "pressor effect" (1949 Amberson et al, J Appl Physiol)
II. MODIFIED HEMOLYSATES
Human filtered hemolysates into humans: renal dysfunction (1951 Miller et al, J Clin Invest)
III. TETRAMERIC HEMOGLOBIN
Human Hb tetramer into dogs: no renal dysfunction (1967 Rabiner et al, J Exp Med)
Human Hb tetramer into humans: renal toxicity, hypertension, abdominal pain (1978 Savitsky et al, Clin Phamacol Ther)
IV. MODIFIED TETRAMERIC HEMOGLOBIN
Human modified Hb into animals: improved survival as low-volume resuscitation agent, but concern for systemic and pulmonary vasoconstriction (1993 Hess et al, J Appl Physiol)
V. POLYMERIZED HEMOGLOBIN
Human glutaraldehyde polymerized hemoglobin (1984 Gould et al, Surgery)
Bovine glutaraldehyde polymerized hemoglobin (1989 Vlahakes et al, Eur J Cardiovasc Surg)
Human o-raffinose polymerized hemoglobin (2000 Carmichael et al, Crit Care Med)

There are considerable differences between various haemoglobin-based products which include different oxygen affinities (P_{50}), haemoglobin concentrations and stabilities. Haemoglobin-based products can use either isolated haemoglobin or synthetically produced haemoglobin.[2] The isolation is typically obtained from donated human blood that has expired before being used. Synthetic haemoglobin-based products are produced from haemoglobin harvested from *E*.coli bacteria strain.[2] Example of haemoglobin-based products is the commercially commercial available polymerized bovine haemoglobin, Hemopure, which has molecules that can be about 1000 times smaller than RBC.

[1]Adapted from Moore EE. Blood substitutes: The future is now. Journal of American College of Surgeons. 2003;196(1): pg 3.

The characteristics of polymerized Hemoglobin solutions is shown in Table 2 with comparison of stored red blood cells.

Table 2. Characteristics of Polymerized Hemoglobin Solutions Versus Stored Red Blood Cells[2]

Characteristic	Hemopure	Hemolink	PolyHeme	RBCs
Hemoglobin (g%)	13 g%	10 g%	10 g%	13 g%
Unit equivalent (g)	30 g	25 g	50 g	50 g
Molecular weight (> 64 KDa)	_95%	_65%	_99%	100%
P_{50} (mmHg)	38	34	29	26
Hill coefficient	1.4	1.0	1.7	2.7
Oncotic pressure (mmHg)	25	24	23	25
Viscosity	1.3 cp	1.1 cp	2.1 cp	(whole blood = 5–10 cp)
Methemoglobin (%)	_10	_7	_8	_1
Half-life	19 h	18 h	24 h	31 d
Shelf-life @ 4° C	≥3y	≥1y	≥1.5 y	42 d
Shelf-life @ 21° C	≥2y	–	≥6wk	<6h

cp = centipoises , P_{50} = O2 tension when hemoglobin-binding sites are 50% saturated

[2]Adapted from Moore EE. Blood substitutes: The future is now. Journal of American College of Surgeons. 2003;196(1): pg 8.

4. METHODOLOGY

4.1 SEARCH METHODS

Literature were searched through electronic databases, which included Pubmed, OVID, Proquest, Ebscohost, EBM Reviews for controlled trials, Cochrane database on systematic review, Cochrane Clinical Trial Registry, Science Direct, Springer Link, and general databases such as Google and Yahoo.

The search strategy used the terms, which are either used singly or in various combinations: "artificial blood" OR "blood substitutes" OR Perfluorocarbons OR "oxygen therapeutics" OR "artificial oxygen carriers" OR "hemoglobin based oxygen carriers", effectiveness OR efficacy, safety OR safe OR "adverse effect*" OR "harm* effect*" OR toxicity, "cost effectiveness" OR "cost analysis" OR econom*. There were no limitations in the search.

4.2 SELECTION OF STUDIES INCLUDED/EXCLUDED

All primary papers, systematic reviews or meta analysis pertaining to safety, effectiveness and cost effectiveness on oxygen therapeutics or oxygen carriers in human were included in this study.
A critical appraisal of all relevant literature was performed and the evidence level graded according to the modified Catalonian Agency of Health Technology Assessment (CAHTA) scale.

