Blood Substitutes: Possibilities with Nanotechnology

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Abstract Nanotechnology deals with molecules in the nanometer (10^{-9}) range and is currently being used successfully in the field of medicine. Nanotechnology has important implications in nearly all the branches of medicine and it has all the capabilities to revolutionize the vast field of medicine in future. Nanotechnological advancements have been used for the preparation of artificial hemoglobin. It is formed by assembling the hemoglobin molecules into a soluble complex. A recent approach includes the assembling of this artificial hemoglobin with enzymes such as catalase and superoxide dismutase into a nano-complex. This complex acts as an oxygen carrier as well as an antioxidant in conditions with ischemia–reperfusion injuries.

Keywords Blood substitutes · Polyhemoglobin · Artificial red cells · Nanotechnology

Introduction

Blood transfusion has been responsible for saving millions of lives each year around the world. Yet the quantity and quality of blood pool available for transfusions is still a major concern across the globe, especially in the developing countries [1]. According to WHO data in the South-East Asian region (SEAR) countries, an estimated annual

15 million units of blood is required, but only around 9.3 million units are collected, leaving behind a huge gap of 5.7 million units of blood [2]. India with its huge population of over 1 billion is lagging way behind in blood collection. India's blood requirement is about 9–9.5 million units per year but blood banks in India are able to collect only about 5–5.5 million units per year. There is a continuous shortage of about 4 million blood units each year in recent years [3]. The situation is similar globally, however the shortage of blood units is more marked in under-developed and developing countries in comparison to the developed countries. WHO recommends that all blood donations should be screened for evidence of infection prior to the release of blood and blood components for clinical or manufacturing use. Screening of all blood donations should be mandatory for HIV, Hepatitis B and C and syphilis [4]. Quality of screening is very significant and critical for these infections in SEAR where number of people living with HIV, Hepatitis B and Hepatitis C is estimated to be 6 million, 85 million and 25 million respectively [2].

Acute shortage and a definite risk of transfusion transmissible infections along with a danger of transfusion related complications bound us to think for some other alternative to blood transfusion. Though, this idea immediately appears to be weird, unpractical and impossible or at least very difficult in application. As blood is unique in itself, its composition, characteristics, functions and properties are irreproducible, but is it really so? It is a well known fact that blood absolves many functions, all vital. However, severe blood loss (due to any cause) endangers life for two main reasons: (1) the drop in circulating blood volume reduces tissue perfusion (ischemia); and (2) the reduction in oxygen transport impairs tissue oxygenation (hypoxia). The circulatory system reacts to these changes

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by vasoconstriction, which further aggravates ischemia and hypoxia. Ultimately, alterations in cell metabolism and functions develop, which finally leads to shock and death. In view of this, an “alternative” is not only a preparation that can replace blood in all of its functions, but an emergency resuscitative fluid that is capable of (a) restoring blood volume, (b) transporting oxygen, and (c) reducing vasoconstriction. Obviously, this fluid must be free of toxic side-effects, antigenicity, agents of any disease such as bacteria and viruses.

If such a blood substitute can be developed, it would presumably play a major role in the setting of trauma care and for elective surgeries. It would also benefit patients with medical conditions who are in need of long-term blood transfusions, such as patients with myelodysplastic syndrome, aplastic anemia, thalassemia, etc. Such a product can also be used as an organ preservative to prevent or decrease reperfusion injury of the donor organs.

