OxyVita: History, Studies, and Future

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Background

OxyVita is one of the polymeric hemoglobin-based oxygen carriers (HBOC), it was invented by the team of Professor Enrico Bucci at the University of Maryland in 1999. OxyVita does not have blood type, just like other HBOCs. It is presently prepared from bovine hemoglobin; however, any mammalian hemoglobin could be used for this purpose. The raw materials are readily available and ubiquitously existing. The zero-link polymerization technology is a well-known technique in biochemistry and the pharmaceutical industry. However, prior to OxyVita, this technique has never been applied for hemoglobin polymerization. During the process of utilizing the zero-link polymerization technique, only activators are used to create a macropolymer of hemoglobin, rather than utilizing linking agents. Therefore, the Zero-link polymerization does not leave any residual chemicals/ polymerization agents/linking agents in the final molecule.

OxyVita is formulated to have an extended shelf life and it can be powderized. Its development and testing process have spanned over 20 years, with significant accomplishments and accolades in the field. The key to the success of the OxyVita product is a well-controlled design and its manufacturing process, allowing the product to be tailored to meet various needs in human and veterinary applications, such as a treatment in hemorrhage, traumatic brain injury, myocardial ischemia, sickle cell anemia, and many other clinical situations, as proposed further within this chapter.

The product has undergone a variety of investigations over the time of its life-span with a significant portion of studies being performed by third party researchers and not funded by the manufacturing company. The developmental evolution of the product has had significant influences of some prominent researchers. These previous research findings have laid the foundation to thoroughly assess the potential of the product and its potential future clinical applications.

Preparation of OxyVita

Detailed description of the process and zero-link polymerization technique can be found in literature [1, 2].

Briefly, the technique includes the following processes:

1. Raw material: bovine blood, but any mammalian blood can be used.
2. Preparation of raw material: centrifugation and lysis of red blood cells (RBC). This material is processed using several runs of centrifugation with 3000RPM - 4000RPM, to ensure the highest purity.
3. The Hb obtained is thereafter β-β cross-linked [bis(3,5dibromosalicyl-adipate)] to stabilize the hemoglobin tetramers.
4. Polymerization is then initiated using the carbodiimide, EDC [1-ethyl-3-(3-dimethylaminopropyl) carbodiimide], which is responsible for the activation of the side chain carboxylate groups on the hemoglobin surface. A complex is formed from the C- terminal on Glu and Asp globin side chain carboxylate groups. These activated species then react with the side chain of the lysyl residues of an adjacent hemoglobin tetrameric molecule to form a stable amide bond (covalent), referred to as a pseudo-peptide bond [3].

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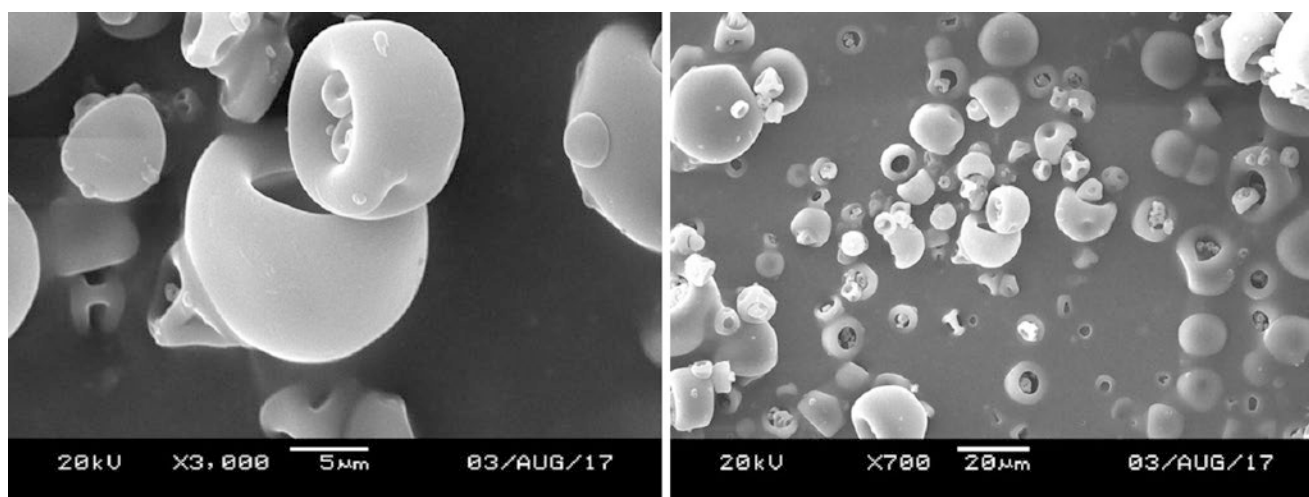
Fig. 25.1 Scanning Electron Microscopy (SEM) of OxyVita powder

