CLASSIFICATION OF BLOOD SUBSTITUTES

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Abstract

Blood substitutes are substances used to replace or supplement the activities of biological blood cellular or acellular components. It is meant to be a transfusion-free option. There are four main categories that blood substitutes fall into: red blood cell substitutes, white blood cell substitutes, platelet substitutes, and plasma derivatives. Red blood cells (RBCs) substitutes can be divided into biological and chemical oxygen carriers. Biological oxygen carriers are haemoglobin-based oxygen carrier (HBOC) and stem cell derived red blood cells (cRBC). Sanguinate is the sole FDA-approved HBOC drug due to its sickle cell reversal, vasodilatory, and noninflammatory qualities while cRBC is utilized to produce universal group of RBCs. It has greater biological connections with natural bloods than chemical oxygen carriers, the second type of RBCs substitutes. When cRBC were transfused into participants, it was found that 63% of the cells continued to circulate in the blood, matching the half-life of a normal RBC, which is 28 days. These showed that red blood cells could be cultivated in a lab and that they also responded well in the human body. A study on stem cell derived red blood cells (cRBC) using growth stimulants, medium cultures, and genetic manipulation to immortalise human erythroid line has yielded mature RBCs. Now, this study is in the clinical trials which portrayed a huge success in the artificial blood field due to its immortal property. Perfluorocarbon (PFC) and polymer-based oxygen carriers are the two subcategories of chemical-based oxygen carriers. Products in this category may not be structurally resemble haemoglobin or RBCs, but they are intended to serve the primary physiological function of blood. Due to the complexity of the cellular parts of the immune system, no alternative to white blood cells (WBC) has been made artificially yet. However, immunotherapy strategies may offer the "functional substitution" for WBC especially in the case of artificial adaptive immunity. There have been approved alternatives for plasma derivatives but none to substitute platelets yet.

Keywords: Blood substitute, Artificial oxygen carrier, Oxygen therapeutic agent, Haemoglobin mimic, Synthetic blood

Introduction

Blood plays several important physiological functions in human body. Amongst them are regulation of body temperature and pH, supply of nutrient to cells, excretion of metabolic waste products such as urea and lactic acid and body defence against pathogen via immune system. However, the most crucial role of blood is delivery of oxygen via cardiovascular system throughout the body for cell respiration. In return, carbon dioxide produced by the cell will be taken up by the blood back to respiratory system for exhalation. This reversible cycles of oxygenation and deoxygenation are carried out continuously by red blood cells throughout the body (1).

The main component of red blood cell that carries that task is haemoglobin. This tetrameric protein has the capacity to bind to four oxygen gas molecules at one go. Such feature makes normal human respiratory system so efficient with the assistance of 20 to 30 trillion red blood cells in adult or five to six million cells in every cubic millilitre of blood (2). Therefore, massive bleeding would compromise the efficiency of oxygen delivery and post life-threatening risk. It is adamant to acknowledge the importance of blood as one of the therapeutic elements to save life. Blood loss due to traumatic injury or diseases such as thalassemia and anaemia may require allogenic blood transfusion for treatment. Research to explore blood alternatives began almost 150 years when several efforts postulated the use of natural compounds such as milk and coconut water to replace blood during wartime. Discovery of Ringer's solution showed that the solution could sustain life but was short-lived and caused severe renal toxicity. Nonetheless, modern medicine has witnessed the emergence of safer blood transfusion practices into human. Collected blood from healthy donor must be screened and processed to ensure compatibility with the recipient's blood prior to transfusion. Knowledge on

blood such as blood group antigen and screening of transfusion transmitted diseases are very important to ensure safe transfusion. Nonetheless, human blood transfusion still relies on the rate of blood donation of country. For instance, fast developing countries only records between 2-3 percent of donors per total population. The figure is still far from the 6% target as in developed nations (3).

Moreover, several risks of transfusion such as incompatible reaction and transfusion transmitted infections like human immunodeficiency virus (HIV) transmission need still need serious attention. These lead to the worldwide searching for suitable alternative of blood especially red blood cells. Research activities to blood substitute or artificial oxygen carriers that are universal, pathogen free, biocompatible, extended shelf-life at room temperature, low cost of production and longer half-life in circulation have been going on for the past 70 years (4). However, there is still no substitution for blood for safe transfusion despite numerous efforts to develop one. Obviously, if it were simple problems and of lesser complexity, it would have been solved long time ago. Wartime has placed requirement for blood substitute or universal oxygen carrying resuscitation fluid for trauma victims at battlefield. Research that has been carried out in both military and public labs at first to develop chemically modified haemoglobin to enhance its effectiveness as cell-free oxygen carrier during the first half. The second half witnessed intensity of civilian labs to develop oxygen rich solutions in 1980's due to scarcity of blood supply, emergence of acquired immune-deficiency syndrome (AIDS) epidemic and commercialisation potential for profit. In 2000's, different approaches were taken to thrust the efforts further by engaging chemical and material sciences as well as molecular cell biology.

With all the interest that have been vested in developing blood substitute, the main challenges remain to be underdevelopment of related fields and lacking understanding on basic biological mechanisms. Ever since Ibn Nafis discovered the pulmonary transit of blood or pulmonary circulation (d. 1288) (5) and William Harvey described blood circulation (1628) (6), modern transfusion medicine has benefited a lot, bringing the searching of red blood substitute nearly at hand. Nonetheless, in 2018 only one product was in phase 1 clinical trials and the final report is yet to be disclosed. This paper aims to provide general classification of blood substitutes of recent updates to illuminate an overall picture of the field. Although a commercial red cell substitute is not in available yet, there are several substitutes to other blood components that are commercially available. We hope to bring all the information on blood substitutes to derive our classification thus far. So far, there is no classification that really looks on the overall spectrum of blood substitutes except on functional classification of plasma substitutes based on experimental and clinical date made in 1969 (7).