Oxygen Carrying Perfluorocarbons

Perfluorocarbons (PFC's) is a class of hydrocarbons produced by substituting single hydrogen of each carbon atom of the benzene ring with fluoride radical in the selected hydrocarbon base. PFC's are chemically inert synthetic molecules and are clear and colorless liquids. They have the ability to physically dissolve significant quantities of many gases including oxygen and carbon dioxide. PFC's, therefore simply dissolves oxygen and carries it in solution throughout the body. They typically carry 4–50 vol% at an arterial oxygen pressure of 160 mm Hg. Their ability to carry oxygen is directly proportional to their concentration in blood and, importantly, to the partial pressure of oxygen in blood. In the tissues there is diffusion of dissolved oxygen, resulting in improved delivery of oxygen to the tissues. PFC's are hydrophobic, and are therefore not miscible with water. On intravenous injection they results in a liquid embolus formation, which can arrest circulation. [5] Subsequently, intravascular administration of PFCs became possible only when a microemulsion of PFC's was made in normal saline [6]. PFC's thus have to be emulsified for intravenous use. The first-generation PFCs, such as Fluosol (Green Cross), used a poloxamer-type Pluronic F-68 as an emulsifier, which has a potential to cause anaphylaxis and complement activation. The second-generation PFCs use egg yolk phospholipids as an emulsifier, which are well tolerated. The most important second-generation PFC is Oxygent, containing 60 g of the perfluorochemical perflubron per 100 mL of emulsion, and also contains a small quantity of perflubrodec (perfluorodecyl bromide-C10F21Br), added as a stabilizer.

Fig. 1 The oxyhemoglobin dissociation curve of blood

With sophisticated technology, it is now possible to generate a stable perfluorocarbon emulsion with exceptionally small particles (median diameter $<0.2 \mu\text{m}$) [7]. After intravenous injection, the droplets of the perfluorocarbon emulsion are taken up by the reticuloendothelial system (RES), and this uptake determines the half life of the emulsion. After the initial uptake of the perfluorocarbon emulsion into the RES, the droplets are then slowly broken down and the perfluorocarbon molecules are taken up by the blood again (bound to blood lipids) and transported to the lungs, where the unaltered perfluorocarbon molecules are finally excreted via exhalation. At present no metabolism of fluorocarbon molecules is known in humans [7–9]. The oxygen transport characteristics of perfluorocarbon emulsions are fundamentally different from those of blood. Blood exhibits a sigmoidal oxygen dissociation curve (Fig. 1), in-contrast, perfluorocarbon emulsions are characterized by a linear relationship between oxygen partial pressure and oxygen content (Fig. 2), as mentioned earlier. Elevated arterial oxygen partial pressures are thus beneficial to maximize the oxygen transport capacity of perfluorocarbon emulsions. In larger vessels due to their small size ($<0.2 \mu\text{m}$ in diameter) perfluorocarbon emulsion particles mainly flow in the peripheral plasma layer [7]. Whereas in the microcirculation, perfluorocarbon emulsion particles perfuse even the tiniest capillaries ($4\text{--}5 \mu\text{m}$ in diameter) where no red blood cells may flow under certain conditions. It is these tiniest capillaries of the microcirculation where perfluorocarbon emulsions exert their greatest effect, because here they augment local oxygen delivery much more than what would be expected from an increase in oxygen content alone of the arterial blood (large vessel with red blood cells) [7]. Another important aspect that determines the efficacy of perfluorocarbon emulsions is the fact that all oxygen carried by the perfluorocarbon is in the

Fig. 2 The oxyhemoglobin dissociation curve of oxygen in contrast to blood

dissolved state, resulting in a higher oxygen partial pressures in the microcirculation and thereby augmenting the driving pressure for the diffusion of dissolved oxygen into the tissue [7]. PFC's are thus very promising for the treatment of thrombosis, atherosclerosis and other vasculo-occlusive diseases, carrying oxygen beyond the occluded area [10]. PFC's have also been investigated for its use as an adjunct to chemotherapy and radiotherapy for solid tumors. They increase the oxygen content in the center of the tumor, thereby rendering the tumor more susceptible to chemotherapy and radiotherapy [11]. Yet another use of PFC's can be as contrast agent in radiology [12], prevention or treatment of sequelae of air embolism, decompression sickness and finally improved organ preservation for subsequent organ transplantation [7].

Still there are a few adverse effects which need attention like limitation of administrable dose to prevent complement activation and hepatotoxicity, retention in RES leading to delayed clearance and need for the patient to breathe 100 % oxygen.