Table 25.1 OxyVita Solution vs OxyVita Reconstituted Powder

OxyVita®C Solution results	Analysis	Reconstituted powder of OxyVita®C, results
6.35g%	g% of Hb Ferric (Fe^{2+})^a	6.30g%
0.35g%	g% of Met Hb Ferrous (Fe^{3+})^a	0.39g%
0.03g%	g% of Ferryl (Fe^{4+})^a	0.04g%
100%	Size Exclusion Chromatography (SEC)^b – % of POLYMER	100%
0.0	Size Exclusion Chromatography (SEC)^b – % of Tetramer (64MDa)	0.0
0.0	Size Exclusion Chromatography (SEC)^b – % of other sizes (below 64MDa)	0.0
312	Osmolarity^c mOsm/kg	335
1.013	Density of solutions^d g/mL	1.045
0.975	Spectral Radio^a A$_{576}$/A$_{540}$	0.965
5.95	Oxygen Affinity^e P$_{50}$	6.59
360	Average Hydrodynamic Radius^f Å	359
17.453	Average Molecular Weight^f MDa	17.337
7.295	pH	7.353
N/A	Reconstitution Time sec.	<60

^{a}UV/Vis HP Spectrophotometer, ^{b}LC with 600 mm × 15 mm column, ^{c}Osmometer –FLEX, ^{d}Oswald densitometer, ^{e}Hemox Analyzer (T-37°C, pH = 7.50), ^{f}Dyna-Pro 801 (Protein Solution)

5. N-hydroxysulfosuccinimide (sulfo-NHS) is introduced to the carbodiimide reaction, and results in the formation of an intermediate sulfo-NHS ester, which then reacts with the amino groups [4]. By altering the relative amounts of sulfo-NHS and EDC within the reaction mixture, the extent of the polymerization process can be manipulated, allowing for better control of the average molecular sizes during the manufacturing process.

6. The powder form of OxyVita Hb (Fig. 25.1) is produced by either lyophilization or spray-drying of the liquid form of OxyVita. After re-constitution, the powder has almost identical characteristics to the solution from which it is derived. The comparison of the characteristics of both liquid and powder forms of OxyVita is presented below (Table 25.1). The powder's shelf -life is 5 years under a wide range of climatic conditions.

The original preparation contained a higher amount of heterogeneous distribution of high molecular weight (MW) species with an average of 25 MDa [5, 6]. OXYVITA, Inc. acquired the license for commercial manufacturing from the University of Maryland, and, thereafter, refined the process, mostly to achieve higher homogeneity, as well as adjusting the average MW of polymer.

The Properties of OxyVita

Understanding the relationship between the structure and the function of oxygen carriers was pivotal in the development of this product. OxyVita belongs to the newer generation of hemoglobin-derived oxygen carriers. Thus, during the development of OxyVita, we have been able to benefit from the lessons learned from the development of earlier products. Specifically, we are more aware of, and paid more attention to, the potential side effects of extravasation, vasoconstriction and oxidative events, which were prevalent in the previous generations of similar products. OxyVita was therefore specifically designed with the aim of avoiding these side effects.

The controlled process of zero-link polymerization allows for specific, pre-defined characteristics, such as a specific MW and hydrodynamic radius of the product, a negative sur-

face charge, precise oxygen affinity (P_{50}), as well as viscosity similar to plasma and a relatively long retention time (Table 25.1).

The characteristics of OxyVita are integral to the product's functional attributes. The polymer is very stable, due to its intramolecular and intermolecular bonds; strong amide bonds connect tetrameric, previously cross-linked hemoglobin molecules into a polymer; on average, there are around 1000 tetramers per polymer. The specific crosslinking on β-β lysines 82 further stabilizes secondary and tertiary structures of the natural bovine hemoglobin, which results in increased conformational stability with a resistance to unfolding. Thanks to this, the heme-iron moieties get increased protection against molecular unfolding, which would otherwise result in heme exposure and iron loss, and consequently the loss of the oxygen carrier's effectiveness in its ability to carry oxygen. Previous studies on the structural integrity of OxyVita and several other natural hemoglobins, proved OxyVita's reduced tendency to unfold. This was done through an investigation of changes in the Soret region (350–450 nm), which is extremely sensitive to alterations within the heme [1, 7, 8].