Classification of Blood Substitutes Based on Blood Components

The term 'blood substitute' literally refers to alternative to each blood cellular components which are inclusive of red blood cells, white blood cells and acellular components such platelet, plasma derivatives and others. But a working classification must consider that blood substitute denotes not only ambitious vision to mimic blood's cellular components but their physiological functionalities. Therefore, classification of blood substitutes must be reflective of this vision as represented in Figure 1. Some reports equate blood substitute to other terminologies such as artificial blood or blood surrogate (8, 9) These terminologies have been narrowed down to represent red blood cells substitute exclusively. Even though haematocrit or packed red cell only makes up almost 45% of the whole blood volume, research focus worldwide has been intensified on red blood cell substitute due to its clinical importance and urging needs. Emergence of HIV post great risk to blood transfusion via transmittable infection of the retrovirus. This has driven the search for red blood cell alternative even further (10). The term red blood cell alternative is interchangeable with others like artificial oxygen carrier, oxygen therapeutic agent, oxygen carrying solution, oxygen rich solution. These terms are more appropriate given the fact that focus on have been heavily on oxygen delivery and volume expansion. Nevertheless, the term 'substitute' is more encompassing to indicate 'alternative' to all cellular nor acellular blood components including their physiological functionalities.

Red Blood Cells Substitutes

Red blood cell substitutes were initially limited to oxygen-carrying solutions known as haemoglobin-based oxygen carrier (HBOC) and perfluorocarbon (PFC) until early 1990's. Recent updates in the filed have witnessed additional alternatives that are different from the former two. Based on these, red blood cell substitutes generally can be categorised into biological based oxygen carriers and chemical-based oxygen carriers. The former utilises either semi or fully biological while the latter are manufactured form fully synthetic chemical materials.

Biological based oxygen carrier

Biological based oxygen carriers can be sub-divided further into haemoglobin-based oxygen carrier (HBOC) and stem cell derived red blood cells or also known as cultured red blood cells (cRBC). The former was the earliest of red blood substitute emerged while the latter only came into the scene in recent years. HBOC is partially biologic while stem cell derived red blood cells are full biosourced.