5. RESULTS AND DISCUSSION

There were not many original papers published on human trials of artificial blood products. Most of the papers published were on development, experimental (laboratories) studies and narrative review. The current status of artificial blood products is shown in Table 1.

Table 3. Artificial blood products and status as of 2006[3]

Product class	Product	Company	Technology	Status
Perfluorocarbons	Oxygent	Alliance	PFC Emulsion	On clinical hold; safety (stroke)
	Oxycyte	Synthetic Blood	PFC Emulsions	Entering Phase II; suspended earlier Phase II
	Oxyfluor	HemaGen	PFC Emulsions	Discontinued; safety
Cross-linked Hb	HemAssist	Baxter		Discontinued; safety
	(ααHb, DCIHb)	US Army		Discontinued; safety
	rHb 1-1	Somatogen	Recombinant Hb	Discontinued; safety (hypertension)
	rHb 2-0	Baxter	Recombinant Hb	Discontinued; safety
Polymerized Hb	Polyheme	Northfield Laboratories	Glutaraldehyde, pyridoxal Hb	Phase III (enrolling)
	HBOC-201 (Hemopure)	Biopure	Glutaraldehyde, bovine Hb	US phase II on clinical hold
	Hemolink	Hemosol	Polymerized Hb	Discontinued; safety (myocardial infarction)
Conjugated Hb	PHP	Apex bioscience	PEG-human Hb	Phase III septic shock
	PEG-hemoglobin	Enzon	PEG-bovine Hb	Discontinued
	Hemospan	Sangart	PEG-human Hb	Entering Phase III

Note:
Hb – haemoglobin
PEG - polyethylene glycol
PFC - perfluorocarbons

[3] Adapted from Winslow RM. Current status of oxygen carriers. Vox Sanguinis. 2006;91:102-110.

5.1 SAFETY

Safety is the main concern related to oxygen therapeutics. As shown in Table 3, most of the oxygen therapeutics products were discontinued due to safety reasons. The only perfluorocarbons (PFC) emulsion approved by FDA for clinical use was Fluosol-DA®. It was indicated for percutaneous transluminal coronary angioplasty (PTCA). However it was withdrawn in 1994 because it is cumbersome and the efficacy was marginal.[vi]

Oxygent®, the second generation perflurocarbons has undergone early clinical and preclinical trials. In the first phase trial among healthy adults, the prevalent of adverse events were higher among the treated group.[xi] Level 3 The clinical trials proceeded to Phase 3, but showed an increase incidence of stroke in treated patients compared with control.[vi]

A variation on the PFC emulsion (e.g Perftoran®) has been developed in Russia and by a US company. Perftoran® is in clinical use in Russia and has recently been approved in Mexico.[vi] None of the HBOC has been approved for clinical use in USA. As shown in Table 3, all the cross-linked Hb was discontinued due to safety reasons. In a multicentre, randomized, single-blind study, infusion of low dose of diaspirin cross-linked hemoglobin (DCLHb) over 3 days adversely affected outcome in acute ischaemic stroke patients. [xii] Level 2 Another RCT conducted among severe traumatic hemorrhagic stroke showed higher mortality for patients treated with DCLHb.[xiii] Level 2

PolyHeme a human polymerized hemoglobin has gone through Phase II trial with no adverse event reported.[xiv] Level 3 A cohort study with historical comparison was published in 2002 showed that PolyHeme increased survival at life threatening RBC Hb level in massive blood loss in the absence of red cell transfusion. Safety issues were not discussed in the paper.[xv] Level 7 In December 2003 PolyHeme began field tests in Phase III trial on emergency patients in USA and is yet to be approved by Federal Drug Administration (FDA).[3]