The molecular size of OxyVita, consistently an average of 17MD, with an average hemodynamic radius of 360 Å and its negative charge on the polymer's surface, prevents extravasation by simply following the rules of physics, specifically, similar charges repel and size exclusion. The polymer is larger than any pores in the vasculature, therefore, it doesn't extravasate. As the polymer stays within the vasculature upon the injection, it doesn't scavenge NO from the external walls of blood vessels, in turn, causing no changes in mean arterial blood pressure (MAP). This has been shown through animal studies reporting the maintenance of the mean arterial pressure, in exchange transfusion and top-load studies, amongst others, while oxygenation of tissue was achieved [9, 10].

The P_{50} of the product is low, at 4–6 mmHg, compared to RBCs and several other products. This design's intent is facilitating oxygen release in microcirculation. Low P_{50} correlates to high oxygen affinity, meaning oxygen release occurs only with the presence of oxygen debt in the tissue, so, not in the macro-circulation.

Selected Studies

Years of in vitro and in-vivo studies with OxyVita brought significant insight into the mechanisms and potential physiologic and pharmacological features of this product. The performed studies have served as an assessment of the safety profile of the product for the potential future application for human and veterinary usage.

Summarizing the published studies conducted, work was performed on assessment of vascular response to infusion of polymer [9], cerebral ischemia and blood flow [11, 12], resuscitation [13], coagulation behavior [14] and redox behaviour [8], among other physiological effects of polymer action. We've learned that upon use of these large polymeric hemoglobin molecules, no hemoglobin was evident in the hilar lymph of rats and no increase in MAP was observed within both anesthetized and awake cats [9]. The study by Mito et al. [11] showed a reduction of cerebral infarct size by 39% in the mouse brain when treated with OxyVita. This beneficial effect seems to be dependent upon the concentration of this high-affinity hemoglobin polymer (P_{50} = 4 mm Hg), with 6% being the ideal concentration, while lower than 3% having no beneficial effect, and the molecular size of the hemoglobin polymers, with intermediate size being the best. It is likely that the imtermediate size could be better adjusted to the mouse's circulatory system [11]. In a DARPA small volume resuscitation study with a rat hemorrhagic shock model induced by bleeding 60% of total blood volume, an OxyVita-augmented hypertonic "cocktail" was proven to be a viable treatment for improved survival and MAP support [13]. Jahr et al. [14] investigated the impact of OxyVita on blood coagulation and found that minimal coagulopathic effects should be expected with the use of OxyVita at the anticipated effective dose of 10 g or 2-3 ml/kg. Bucci et al.[4]concluded that the extravasation and significant MAP increase were avoided when using the hemoglobin polymer with an average MW of 25 MDa and P_{50} of 18-30 mmHg. Their study showed that OxyVita delivered oxygen to tissue, providing either vasodilation or vasoconstriction according to oxygen needs *in vivo*. These studies also showed that cell free hemoglobins are potentially more efficient oxygen carrier than RBCs for delivering oxygen to tissue. Harrington et al. [7] conducted a redox behavior comparison study of OxyVita and natural acellular hemoglobin, *Lumbricus terrestris* Hb and *Arenicola marina* Hb, confirming that structural integrity in the reduced state (heme-Fe^{+2}) of OxyVita Hb is evident by its greater resistance to molecular unfolding than either of these natural hemoglobins; the reduction of met (heme-Fe^{+3}) OxyVita Hb to oxyHb occurs slowly in the presence of either ascorbic acid (70% reduction in 560 minutes) or β-NADH (40% reduction in 90 minutes). Wollocko et al. [8] studied the impact of oxidative stress by comparing OxyVita to myoglobin and natural bovine Hb, when exposed to unfolding agents. Little or no change in the Soret Maxima, small decreases in absorbance signal and a high value of unfolding midpoints demonstrated the structural integrity and strong resistance to molecular unfolding, thus limiting OxyVita's oxidation within the circulatory system. Song et al. [10] compared vasoactivity and tissue oxygenation resulting from top-load infusions of high molecular weight polymers of OxyVita (oxygenated) and OxyVitaC (COform) using the rat model. Neither product caused vasoconstriction in the rat spinotrapezius muscle in the study, while maintaining tissue oxygenation.