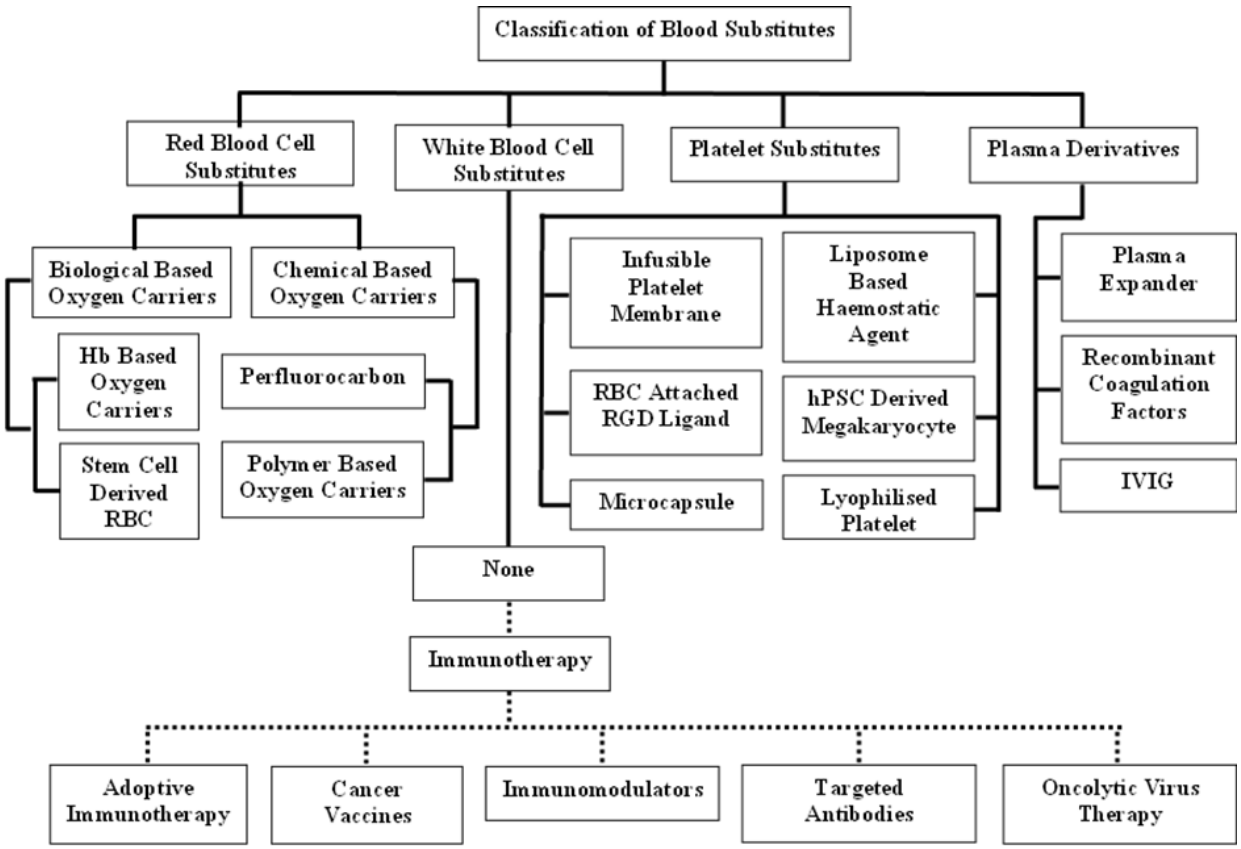

Figure 1: Classification of Blood Substitutes

Haemoglobin based oxygen carriers

Haemoglobin based oxygen carrier (HBOC), are made from chemically modified and molecularly stabilised haemoglobin. Haemoglobin is tetrameric protein with four globin subunits: two α and two β chains (11). Each subunit contains haem containing iron cored porphyrin unit and polypeptide chain. The central cavity allows oxygen to bind to the metal atom and induce conformational change to the protein for increase in oxygen affinity for progressive addition of next oxygen molecules (12). Thus, each haemoglobin is capable to carry four oxygen gas molecules (13). The cooperative process is also bound to several other factors such as temperature, pH, partial oxygen pressure and allosteric effects of 2,3-bisphosphoglyceric acid. The haemoglobin can be sourced from human and animals such as cattle and swine. Free haemoglobin can still maintain its capacity to deliver oxygen without its cell membrane, but it is highly toxic to normal tissues (14). Amongst the toxicity issues are vasoconstriction due to nitric oxide scavenging by endothelial and formation of radicals resulted from oxidative state of haem and globin (15). More, cell free haemoglobin is free from 2,3-DPC making oxygen delivery less efficiency (16). Therefore, modification of the haemoglobin is required to optimise the affinity of the HBOC to bind oxygen as well as lowering the risk of its degradation during oxygen delivery within circulation. This modification could be summarised in the tabulated data, Table 1.1.

The first generation HBOC is known as stroma-free haemoglobin (SFH). It was prepared by lysis of red blood cells to give soluble haemoglobin before centrifuged to remove all the stroma. It was reported that the preparation of SPH yielded 7g/dl in 500 ml haemoglobin solution with normal concentration of physiologic electrolytes (Na+, K+, HCO3), pH (7.1-7.4) and osmolality. Only 7 to 12% of methaemoglobin concentration of the total haemoglobin was observed after four weeks of 4°C storage (17). The preparation of SPH via ultra-crystallisation resulted to likelihood of denaturation of haemoglobin during storage as compared to ultrafiltration. Degradation of haemoglobin resulted from isolation and purification processes required for further modification techniques such as cross-linking, conjugation, polymerisation, and encapsulation to stabilise the protein and reduce side effects such as vasoconstriction (18).

The second generation HBOC utilised chemical modification of haemoglobin by conjugating the SPH to form pyridoxilated haemoglobin-polyethylene conjugates (PHPCs). This technique was introduced to address issues surrounded the SPH such as exaggerated oxygen gas affinity, limited circulation time or half-life and renal toxicity (19). SPH, the free haemoglobin was pyridoxylated by addition of vitamin B6 to balance oxygen affinity before conjugated with α-carbomethoxy-ω-polyethylene to gain higher molecular

weight ensuring longer circulation (20). Amongst the second generation HBOC products are Hemopure and PolyHeme. Hemopure or haemoglobin-glutamer-250 (HBOC-201) developed by Biopure Corp was derived from bovine haemoglobin polymerised with glutaraldehyde had been introduced to medical application and showed effectiveness with good tolerance by patients (21). HBOC-201 was produced from highly purified bovine haemoglobin and the third-generation product. Its previous two other solutions; Hemopure-1-Solution (HIS) was made up of 50% tetrameric haemoglobin while Hemopure-2-Solution (HBOC-301, Oxyglobin) was the next generation but lower average molecular weight. HIS had been used in Phase 1 clinical trial and ended due to intolerable gastrointestinal problems. Oxyglobin was only approved for veterinary use only. HBOC-201 has undergone extensive animal and human trials. Although no Hemopure products were approved by US-FDA, HBOC-201 was approved for clinical application in South Africa in 2001 by their Medical Control Council (22).

PolyHeme was a Northfield lab's product that has entered phase III clinical trial in 2006 before it was found to provide insignificant benefit to patient's health (23). PolyHeme or SPH-P was first developed as poly stroma-free haemoglobin. Its developer, North claimed that PolyHeme was free form any vasoconstrictive effects due to its relatively larger polymerised haemoglobin as compared to other HBOC products. It is a product of crosslinking between stroma-free human haemoglobin from expired RBC with glutaraldehyde prior to pyridoxylation. PolyHeme entered two Phase II and one Phase III clinical trials but eventually the program was ended after US-FDA refused its approval in 2009. There are also other products such Oxygent and Hemopure that were reported to cause side effects such as stroke and cardiovascular failure (24). Another HBOC product that is free from polymer is known as Oxyvita but failed to enter clinical trial due to unsuccessful animal testing. Report stated that healthy rats that were tested with the product suffered from hypertension which then caused cerebral ischemia (25, 26).

The third generation HBOC utilised crosslinking α chains of haemoglobin sourced from bovine blood with bis (dibromosalicyl) fumarate known as αα-haemoglobin. This HBOC was first developed by US Army Lab before Baxter Healthcare developed their own Hemassist in 1985. This version sourced haemoglobin from expired human red blood cells before crosslinking the tetrameric protein with diaspirin-crosslinked haemoglobin (DCLHB). The haemoglobin was pooled, washed sterile saline to remove plasma, subjected to hypotonic lysis and filtered out to remove the membrane debris before crosslinked with bis(3,5-dibromosalicyl) fumarate. The final yield was reported between 55% to 58%, (27) storable at -20oC for up to one year (28). Hemassist and αα-haemoglobin were furthered to animal and clinical trial phase 1. Despite αα-haemoglobin unpromising performance, Hemassist was the first HBOC product to advance to Phase II and III. However, in 1998 Hemassist was found to be less effective than standard care and provided no significant assistance to haemorrhagic shock patients plus some adverse effects such as vasoconstriction, before it was finally terminated (24, 29) It was found out that Hemassist failed to regulate oxidative state of porphyrin iron in haem unit (27).

Another example of third generation HBOC is Hemolink developed by Hemosol Inc. The product used raffimer as crosslinker to crosslink haemoglobin with activated sugar, O-raffinose. It was tested in phase I and phase II clinical trials for patients underwent open heart surgery but no report on phase III trial was available to public (30-32). Oxidation of iron in free haemoglobin to form methaemoglobin disrupt regulation of physiological functions especially to vascular endothelium. Oxidised iron as the results of autooxidation turns the metal atom into irreversible 'rusty' iron states causing oxidative stress to circulatory plasma (33). Formation of free radical that is highly oxidative not only damage normal cells, but also other biochemical reactions (34).