Hemopure is Biopure's brand name for hemoglobin glutamer-250 (bovine), an oxygen therapeutic derived from cow's blood that is designed to deliver oxygen to tissues as a substitute for red blood cells. In a multicentre, randomized single–blinded trial among 81 patients going for elective operation, serious adverse events were reported which include mast cell degranulation and cardiac complications.[xvi] Level 2 Another RCT double blind study also showed major adverse events and increased in mean arterial blood pressure in patients receiving Hemopure.[xvii] Level 2 Biopure had submitted Biologic License Application (BLA) to FDA in 2002 to use Hemopure in the treatment of acutely anemic patients undergoing orthopedic surgery and an IND application in 2003 to conduct clinical trials of Hemopure on human trauma victims in hospitals.[22] However FDA has imposed clinical hold barring the company from initiating any clinical trials in connection with the trauma IND because of safety concerns arising out of data relating to the BLA clinical trials.[22xxii] However, Hemopure has been registered for routine clinical use in South Africa for the treatment of

acute adult surgical anaemia in 2001.[iii] A non controlled clinical series among 336 adult patients who received Hemopure in South Africa reported that 5% of the patients had increased in blood pressure more than 30 mmHg, 6 cases had cardiac complications, 5 cases had deterioration of renal function and 3 patients died of uncompensated anemia.[xviii] Level 8

Sangart's Hemospan® an oxygen –carrying plasma expander, intended to promote tissue perfusion and oxygenation is currently in clinical trials in Europe and USA. Hemospan® has completed Phase I testing, a Phase II clinical trial in elective orthopedic surgery patients. The studies revealed that the patients who received up to 100 ml Hemospan® did not experienced hypertension or gastrointestinal pain. A Phase III trial has been proposed for a similar population of elective surgery patients to begin in mid-2006.[xix]

Based on the literatures retrieved, there were sufficient evidences to caution regarding the safety of oxygen therapeutics products. Further research is needed to develop safe products for human usage and also to determine the safety of the products.

5.2 EFFICACY/EFFECTIVENESS

As shown in Table 3, none of the oxygen therapeutics has been approved for clinical use except for Fluosol-DA, but it was eventually removed from the market. Very few products have gone through Phase III trial to assess the efficacy.

Artificial blood products are produced to replace the transfusion of banked red blood cells. Efficacy studies on these products should be able to show superiority of the products such as in reducing the occurrence of organ failure. Efficacy study on DCLHb showed no significant effect on the occurrence of organ failure.[xx] Level 2 Clinical case series on South Africa experience with Hemopure showed that 88% of the patients were able to achieved blood exclusion, however the report revealed that there were occurrence of adverse events, limited usage of the products and training is crucial.[xviii] Level 8 In a RCT to evaluate the ability of HBOC-201 to substitute for RBC transfusion in the treatment of moderate anemia resulting from blood loss and hemodilution after cardiac surgery revealed that 34% cases achieved blood exclusion however safety issues should also be concerned.[xvii] Level 2

Phase III clinical trials on PolyHeme and Hemospan is still ongoing and results are yet to be published.[xxi,xix] In conclusion, there were insufficient evidence on the efficacy of oxygen therapeutics what else the effectiveness of the products. Further research is needed to ensure the effectiveness of these products.

5.3 COST-EFFECTIVENESS

There was no literature retrieved on cost effectiveness of oxygen therapeutics. However, serious investments were made by industry, the military, and National Institutes of Health (NIH)-sponsored researchers to develop artificial blood. USD 2.2 billion were spent (quoted by Professor John Hess, Professor of Pathology and Medicine, University of Maryland, WHO Expert Advisory Panel Member) but in spite of all the efforts, none of these products is currently approved for used in North America or Europe.

5.4 ETHICAL AND LEGAL IMPLICATIONS

There were legal issues that need to be taken into consideration in evaluating oxygen therapeutics. The US Securities and Exchange Commission had filed lawsuits against Biopure Corporation, the developer of Hemopure, its former Vice Chairman of Board of Directors and Senior Technology Officer of Biopure Corporation of Cambridge, Carl Rausch and other three top executives for misleading public statements about the company's effort to obtain FDA approval for its primary product , a synthetic blood product while at the same time Biopure was raising millions of dollars from investors in September 2005.[xxii] The final judgement has been released in September 2006[xxiii] and February 2007.[xxiv] Further in depth evaluation on legal implications by legal expertise is recommended.