Introduction to Hemoglobin in Brief

Hemoglobin (Hb) contains iron (Fe^{2+})-protoporphyrin IX (the heme) as the prosthetic group that reversibly combines with O_2 and several other gaseous and non-gaseous ligands, along with a protein moiety 'globin'. The 'globin fold' of Hb is built by helices which provide a deep crevice where heme is bound. Hb is a tetramer constituted by two different subunits named α and β with the quaternary formula $\alpha_2 \beta_2$. The tetramer has a symmetry axis and can be considered as a dimer of $\alpha \beta$ dimers, called the $\alpha_1 \beta_1$ and $\alpha_2 \beta_2$. The $\alpha_1 \beta_1$ dimer (and the symmetrical $\alpha_2 \beta_2$) may be considered the basic structural unit, and dissociation of the tetramer into dimers involves cleavage along the symmetric interfaces α_1

β_2 and $\alpha_2 \beta_1$ [13]. The overall dissociation into subunits may be represented as follows:

In solution, the α subunits are mostly monomers while the β subunits aggregate to form the β_4 homotetramer [13]. The dissociation of Hb into dimers causes it to be filtered by the kidney, as occurs in hemolytic crisis, this shortens the half-life of the protein in circulation and also causes renal toxicity. Ferrous (Fe^{2+}) Hb with no ligand on the distal side of the heme is called unligated (or deoxy) Hb. DeoxyHb may also be prepared by reduction of the ferric derivative using borohydride, dithionite, ascorbate, or other reductants [13]. The Fe^{2+} is subject to slow spontaneous oxidation in the presence of O_2 , yielding ferric (Fe^{3+}) or metHb. In blood the rate of autoxidation is about 3 % per day, but the oxidized iron-porphyrin is reduced to its active form by a reductase enzyme. Within the red blood cell, this efficient enzyme system ensures that the iron atom remains in the reduced (Fe^{2+}) state, a requirement for binding O_2 . Without such mechanisms iron would oxidize to Fe^{3+} , releasing an electron. Ferric hemoglobin does not bind O_2 and may catalyze toxic reactions. The O_2 affinity of Hb is lowered by several substances that combine at sites other than the heme and are collectively called as allosteric effectors or heterotropic ligands. Physiologically, the most important heterotropic ligands are H^+ , Cl^- , CO_2 and 2,3-diphosphoglycerate (2,3-DPG) [14]. 2,3-DPG in humans exerts the most effective control of O_2 affinity [15]. It is produced by the erythrocytic enzyme glycerate bisphosphate mutase, which uses as a reagent the glycolysis metabolite glycerate 1,3-diphosphate. 2,3-DPG binds to a specific site between the α chains and lowers the O_2 affinity of Hb by a factor of 3.5 [16]. The oxyhemoglobin dissociation curve (Fig. 1) has a characteristic sigmoid shape due to the cooperative effect that exists between the multiple oxygen binding sites on the hemoglobin molecule. Along with (2,3-DGP) factors that modify the ability of hemoglobin to bind oxygen include body temperature and pH of blood [17].

For over 50 years, efforts directed to the development of a blood substitute have focused on hemoglobin, because this is the only substance capable of picking up enough oxygen from atmospheric air to serve as a physiological oxygen carrier. In addition, hemoglobin exerts the same colloid-osmotic pressure as serum albumin and can, therefore, serve as a plasma volume expander. As discussed above the problems with Hb can be summarized as:

- (i) Instability of the Hb molecule with tendency to extravasation and rapid renal excretion.
- (ii) High oxygen affinity of hemoglobin in solution which interferes with oxygen release to the tissues.

- (iii) Tendency of hemoglobin to undergo autoxidation with generation of met-Hb (non-functional Hb) and free radicals.
- (iv) Toxicity due to the contamination of hemoglobin with environmental bacterial endotoxins, and stromal phospholipids.

Hemoglobin Based Oxygen Carriers (HBOC's)

Hemoglobin-based substitutes use hemoglobin derived from several different sources: human, animal, and recombinant DNA technology developed hemoglobin. Human hemoglobin is obtained from donated blood that has reached its expiration date and from the small amount of red cells collected as a by-product during plasma donation.