To overcome the shortcomings of the previous HBOC, polymerised haemoglobin was designed. The term was HBOC was eventually replaced to oxygen therapeutic agent (OTA). This was achieved by PEGylation of haemoglobin such as the use of maleimide to modify the protein in Hemospan or carboxylated haemoglobin such as Sanguinate. The products were respectively developed by Sangart and Prolong Pharmaceuticals aimed to improve oxygen delivery functions. Hemospan or also known as MP4 was developed to overcome the issues vasoconstriction and hypertensive effects of previous products. The product managed to deliver oxygen to ischemic tissue (35, 36) and was reported of no significant adverse reaction in patients underwent major surgery (37). Despite Hemospan entered phase II and phase III trials in US and Europe respectively, no data on efficacy and clinical safety was made available (38). Sanguinate was a PEGylated bovine haemoglobin with carbon monoxide hybrid studied to improve vasodilatory and non-inflammatory properties (33). It was reported to improve perfusion of myocardial ischemia in rats (34). In vitro studies also demonstrated the potential of Sanguinate to reverse sickling of sickle cells. The product was approved as drug for treating sickle cell disease by FDA (39, 40). Other HBOC products that worth mentioned are ErythroMer, Hemo2Life, OxyVita, a liposomal encapsulated human haemoglobin like HbVesicles and HemoAct that involved human haemoglobin and albumin molecules (41-45).

Table 1.1: Haemoglobin based oxygen carriers

Production stage	Product	Producer	Source	Derivation	Application/Trial	Adverse effect	Approval Status	Ref.
First generation	Stroma-free haemoglobin (SFH)		Bovine haemoglobin	Lysis of RBCs with removal of stroma	Preclinical stage	SFH's crystallization process led to vasoconstriction and contractility-depressant activity.		17-18
Second generation	Hemopure Haemoglobin-glutamer-250 (HBOC-201)	Biopure Corporation	Bovine haemoglobin	Haemoglobin polymerised with glutaraldehyde	Preclinical stage and human clinical trial.	There are perioperative complications occurred but were not considered to be related to HBOC-201.	USA-FDA denied approval. Received approval from South Africa for use in adult surgery patients to treat acute anaemia and reduce allogeneic blood use.	21-22
	Hemopure-1-Solution (HIS)	Biopure Corporation	Bovine haemoglobin	50% tetrameric haemoglobin	Phase I clinical trial	Terminated due to gastrointestinal problems.		22
	PolyHeme (SPH-P)	Northfield Laboratories	Expired human RBCs	Polymerized pyridoxilated haemoglobin	Enter two Phase II and one Phase III clinical trial	Failed in clinical trial led to high mortality cases.	FDA denied approval	23
	Oxygent	Alliance Pharmaceutical Corporation	Perfluorochemical (PFC) emulsion	PFC with two active ingredients: perfluorooctyl bromide and perflubrodec.	Various preclinical stages and clinical trial	The USA terminated second trial because of possible increase in stroke rates and causing mild thrombocytopenia		17
	Oxyvita	OXYVITA, Inc	Bovine blood	Red cell lysis using Phosphate buffer in haemoglobin isolation.	Preclinical stage: Unsuccessful	Causing hypertension in rats, which resulted in brain ischemia.		25-26

Continuation of Table 1.1

Production stage	Product	Producer	Source	Derivation	Application/Trial	Adverse effect	Approval Status	Ref.
Third generation	Hemopure-2-Solution/ Oxyglobin (HBOC-301)	Biopure Corporation	Bovine haemoglobin	13 g/dl of polymerized SPH that is suspended in a modified lactated Ringer's solution.	Preclinical trial		The only veterinary FDA-approved HBOC	21-22
	αα-haemoglobin	Letterman Army Institute of Research (LAIR) USA	Bovine blood	α chains' haemoglobin crosslinked with bis(dibromosalicyl) fumarate	Preclinical trial and Phase I Clinical trial	Report from US Army stated the product caused vasoconstriction.		24
	HemAssist/ DCLHb	Baxter Healthcare	Expired human RBCs	SFH crosslinked with bis(3,5-dibromosalicyl) fumarate	Preclinical trial and Phase I, II, and III Clinical trial	Terminated due to vasoconstriction.		24, 27-29
	Hemolink	Hemosol Inc	Human haemoglobin	haemoglobin crosslinked with activated sugar, O-raffinose	Phase I, and Phase II Clinical trial	Product halted because of an increase in cardiac arrests.		24, 30-32
	Sanguinate	Prolong Pharmaceuticals	Bovine haemoglobin	PEGylated haemoglobin with carbon monoxide hybrid	Undergoes two Phase I studies and Pass Phase II Clinical trial.	**Positive Advancement** Phase I trials shown positive reaction with sickle cell anaemia, and other Phase-II studies testing on SCD.	FDA approved as Orphan Drug Designation (drug for sickle cell disease)	33-34, 39-40
	Hemospan (MP4)	Sangart Inc	Human haemoglobin	Oxygenated polyethylene glycol–modified haemoglobin	Preclinical studies. Phase II, and Phase III Clinical trial	Prevent hypotension and ischemia in Preclinical studies (haemorrhage) and decrease hypotension in surgical patients.		35-37

Despite several downfalls of HBOC and other products, the search for red blood cell substitute or artificial oxygen carrier continue to flourish. Research activities on the subject venture into nanomaterial for the development of oxygen carriers mainly such as involve biocompatible polymeric material (46). Nonetheless, the uncertainties of synthetic or chemical-based oxygen carrier and semi-biological product like HBOC have triggered the exploration towards a more natural alternative which is stem cell derived red blood cells or cultured red blood cells (cRBC) in laboratory.