Other than that ethical issues such as informed consent of the recipient should be taken into consideration since oxygen therapeutics usually given during acute severe hemorrhagic condition.

6. CONCLUSION

Based on the literatures retrieved, oxygen therapeutics is still in experimental stage. None of the products has been approved by FDA for clinical use in US except for Fluosal-DA. There were sufficient evidences on the harmful effect of oxygen therapeutics products. More research is needed to develop a safe products for human use.

Artificial blood may be useful in areas where there is **shortage in blood supply** or where safe blood is scarce as in South Africa where 40% of the population has HIV/AIDS, thus disease free blood for blood transfusion is difficult. Artificial blood may also be beneficial in battlefield scenarios, where it is often impossible to administer rapid blood transfusion. However 100,000 units of universal blood transfusion given during the Vietnam war were without any fatal transfusion reaction.[ii]

It is postulated that great benefit would be derived from rapid treatment in trauma situations using artificial blood. These blood substitutes do not contain any antigens that determine

blood type thus they can be use across all types without immunologic reactions. However serious adverse events have been reported.

7. RECOMMENDATION

There is insufficient evidence to support the use of artificial blood. Some are used as under "investigational" use only, namely trial stage, while many others are discontinued. None of the products have shown to be effective outweighing the harmful effects despite billion dollars spent for development and research. Hence, we could not support the proposal to set up a facility to produce artificial blood in Malaysia

In Malaysia, there is no great need for use of artificial blood since there is no serious shortage of blood supply. In 2001, out of 441788 units of blood collected, 297094 units were transfused.[xxv] Although blood transfusion is not without risk, it is very safe with the current strategies. If more or safer natural red cells are needed, then, we need to develop the social and technical capacity to supply them since this is a cheaper option. The current evidence of toxicity and limited indication of artificial blood prevent its use in clinical settings. It is anticipated that the cost to establish a facility to manufacture these products would be very high. In developing country like Malaysia, cost is sensitive since the need is greatest in other areas of health care services.

8. ACKNOWLEDGEMENT

This review is prepared in consultation with Dato' Dr. Yasmin Ayob and the Health Technology Assessment Unit would like to express its appreciation to her contribution.

9. REFERENCES

[i]. Winslow RM. Blood substitutes. Current opinion in hematology. 2002;9:146-151.

[ii]. Hess JR, Reiss RF. Resuscitation and the limited utility of the present generation of blood substitutes. Transfusion Medicine Reviews. 1996;X(4):276-285.

[iii]. Jahr JS, Walker V, Manoochehri K. Blood substitutes as pharmacotherapies in clinical practice. Current opinion in anaesthesiology. 2007;20:325-330.

[iv] . Schubert A, Hemoglobin-based oxygen carriers, blood substitutes, and their relevance to high-volume blood loss. Seminars in Anaesthesia, Perioperative Medicine and pain, 2001;20(1):78-84.

[v]. Blood substitutes. Wikipedia (website). Retrieved from http://www.en.wikipedia.org/wiki/Blood_substitutes on 4 June 2007

[vi]. Winslow RM. Current status of oxygen carriers ('blood substitutes'):2006. Vox Sanguinis, 2006;91:102-110.

[vii]. Bernama. Malaysia may become world's largest producer of artificial blood. The New Straits Time (online). 2007;July 2007 . retrieved from http://www.nst.com.my/Current_news/NST?Tuesday/Newsbreak/20070703152211/article/pppul l_index on 6 July 2007

[viii] . Hess JR. Blood substitutes. Seminars in Hematology. 1996; 33(4): 369-378.

[ix]. Goorha YK, Deb P, Chatterjee T et al. Artificial blood. MJAFI, 2003; 59: 45-50.

[x]. Inayat MS, Bernard AC, Gallichio VS et al. Oxygen carriers: A selected review. Transfusion and Apheresis Science, 2006;34:25-32.