HBOCs from expired human blood or fresh bovine blood have to undergo numerous modifications to make them safe and effective oxygen carriers. The RBCs are first lysed to release their hemoglobin, and then the stroma is removed by a variety of methods, including centrifugation, filtration and chemical extraction. The stroma-free hemoglobin is then purified and undergoes modifications to cross-link, polymerize or conjugate it to other compounds. Without these modifications, the oxygen affinity of the stroma-free hemoglobin is too great to facilitate oxygen release in the tissues. Also, when it is outside the RBC, hemoglobin rapidly dissociates into 32 kDa $\alpha\beta$ dimers and 16 kDa α or β monomers, both of which are rapidly filtered in the kidney and can precipitate in the loop of Henle, resulting in severe renal toxicity. The dimer haem iron is oxidized more easily than in the tetramer, leading to molecules unable to bind oxygen. Moreover, the formation of ferric ions triggers a cascade of reactions that generate reactive oxygen species and reactive nitrogen species, the molecular basis of oxidative damage.

Dimers extravasate and scavenge nitric oxide (NO), the key controller of vessel tension produced by the endothelial cells, thus causing hypertensive effects. The vasodilating effect of NO is critical in the regulation of the vascular tone. Given the negative effects associated with the presence of dimers free in the plasma, the research on HBOCs has been primarily focused on the development of hemoglobin tetramers that either do not dissociate into dimers or, if they do, do not undergo ultrafiltration and extravasation because their size is artificially increased. This goal is achieved via either genetic or chemical modifications of hemoglobin and four different HBOCs have been considered: cross-linked hemoglobin, polymerized hemoglobin, hemoglobin conjugated to macromolecules and encapsulated hemoglobin. Although there is an increasing concern that the worsening

shortage of blood donors will eventually limit the availability of human hemoglobin for processing [18].

Cross-Linked Hemoglobin

Diaspirin cross-linked hemoglobin (DCLHb) is the prototype molecule of this category of blood substitutes. It consists of cross-linking between the two α -chains that lend stability to the molecule. The cross-linking agent is bis (dibromosalicyl) fumarate (DBBF). DCLHb made from outdated human blood has a shelf life of approximately 9 months when frozen and 24 h when refrigerated. The intravascular half-life is 2–12 h and is dose dependant. Clinical trials proved that this compound did indeed raise blood oxygen content. However, it also caused intense vasoconstriction resulting in increased systemic pressure, reduced cardiac output, and increased vascular resistance. Hence, no net benefit was derived from this product. A great deal of work on DCLHb was initially performed by the US Army. However, due to significant adverse effects associated with it, the army discontinued further development of this molecule. Subsequently, Baxter Healthcare halted further development of DCLHb in 1998 after the product failed trials in patients with stroke and trauma [19].

Polymerized Hemoglobin

Polymerization of Hb through intermolecular cross-linking increases the size of molecules through the formation of Hb oligomers. In the process multiple Hb proteins are linked together through the use of dialdehydes, such as glutaraldehyde and glycoaldehyde. The increase in size as a result of polymerization prevents the rapid excretion of the molecule, prolonging the Hb plasma half-life. PolyHeme is a prototype glutaraldehyde cross-linked polymer of human Hb in which two or more tetramers are covalently linked. A pyridoxal molecule is incorporated into each tetramer of PolyHeme to facilitate oxygen offloading. It has a half-life of 24 h, a shelf life longer than 12 months when refrigerated. Currently, PolyHeme is being tested in phase III pre-hospital trauma study. Results of a trial published in 2002 showed that 75 % of patients with red cell hemoglobin levels less than 1 gm% survived traumatic injury after receiving PolyHeme as compared to 16 % of historical controls at the same hemoglobin level [20].