Stem cell derived red blood cells

This second sub-category of red blood cell substitute involves ex vivo production of red blood cells from stem cells (47-49). Experiments on stem cell to produce universal group red blood cells have been going on for the past 20 years. A group of scientists led by Donald Kohn have developed a model of in vitro manufacturing of human red blood cells from hematopoietic progenitor cells (HPC) in 1998. Their work was an initial effort that used recombinant growth factor to promote full maturation of red blood cells from HPC. Enucleated adult red blood cells were then isolated for further detail analyses and studies. This in vitro technique to produce red blood cells from stem cells has gained interest from so many researchers in the field of medicine and cell biology (50).

In 2005, Luc Douay and his team reported their large-scale production of mature red blood cells in laboratory through various sources of hematopoietic stem cells (HSC) (51). They created microenvironment in the culture media that was full of different cytokines, growth factor and other reagents to produce red blood cells that reached full maturity upon differentiation and amplification phases. Subsequently in 2008, Robert Lanza's group reported their successful characterisation of biological properties and enucleation of red blood cells sourced from human embryonic stem cells (hESC). They proved that functional red blood cells could be derived from hESC in large scale up to 1010 to 1011 cells per six well plate. More, it was also noticed for the first time that the cultured red blood cells could matched the oxygen equilibrium curve of natural red blood cells and responsive to the changes in pH and 2,3-DPG. Even though, hESC derived red blood cells still present with foetal and embryonic globin and embryo, the cells still capable to express adult β-globin chains on further maturation in-vitro. During the process, red blood cells underwent series of maturation phases than include progressive size reduction, increase glycophorin A expression and chromatin as well as nuclear condensation. The rate of nuclei extrusion was more that 60% to yield red blood cells with diameter between 6 to 8 μm, indicating the large-scale feasibility of hESC to differentiate and mature into functional erythrocytes (52).

Luc Douay et al in 2011 continued their previous work to further prove the capacity of cultured red blood cells they produced for clinical application (53). The experiment identified the capacity of mature red blood cells produced in-vitro to survive in human blood circulation. The cRBC demonstrated capacity to bind, deliver and release oxygen gas within human physiological systems. Besides that, the cRBC also expressed blood group antigens on the surface of cell membrane. The transfused cRBC into human subjects revealed that 63% of the cells to remain in blood circulation to match 28 ± days of natural red blood cell's half-life. These findings not only proved that red blood cells could be cultured in laboratory but also functioned well in human body. Up to that point, the scientists are confident that large scale ex-vivo production of red blood cells in laboratory from various sources of stem cells is feasible. Nonetheless, the main obstacle is the process of cell culture to promote cell differentiation and amplification required the use of various reagents and growth factors making large-scale production of red blood cells very costly and complex.

Several efforts to simplify and optimise the culturing techniques of red blood cells such as the work of Joanne Mountford form the University of Glasgow. Between 2011 until 2017, there were a few research that focused on various levels of stem cell differentiation, amplification, and maturation especially from induced pluripotent stem cells (iPSC), HSC and hESC (54-62).

Number of growth factors, media cultures and genetic modification and other aspects have been studied to produce functional red blood cells efficiently. That includes that work of David Anstee, Allison Blair, Jay Frayne, and others from University of Bristol who successfully produced immortalised erythroid line from human for the first time to derive mature red blood cells (63). Their research collaboration with National Health Service (NHS) on stem cell derived red blood cells have been advanced to clinical trial phase involving 20 to 25 human subjects in the UK. The clinical trial is so much waited for by all scientist worldwide and the results is expected in few years' times (64). Could universal group red blood cells cultured from stem cells in-vitro be the key answer to the challenges of natural red blood transfusion? It is expected for the cRBC to equate the functions of natural RBC due to its biological interaction advantages as compared to the second category of red blood cell substitutes; chemical-based oxygen carriers.

Chemical based oxygen carriers

Chemical based oxygen carriers can be divided further into two sub-categories which are perfluorocarbon (PFC) and polymer-based oxygen carriers. Products in this category might not resemble the structure of haemoglobin or red blood cells but aimed to serve the main physiological function of blood, oxygen delivery.

Perfluorocarbon

PFC is an organic chemical solution that was firstly tested on rat lab in the infamous experiment by Leland Clark and Frank Golan in 1966 (65). The fluorine-rich hydrocarbon solution has the capacity to dissolve oxygen gas molecule. A group of scientists led by Robert Geyer have tested the solution on animal in 1968 (66). Molecules of PFC solution is far smaller that red blood cells but capable to carry more oxygen gas than haemoglobin. The chemical structure of PFC is completely different from red blood cell or haemoglobin. Nonetheless, hydrophobic nature of PFC requires emulsification to ensure its optimum interaction with physiological system of human body especial cardiovascular system. Amongst the PFC products that have been developed, tested, and applied were Fluosol-DA, Oxygent, Oxycyte and Perftoran.

Fluosol-DA was first generation of PFC emulsion developed by Green Cross Corporation in Osoka, Japan. The first accepted PFC-based red blood cell substitute is an oxygen carrying emulsion of 7 to 3 ratio of perfluorodecaline and perfluorotripropylamine with the addition of an emulsifier made of short-chain linear polymers known as pluronic F-68. The capacity of Fluosol-DA to carry oxygen at 20% solution was only 7.3% at 37°C which is much lower than red blood cells. The solution could also carry 35% of oxygen content in whole blood at 14g/dl hb level (67). The first report on its commercial use was in Japan with infusions to patients underwent severe gastrointestinal bleeding and surgery related blood loss (68-69). Its clinical trials had proved the concept of injectable oxygen therapeutic for tissue oxygenation. However, Fluosol-DA was reported to cause complications such as pulmonary reactions due to complement activation by its emulsifying agent. Failure to demonstrate its efficacy in clinical trial has stopped its clinical application. Fluosol-DA was the first approved by US-FDA for clinical use in 1989 before it was withdrawn in 1994 due to renal toxicity effects (70).