[xi] . Noveck RJ, Shannon EJ, Leese PT et al. Randomized Safety Studies of Intravenous Perflubron Emulsion. II. Effects on Immune function in Healthy Volunteers. Anesth Analg, 2000;91:812-822

[xii] . Saxena R, Wijnhoud AD, Carton H et al. Controlled safety study of a hemoglobin-based oxygen carrier, DCLHb in acute ischaemic Stroke. Stroke, 1999;30:993-996.

[xiii] . Sloan EP, Koenigsberg M, Gens D et al. Diaspirin Cross-linked hemoglobin (DCLHb) in the treatment of severe traumatic hemorrhagic shock: a randomized controlled efficacy trial. JAMA, 1999:282(19): 1857-1864.

[xiv]. Gould SA, Moore EE, Hoyt DB et al. The first randomized trial of human Polymerized Hemoglobin as a blood substitute in acute trauma and emergent surgery. J Am College of Surgeons, 1998;187(2):113-120

[xv]. Gould SA, Moore EE, Hoyt DB et al. The Life-sustaining capacity of human Polymerized Hemoglobin when red cells might be unavailable. J Am College of Surgeons, 2002;195(4): 445-455.

[xvi] . Sprung J, Kindscher JD, Wahr JA et al. The use of bovine hemoglobin Glutamer-250 (Hemopure®) in surgical patients: results of a multicenter, randomized, single-blinded trial. Anest Analg, 2002;94 :799-808.

[xvii]. Levy JH, Goodnough LT, Greilich PE et al. Polymerized bovine hemoglobin solution as a replacement for allogeneic red blood cell transfusion after cardiac surgery: Results of a randomized, double-blind trial. J Thorac cardiovasc Surg, 2002; 124: 35-42.

[xviii]. Levien LJ. South Africa: clinical experience with Hemopure. ISBT Science Series, 2006; 1: 167-173.

[xix]. Winslow RM. Hemospan: Scientific foundations and clinical development. ISBT Science Series, 2006; 1: 161-166.

[xx]. Kerner T, Ahlers O, Veit S et al. DCL-Hb for trauma patients with severe hemorrhagic shock:the European "on-scene" multicenter study. Intensive Care Med, 2003;29:378-385

[xxi]. PolyHeme Pivotal Phase III trial. Northfield laboratories INC (website). Retrieved on 27 August 2007 from http://www.northfieldlabs.com/amb_trial.html

[xxii]. Securities and Exchange Commission v. Biopure Corporation, Thomas Moore, Howard Richman and Jane Kober. United States District Court; District of Massachusetts 2005, 05 CA 11853 WGY.

[xxiii]. SEC v. Biopure Corporation, et al., Civil Action No 05-11853-PBS(D. MA). SEC settles Civil Injunctive Action Against Biopure Corporation and its general counsel. US Securities and Exchange Commission (website). Litigation release No. 19825, September 12, 2006. Retrieved on 28 August 2007 from http://www.sec.gov/litigation/litreleases/2006/lr19825.htm

[xxiv]. SEC v. Biopure Corporation, et al., Civil Action No 05-11853-PBS(D. Mass). SEC settles Civil Injunctive Action Against Former CEO of Biopure Corporation. US Securities and Exchange Commission (website). Litigation release No. 20010, February 21, 2007. Retrieved on 28 August 2007 from http://www.sec.gov/litigation/litreleases/2007/lr20010.htm

[xxv] . National Blood Centre. Kutipan dan Penggunaan Darah di Malaysia 2001. Retrieved on 28 August 2007 from http://www.pdn.gov.my/jadual.html

9.1. Appendix I- Levels of evidence scale

Level	Strength of evidence	Study design
1	Good	Meta-analysis of RCT, Systematic review
2	Good	Large sample RCT
3	Good to fair	Small sample RCT
4		Non-randomised controlled prospective trial
5	Fair	Non-randomised controlled prospective trial with historical control
6	Fair	Cohort studies
7	Fair	Case-control studies
8	Poor	Non-controlled clinical series, descriptive studies multi-centre
9	Poor	Expert committees, consensus, case reports, anecdotes

SOURCE: ADAPTED FROM CATALONIAN AGENCY FOR HEALTH TECHNOLOGY ASSESSMENT (CAHTA), SPAIN