Bovine hemoglobin is also a potential source of hemoglobin, which after modification can be used in human subjects. Its production is based on reacting pure bovine hemoglobin with three chemicals: o-adenosine 5'-triphosphate (o-ATP), o-adenosine, and reduced glutathione (GSH). This process chemically modifies the bovine

hemoglobin to generate “beneficial activities”. The o-adenosine can counteract the hemoglobin properties that cause narrowing of blood vessels as previously seen with other substitutes. The GSH with adenosine attached to it has been shown to reduce the inflammation that is often caused by free hemoglobin. Hemopure (inter-molecular cross-linked cow hemoglobin) is smaller in size (upto 1,000 times smaller than a typical red blood cell) and has less viscosity than human red blood cells. It can carry more oxygen at a lower blood pressure than red blood cells and can also carry oxygen through partially obstructed or restricted blood vessels, where RBC's cannot reach. Hemopure was licensed for compassionate use in humans in South Africa since 2001, but US Food and Drug Administration has imposed a ban on further clinical testing of the product on human test subjects in the United States.

Recombinant Hemoglobin

It is cross-linked hemoglobin made by genetically altered organisms such as the bacteria *Escherichia coli* or *Saccharomyces cerevisiae* yeast. Recombinant hemoglobin is obtained by inserting the gene for human hemoglobin into bacteria and then isolating the hemoglobin from the culture. A few parts of the amino acid sequence of hemoglobin produced are replaced to prevent dissociation into dimers and to help maintain adequate oxygen affinity. This altered human hemoglobin coding segment DNA can be inserted into the bacteria or yeast DNA allowing for the manipulation of the gene itself to create variant forms of hemoglobin. 1 unit of hemoglobin solution can be produced from 750 l of *E. coli* culture [18]. This recombinant hemoglobin leads to adverse effects causing vasoconstriction, GI distress, fever, chills, and backaches, therefore trials have been discontinued.

Role of Nanotechnology

Nanotechnology refers to the application of scientific knowledge in any field, with the basic characteristic of the particles used in the range of 1–100 nm (nm). Nanotechnology has already proved its utility in the field of medicine (Nanomedicine), cancer diagnosis and treatment, treatment of infections, molecular and genomic modifications, to compile in few words nanotechnology is being used in all the branches of medicine.

Nanotechnology can also be used to develop artificial Hb. The first nanobiotechnological approach reported in the literature is the cross linking of Hb into polyHb (PolyHb) of nanodimension thickness [21, 22]. If the emulsion is made even smaller, then the whole artificial

product with its PolyHb (comprising 4–5 Hb molecules) becomes a nanodimensional structure. Glutaraldehyde can be used to crosslink Hb into soluble PolyHb of nanodimension [23]. From this PolyHb nanodimension artificial cells can be formed, either in the form of lipid-vesicles containing Hb or biodegradable polymeric membrane artificial cells containing Hb [24, 25].

Even in the form of tetramers Hb molecule can cross the intercellular junction of blood vessels to cause adverse vasopressor effects. To overcome this problem, principle of nanobiotechnology can be used to assemble Hb molecules into nanodimension polyHb [21–23]. Bifunctional agents can cross-link the reactive amino groups of Hb to assemble Hb molecules into polyHb (polyHb). Sebacyl chloride was the first bi-functional agent used to form membrane of nanodimension thickness [21, 22]. Another bi-functional agent, glutaraldehyde was the first to be used to cross-link Hb and trace amount of catalase (CAT) into soluble nanodimension structure [23]. In the glutaraldehyde procedure, cross-linking is adjusted so that the cross linked PolyHb is in a soluble state. This cross-links protein molecules to one another (intermolecular), it can also cross-link the protein internally (intramolecular). PolyHb preparation by nanotechnology must contain less than 2 % of tetrameric Hb, otherwise, infusion can result in vasopressor effects as the tetramer crosses the intercellular junctions of blood vessels to bind nitric oxide needed for normal vasoactivity. This cannot be done just by increasing the degree of polymerization alone since this would result in very large polyHb that is not stable in solution. To prevent this situation, polyHb containing about 10 % of tetramers is prepared, and the tetramers are then removed by size exclusion chromatography and filtration using plasmapheresis membranes [24]. PolyHb is safe and does not cause any complement activation, adverse effects, toxicity as seen with monomers, dimers or even tetramers. Gould's group has carried out Phase III clinical trials on 171 patients who received PolyHeme during surgery to a historical control group of 300 patients who refused red cells for religious reasons. The PolyHeme patients received up to 20 units and the study compared their 30 day mortality rates against the control. PolyHeme maintained total hemoglobin concentration in the 7–10 g/dL range and the mortality rate in this group was 25 %, compared with a mortality rate of 65 % in the historical control group, with no reported side effects [26]. The supply of Hb from outdated donor blood can be a limiting factor, a glutaraldehyde cross linked bovine polyHb (Hemopure) has been developed and tested in phase III clinical trials [27]. Therefore nanotechnology gave the breakthrough that was needed to develop artificial blood and was eagerly awaited and tremendously researched the world over for decades, but the task is not over, it had just begun.