The second-generation PFC products that have been extensity explored were perflubron and perfluorodecalin (4). Oxygent, a trade name for perflubron was developed by Alliance Pharmaceutical Inc. The product was formulated with two active compounds which are perfluorooctyl bromide and perflubrodec to produce PFC at 60g/dl. Addition of perflubrodec in small amount to stabilise the particle during storage (71). The product was emulsified with egg-yolk phospholipid to yield a product storable at 2-8°C for 24 months or weeks at room temperature. Toxicology studies from trials on animal and human in the beginning showed promising results with well tolerated emulsion without any adverse effect (72). Except some changes in clotting factors, no report was found on specific interaction between transfused Oxygent and blood components (4). In fact, phase III trial in Europe revealed that cardiac surgery patients received lesser red blood cell transfusion as compared to the control group by 10%. However, Oxygent was terminated due to some side effects such as increased risk of stroke and reduced platelet count (mild thrombocytopenia) as well difficulties in determining effective dose of the product (19). Another second-generation PFC product was Oxycyte which was developed Synthetic Blood International. This product was like Oxygent utilised egg-yolk phospholipid as emulsification agent (73). It was entered at phase II trials for testing on traumatic brain injury patients, but no report was available. Oxycyte suffered the same fate as Oxygent when its second trial was suspended (74). Part of the reasons was high-cost inputs and increased in stroke cases in large multicentred studies (73).

Russia had also developed PFC product known as Perftoran in 1997 by Russian Academy of Sciences. The product was approved for clinical application and reported to post less side effects than Fluosol (75). Moreover, Perftec (commercial name of Perftoran) was also approved for clinical use in Mexico in 2005 (76). The same product rebranded as Vidophor is still awaiting clinical trials in the US (20 Friedman). It would be difficult for PFC to be approved for now due to lacking clinical evidence. Some manufacturers did not have faith in the product's clinical and commercial future, demanding a new approach to synthetic oxygen carrying compound such as polymer based artificial oxygen carrier (17).

Polymer based oxygen carriers

The second sub-category is polymer-based oxygen carrier. Polymer has attracted wide attention especially after several success in biomedical applications such as drug delivery and diagnostic imaging due to its valuable properties (77, 78). Amongst these properties are biocompatibility, biodegradability, broad spectrum of polymers with tailorable features, making polymer one of the suitable candidates for development of red blood cell analogue (79-81). Common polymers that are usually selected to construct artificial oxygen carrier include poly (ethylene glycol) (PEG), poly(caprolactone) (PCL) and poly (lactic acid) (PLA) (82). However, it does not limit exploration of other polymer classes for such purpose.

In 2007, Lance Twyman of the University of Sheffield reported successful synthesised of 'plastic' blood from iron-porphyrin cored hyperbranched polymer (83). The synthetic model was free form natural or biological components (non-immunogenic) unlike HBOC, that could risk transfusion reaction. US Military research institute such as Dendritech Corp had seriously look to develop polymer like dendrimer as potential artificial oxygen carrier (84). Even though several polymer-based oxygen carriers such as polymeric micelle and polymeric vesicle were reported to have the capacity of binding oxygen reversibly (80, 85), more investigations are required. Amongst them are optimisation of physiological function, cell biological interaction and improving half-life and shelf-life of the products (86-91).

White Blood Cells Substitutes

To date, no alternative to white blood cells (WBC) and

major signalling protein components of the immune system have been synthetically produced. This is mainly due to the complex nature of cellular components of the immune system in human body. Most of the cells involve in series of defence mechanism either via adaptive or innate immunity (92). The main tasks include to fight against disease causing agents such as virus or bacteria, to recognise and neutralise harmful foreign substances from external environment and to fight against diseases that cause changes within human body such as cancer (93, 94). The 'nearest' alternative to cellular components of immune system and the main signalling proteins to boost immune response is through development of immunotherapy strategies mainly for cancer treatment (95, 96). These may offer interesting 'functional substitution' to WBC or cellular components of body defence especially in artificial adaptive immunity. Components such as macrophages, monoclonal antibodies, cytokines are produced via recombinant technology (97). There are five main types (not categories) of immunotherapy; adoptive cell therapy, vaccine, immunomodulators (correcting dysregulated immune system by cytokines), targeted antibodies (monoclonal antibodies) and oncolytic virus therapy (98, 99).

Immunotherapy

Immunotherapy is a form of cancer treatment that aids the immune system in its battle against cancer. The immune system assists the body's defence against infections and other illnesses where it discovers and eliminates abnormal cells, so it prevents or inhibiting the formation of numerous malignancies. Immune cells, for example, are sometimes found in and surrounding cancer cells. These cells, known as tumour-infiltrating lymphocytes (TILs), indicate that the immune system is responding to the cancer. Individuals whose cancers have TILs typically fare better than those whose doesn't (100, 101). Immunotherapy improves the immune system's ability to fight the cancer cells.

Adoptive immunotherapy

Adoptive immunotherapy involves harvesting of immune cells from donor before reprogramming them for specific role or target. The cells are expanded in the lab to a certain therapeutic dosage before re-infuse to patient for treatment. The therapy is also referred to as cellular immunotherapy that utilises immune cells such as killer T cells to treat cancer. Several approaches in adoptive immunotherapy involve genetically engineered immune cells via gene therapy to bolster cancer fighting cells. The deployment of the immune cells with natural ability to bind to cancer makers or surface antigen of cancer cells are possible in various therapeutic ways; tumour-infiltrating lymphocyte, engineered T cell receptor, chimeric antigen receptor T cell and natural killer cell (102-104).

Cancer Vaccines

Vaccine is undeniably proven to prevent virus and bacteria cased diseased. It is typically work effective when individual is given the vaccine prior to being infected by the pathogens. Besides preventive vaccine, the latest vaccine for cancer is another form of immunotherapy that is useful to sensitise immune system against cancer cells by introducing 'cancer-cells resemblance' or 'antigens' so that immune cells identify and kill the tumour cells. The identification and elimination of cancer cells are executed by memory T cells and natural killer cells. But these processes are not as simple as killing the foreign viruses or bacteria since cancer cells mimic normal and healthy cells. Every person with cancer may develop individual and unique distinguishing antigens. Hence, vaccine for cancer treatment can be in the settings of preventive cancer vaccination, therapeutic cancer vaccination and personalised neoantigen vaccination (105-107).

Immunomodulators

Immunomodulation uses molecules to act on regulation pathways of immune system. Up and down regulation of immune response are useful in improving its ability to assault and eliminate cancer cells. There are generally four categories of immunomodulators: checkpoint inhibitors, cytokines, agonist, and adjuvants (108). Checkpoint inhibitors act to reverse the manipulative shutdown of immune response by cancer cells so that new response against tumour can be established such as CTLA-4-blocking ipilimumab for treating melanoma (109). Checkpoint inhibitor is the most widely used and successfully developed immunomodulators thus far (110-111). Cytokines are responsible for maturation, growth, and sensitivity of immune cells. So far, there are only four cytokines for immunotherapy for treatment of kidney cancer, leukaemia, melanoma and sarcoma (111). Cytokines are messenger molecules that regulate immune cell maturation, growth, and responsiveness. The four currently US-FDA-approved cytokine immunotherapies—for the treatment of subsets of patients with kidney cancer, leukaemia, lymphoma, melanoma, and sarcoma (112). Agonists are promoting molecules that activate adaptive immune responses via either killer T cells activation or stimulation of cellular components in innate immune response like dendritic cells (113). Meanwhile, adjuvants promote activation of innate immune system pathways to stimulate general adaptive immune responses. One US-FDA approved application for adjuvant immunotherapy is for treatment of squamous cell carcinoma (114).

Targeted Antibodies

Immunotherapy that utilises targeted antibodies aims to disrupt activity of cancer cells and alert immune response to recognise and kill them. Antibodies are produced by B cells for protection against various pathogens/ threats such as bacteria, viruses, and cancer cells via precise target of cancer antigen surface markers (115). Customisation of antibodies against specific cancer

target in the lab produce monoclonal antibodies such as naked monoclonal antibodies, antibody-drug conjugates, and bispecific antibodies (116).

Oncolytic virus therapy

Oncolytic virus immunotherapy that utilises viruses to kill cancer cells upon targeted infection. Viruses are introduced to infect and enter cancer cells to use the genetic information and copy themselves before spreading to uninfected cells. Viruses have been used to target tumour upon genetic modification to form oncolytic viruses (117, 118). This approach takes advantage on the impaired antiviral defence of tumour cells that makes them susceptible to infection. Modification of the viruses to make them less infectious to healthy cells and yield therapeutic threshold as well as inducing immune boosting effects once cells are infected (119-120). In 2015, US-FDA approved the first oncolytic virus immunotherapy to treat melanoma (121).

Platelet Substitutes

To date, there are not many progresses have been recorded on platelet substitutes in answering issues like prolonged shelf-life, inactivation of microbes and haemostasis capability of platelets (122). Regardless, several types of platelet substitutes have been experimented such infusible platelet membrane (IPM) (123, 124), red blood cells attached with haemostatic ligand group (RGD) (35), microcapsule (125, 126), liposome-based agent (127, 128), human pluripotent stem cell (hPSC) derived megakaryocytes (127, 129), and lyophilized platelet (130). But there are also other commonly available alternative agents in clinical practices to prevent or deal with bleeding such as thrombopoietin (TPO), platelet-rich plasma (PRP) and some drugs (131, 132).

Infusible platelet membrane

Infusible platelet membrane (IPM) or platelet-derived microparticle is harvested from expired human platelets that still retain their glycoprotein 1b receptor with presence of factor 3 activity (133). The harvested platelets need to be washed and heated for viral deactivation before sonicated to isolate microvesicle fractions prior to lyophilisation. Nonetheless, IPM is absent of other components such as factor V, serotonin, maker enzyme, GP IIb/IIIa, and HLA class I and II (134). IPM recorded reduction of bleeding time in animal and human trial with aspirin ingestion with no detection of platelet antibodies. Research shown that maximum decrease in the percentage of bleeding time was obtained after 2 hours admission of 2 mg/kg with platelet count of 50 to 70×10^3 µL, confirming the promising application of IPM as a substitute for platelets in the treatment of thrombocytopenia in humans (135). Besides, the combination of platelet membrane and other biochemical substances such as liposomes has potential as vascular delivery and smart drug delivery. A study found that merging natural platelet membranes with artificial liposomes results in a platelet-mimetic hybrid liposome (P-Lipo) for atherosclerosis targeted therapy. P-Lipo demonstrated outstanding physicochemical qualities, a high drug loading capacity, and a consistent drug release profile with great tolerability (127).

RBC attached with RGD ligand

Another approach is to attached red blood cells with haemostatic ligand group known as ARG-LYS-ASP or RGD peptides. RGB is a cell adhesion motif found on numerous extracellular matrix (ECM) and plasma proteins. It is vital in cell recognition and cell adhesion, and it has been utilized in targeted therapy and tissue engineering via recombinant methods and several chemical ways (136). RGD is a form of ligand for certain integrins that plays important role in platelet aggregation. RGD bearing RGD ligand or fibrinogen is haemostatically active since one fibrinogen has more than one RGD sequence to stimulate platelet bridging and form aggregates.