Ischemia–Reperfusion Injuries

In ischemic injuries there is development of oxygen free radicals [28], because ischemia stimulates the production of hypoxanthine. When the tissue is perfused again with oxygen, hypoxanthine is converted into superoxide. Superoxide results in the formation of oxygen radicals, which can cause tissue injury. Prof TMS Chang developed the idea to assemble Hb with CAT and superoxide dismutase (SOD) into nanodimension PolyHb–CAT–SOD complex that combines both, the oxygen carrying function as well as the ability to remove oxygen radicals (just as most enzymes in the body function as multienzyme system with cofactor recycling). After basic research on artificial cells containing multienzyme system [29], their possible utility in proper functioning of the artificial cells was studied. The artificial cells containing three different enzymes carbonic anhydrase, CAT and SOD can convert metabolic wastes (e.g. urea and ammonia) inside the artificial cell into essential amino acids [30]. The needed cofactor, NADH can be recycled and retained inside the artificial cells by cross-linking it to dextran or by using a lipid-polymer membrane. All the multienzyme systems present in the natural red blood cells can be included inside nanodimension artificial red blood cells [31]. Human SOD and CAT are available in the recombinant form and safe for use in human. However, heterologous source of these enzymes may result in immunological reactions. During the cross-linking process, there is minimal formation of metHb when Hb is cross-linked with SOD and CAT [24]. However, when cross-linking is carried out with Hb alone, there is marked formation of metHb during the preparative procedure. This shows that cross-linking Hb with SOD and CAT may also provide oxidative protection during the preparation of polyHb. Oxygen radicals can oxidize the Fe^{2+} -heme component of Hb to Fe^{3+} -heme that can stimulate the production of superoxide ($\text{O}_2^{\cdot -}$) [28]. Furthermore, excessive oxidative damage to Hb leads to the release of iron from heme. In the presence of free iron, ($\text{O}_2^{\cdot -}$) and H_2O_2 rapidly react to produce hydroxyl radical ($\cdot\text{OH}$) (i.e. Fenton's reaction).

Hydrogen peroxide can also react with Hb to produce ferrylHb. These can promote cellular injury by reacting with carbohydrates, nucleic acids, and proteins, and these reactive species can also cause lipid peroxidation. The production of oxygen free radicals and lipid peroxidation in ischemic injuries can also be prevented with the use of PolyHb–CAT–SOD. Another beneficial effect is increased

half-life, when given alone enzymes stay in the circulation for a very short time period and there is rapid removal of free SOD and CAT (10 and 20 min., respectively). In the form of PolyHb–CAT–SOD the circulation half life is 24 h, and with increased activity of these enzymes [24].

Furthermore, unless cross-linked to Hb, these enzymes are not located in close proximity to Hb, and thus are less likely to give adequate protection from Hb initiated oxygen radicals. The attenuation in hydroxyl radical generation with PolyHb–SOD–CAT shows promise for its potential role as a protective therapeutic agent in clinical situations of ischemia and oxidative stress as in stroke, myocardial infarction, sustained severe hemorrhagic shock, organ transplantation and in cardiopulmonary bypass.