Microcapsule

Fibrinogen-coated albumin microcapsule or microsphere (FAM) is a form of formaldehyde fixed platelet bearing fibrinogen to enhance platelet aggregation (137). Over 30 years ago, platelet microparticles—micro vesicles of platelet membranes were originally researched as potential platelet replacements. These platelet microparticles are present in platelet concentrates, fresh-frozen plasma, and cryoprecipitate and develop spontaneously during the platelet's storage (122) Two of FAM preparations recorded effective haemostasis in animal model, but none managed to further into human clinical trials. Research on fibrinogen-coated nanospheres (FCNs) consisting of human albumin polymerized into a 100-nm sphere and coated with fibrinogen can reduce thrombocytopenia (TCP)-related bleeding. FCN therapy has the potential to be a safe and successful method for thrombocytopenia-related haemorrhage prevention or treatment. Improvements in bleeding times and platelet aggregation demonstrate that FCNs have an impact on primary haemostasis, and subsequent in vitro experiments indicate that FCNs only interact with active platelets (138).

Liposome based haemostatic agent

Liposome based haemostatic agent uses two approaches. The first one is by constructing liposome bearing platelet glycoproteins as artificial platelet. The platelet-some, a liposome-based substance, was explored as a platelet substitution. Plateletsomes with dimensions ranging from 10 to 200 nm were created by integrating a deoxycholate extraction of platelet membrane including GPIb, integrin IIb3, and GPIV into unilamellar lipid vesicles made of sphingomyelin, phosphatidylcholine, monosialylganglioside, or egg

phosphatide (139). The second approach is by intravenous infusing of procoagulant liposomes with activated coagulation factors such as Factor X. The latter demonstrated haemostatic effectiveness upon trial on haemophiliac dogs but also exhibited intolerable toxicity issues.

Human pluripotent stem cell (hPSC) derived megakaryocyte

The latest development of platelet substitute involves derivation of megakaryocyte from hPSC sources. The in-vitro production of the platelet precursor requires chemical modification and forward reprogramming strategies. It was reported that the purity of the product was almost 90%, yielding 2.0 x 105 of mature megakaryocytes per input hPSC. In 2018, it was reported the production of megakaryocytes from iPSC in vitro and in vivo upon utilisation of specialized bioreactor to increase the final yield of megakaryocytes by 10-fold (18.7 x 107 per iPSC unit).

Lyophilised platelet

Lyophilised platelet isn't really a synthetic product, rather is in an alternative preparatory approach to answer the issue of platelet's short shelf-life. Washed platelets are treated with 1.8% paraformaldehyde and frozen in 5% albumin before lyophilized (140). Rehydration of platelets have similar structure to their original form but with slight decrease in glycoproteins concentration. The product was also reported to exert shorter haemostatic activity in vivo (4-6 hours) in RES blocked thrombocytopenic animal model.

Plasma Derivatives

Besides the cellular components such as RBC, WBC and platelets, blood also contains other important non-cellular components in plasma. 95% of plasma is liquid (water) and the remaining are dissolved proteins such as serum albumin, globulins and fibrinogen, clotting factors, glucose, and gases. The main role of plasma is maintenance of intravascular osmotic effects such as electrolyte balance and defence against infection (141). Ideally, plasma substitute must encompass of all its derivatives. However, the alternatives to the derivatives are only well established in fluid replacement therapy such as plasma expander, coagulation factors and intravenous immunoglobins (IVIG).

Plasma expander

Fluid replacement therapy may use different volume expander indicated in different conditions (142). Amongst the common plasma expanders are colloid such as albumin or fresh frozen plasma (FFP) (143), crystalloid (144), dextran, hydroxyethyl starch (HES) (145), Ringer's solution and saline (146). However, none of them is capable to deliver oxygen within intravascular circulation. That shortcoming became one of the reasons for development of an ideal blood substitute that have oxygen carrying capacity besides correcting blood volume, maintaining osmotic pressure and perfusion.

Recombinant coagulation factors

Besides plasma fractionation, production of components such as coagulation factors can be made by recombinant technology. Equivalent proteins such as coagulation factors are expressed upon genetic modification of related cell lines. Amongst coagulation factors that have been successfully manufactured by recombinant technology are Aprolox (Factor IX), NovoSeven (Factor VIIa), NovoEight (Factor VIII), Tretten (Factor XIII A Subunit), Coagadex (Factor X) and Vovendi (Von Willebrand Factor) (147-150).

Intravenous immunoglobins (IVIG)

In theory, production of intravenous immunoglobulin (IVIG) utilises similar recombinant technology to produce coagulation factors (151). More, aptamer technology and non-immunoglobulin are also possible to produce synthetic antibodies (152, 153). To date, no synthetic IVIG product is commercially available and approved for clinal use. All the IVIG products in the market are sourced from healthy and screened plasma donors.

Conclusion

The blood substitutes development is receiving more and more attention due to the demand on blood transfusion and issues about blood-borne diseases. This requires a new classification of blood substitutes to garner the whole perspectives of the available products and the on-going research and development milestones. Despite the numerous discoveries that have been made, the FDA has only approved one haemoglobin-based oxygen-carrier; Sanguinate for clinical application due to its' ability to reverse sickling of sickle cells. Adverse effects such as gastrointestinal distress, and vasoconstriction that led to failure in clinical trials are two of the many reasons why they did not meet approval standards. The fact that there is just one FDA-approved product in this study indicates that there is a significant obstacle to the formulation and application of promising and effective blood alternatives. However, there is one stem cells exploration that efficiently create functional artificial RBCs. Researcher from Bristol University successfully immortalised human erythroid line to yield mature RBC from the exploration of growth factors, medium cultures, and genetic manipulation. Their research cooperation with the NHS on RBCs produced from stem cells has reached the stage of human clinical trials in the United Kingdom, and the results are eagerly anticipated by scientists worldwide. This demonstrates the field's vast potential. The highest demand has always been for red blood cells but the increase in prevalence of cancer and infectious diseases require for alternatives blood components such as antibodies as the therapeutics. Future advancement in

science and technology may help develop functional and safe cellular and acellular blood substitutes whose manufacturing will reduce the global blood shortage.

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Competing Interests

The authors declare no conflict of interest.

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