Artificial RBC's

With the successful development of synthetic Hb and later combining it with useful and functional enzymes that are present in natural occurring RBC's, efforts started to develop a fully artificial red blood cell. There were many earlier attempts to make artificial RBC's but were unsuccessful because the circulation time of such artificial cells was too short, to be of any therapeutic use. These artificial RBCs, even with diameters of down to one micron, survived for a short time in the circulation after intravenous injections. Studies showed that the long circulation time of natural RBCs is due to the presence of neuraminic acid on the membrane [22]. This led to the idea of changing surface properties of the artificial RBCs [22].

Lipid Membrane Artificial Cell

With the help of nanotechnology larger artificial cells can be prepared with a lipid membrane in the form of lipid-protein and lipid-polymer membrane [22]. Some researchers also reported the preparation of submicron (0.2 μ diameter) artificial RBC's using lipid membrane vesicles to encapsulate Hb [32]. Although, this increased the circulation time significantly, but it was still rather short. Along with a short circulation time, the large amount of lipid used to encapsulate the artificial cell can decrease the phagocytic function of the RES. In traumatic hemorrhagic shock, the RES needs to be efficient in order to remove the contaminating microorganisms. Furthermore, on reperfusion of the ischemic tissue by these lipid coated cells, the membrane lipids may induce lipid peroxidation leading to an unwanted increase in reactive oxygen free radicals which already were responsible for the ischemia reperfusion injury. One more problem is that of oxidation of Hb in the presence of O_2 to metHb. The lipid membrane

is impermeable to reducing agents present in the plasma like glucose, ascorbic acid and glutathione which are necessary for the methemoglobin reductase system inside red blood cells to convert metHb back to Hb. Many investigators have carried out research to improve the preparation and increase the circulation time of the artificial hemoglobin and convert metHb to Hb again. The most successful approach is to incorporate polyethyleneglycol (PEG) into the lipid membrane of artificial RBC resulting in an increased circulation half-time of more than 30 h [33].

Nanotechnology Based Biodegradable Polymeric Membrane Based Artificial RBCs

Biodegradable polymer, polylactic acid (PLA), for the microencapsulation of Hb, enzymes and other materials was started to be used in place of lipid membrane [34]. More recently, nanotechnology based artificial RBCs were prepared of about 100 nm mean diameter using PLA, PEG-PLA membrane and certain other biodegradable polymers [35–37]. The Hb nanocapsules, prepared with PLA shows that they are spherical and homogeneous. Their diameter ranges from 40 to 120 nm, polymer membrane thickness is 5–15 nm, and most of the lipid membrane in Hb lipid vesicles is replaced with biodegradable polymeric membrane material. This marked decrease in the lipid component would lessen effects on the RES and lessen lipid peroxidation in ischemia–reperfusion. A number of enzymes like carbonic anhydrase, CAT, SOD and the metHb reductase system, normally present in RBC have been encapsulated within polymeric membrane based artificial RBC and these enzymes retain their activities inside [24]. For example the metHb reductase system incorporation into the nano artificial RBC showed that this can convert metHb to Hb. In PLA nano artificial RBC, the membrane can be made permeable to glucose and other small molecules, this allows the metHb reductase system to function optimally. The inclusion of RBC enzymes prevents MetHb formation even at 37 °C and they can also convert MetHb to Hb at 37 °C. In addition, unlike lipid membrane, the nanocapsule membrane allows plasma factors like ascorbic acid to enter the nanocapsules to prevent MetHb formation [24].

Conclusion

Blood transfusion is a potentially lifesaving measure, but transfusion has its own drawbacks and is associated with short and long term complications. Use of blood components is fastly increasing and their use is more rationale,

but donor shortage is an ever ending problem with either blood or blood component transfusion. Nanotechnology has rejuvenated the idea of an artificial blood substitute. This approach is being used to develop artificial red blood cells with their inherent enzyme and other supportive systems. In future, it could be possible that a complex system may be developed which can replace blood and can also obviate all the shortcomings of blood transfusion.

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