Traumatic Brain Injury (TBI)

Abutarboush et al. [15] undertook the challenge of evaluating the effects of OxyVita on systemic blood pressure and cerebral pial arteriole diameters in healthy rats. The interest of looking into the effects on cerebral pial arterioles was driven by the search for a future therapeutic agent able to safely deliver oxygen to the brain tissue in patients with TBI. Four incremental dosing regiments were used in the study, 2 mg/kg, 25 mg/kg, 50 mg/kg and 100 mg/kg. The control group received saline (0.9%NaCl) and Hextend as a treatment. The measurements of blood pressure were taken at planned intervals, and the sizes of the pial arterioles were observed through intravital microscopy through a cranial window.

The heart rate (HR) did not change significantly during the experiment in the OxyVita group, and increased slightly in the saline group. The study was designed as four, 30-minute infusions, with 10 minute intervals between them. As OxyVita retention time in the circulation is 3 hours, as compared to about an hour or less for saline, the short 10 minutes interval between infusions was not sufficient for the MAP to return to the base line in OxyVita treated animals, and therefore as a result of cumulative effect, the OxyVita group showed an increase in MAP. This is consistent with the previous findings in topload model when an increase in MAP was seen without vasoconstriction in skeletal muscle arterioles [10].

The measurements with intravital microscopy were conducted on small (<50um) and medium (50–100um) pial arterioles, and no vasoconstriction was observed in either of the vessels as a result of injection of four doses/cumulative doses of OxyVita. The injection of vasoconstrictive aqueous barium chloride was performed at the end of the experimental work to confirm proper responsiveness of the arterioles during the experiment.

This study unveiled a very promising future for an application of OxyVita in patients with TBI.

Whole Blood Approach

The main goal of developing oxygen therapeutics was to address the world's critical need for blood transfusion alternatives. The hope of the industry is to develop an artificial blood product with the features of being donor independent, unaffected by contaminations to the blood supply and easy stockpiling for emergency or other clinical indications.

It is recognized that the OxyVita product improves oxygen delivery capacity of blood in in-vitro and animal studies, potentially replacing the function of RBCs. OxyVita can be formulated into a powder form (Fig. 25.1), which is benefi-

cial because it will offer the product with a much longer shelf-life and no strict storage requirements. The powderization process involves the following steps: washing in a buffer solution containing 1.5% of sucrose and trehalose, followed by a treatment with OXYVITA's proprietary molecular protectant, and then lyophilization or spray-drying. The protectant preserves the structural and functional integrity of the components of the powderized hemoglobin polymers, yet allowing for easy reconstitution. Figure 25.2 presents a comparison of the product's Size Exclusion Chromatography before and after spray-drying.

Experimentation with the powderization technology led to its application to other blood components, plasma and platelets. There is an enormous demand for a much longer platelet shelf-life. Platelets generally have only 5 days shelf-life. Previously conducted experiments have shown that platelets collected from a donor and lyophilized on day 4 after collection, using the same methodology as for lyophilizing OxyVita, can be stored at room temperature for 30 days. Thereafter, stored platelet activity was compared to fresh donor platelets, using an aggregometer, PAP-8, with two agonists for the analysis, ADP and collagen [16]. The reconstituted platelets, on day one after the powderization, presented the exact same activity as fresh platelets, and after 30 days of storage at room temperature, their activity decreased by 7% compared to fresh donor platelets. The studies were repeated after 60 days of storage, a decrease of platelet activity by 14% was documented. This data may be extrapolated to lyophilized platelet activity of not less than 58% of fresh platelets, after 6 months.

Previously conducted studies performed combining OxyVita with dried plasma and platelets, utilizing OXYVITA's in-house prepared platelets [16], demonstrated that this coexistence is possible, with no interference, serving as a proof of concept for a possibility of complete blood transfusion alternative product in the future.

Future Direction

Research on OxyVita continues, with many directions being contemplated or explored. Although the main purpose of this product is a safe and effective alternative for the blood transfusion, through the years of research, many other potential applications have arisen. These include applications that are not currently considering a blood transfusion as a primary mode of treatment- such as myocardial infarction (MI), stroke, carbon monoxide poisoning, sickle cell anemia (SCD) - amongst others.

Some of the studies are presented below. Their scope is briefly described.
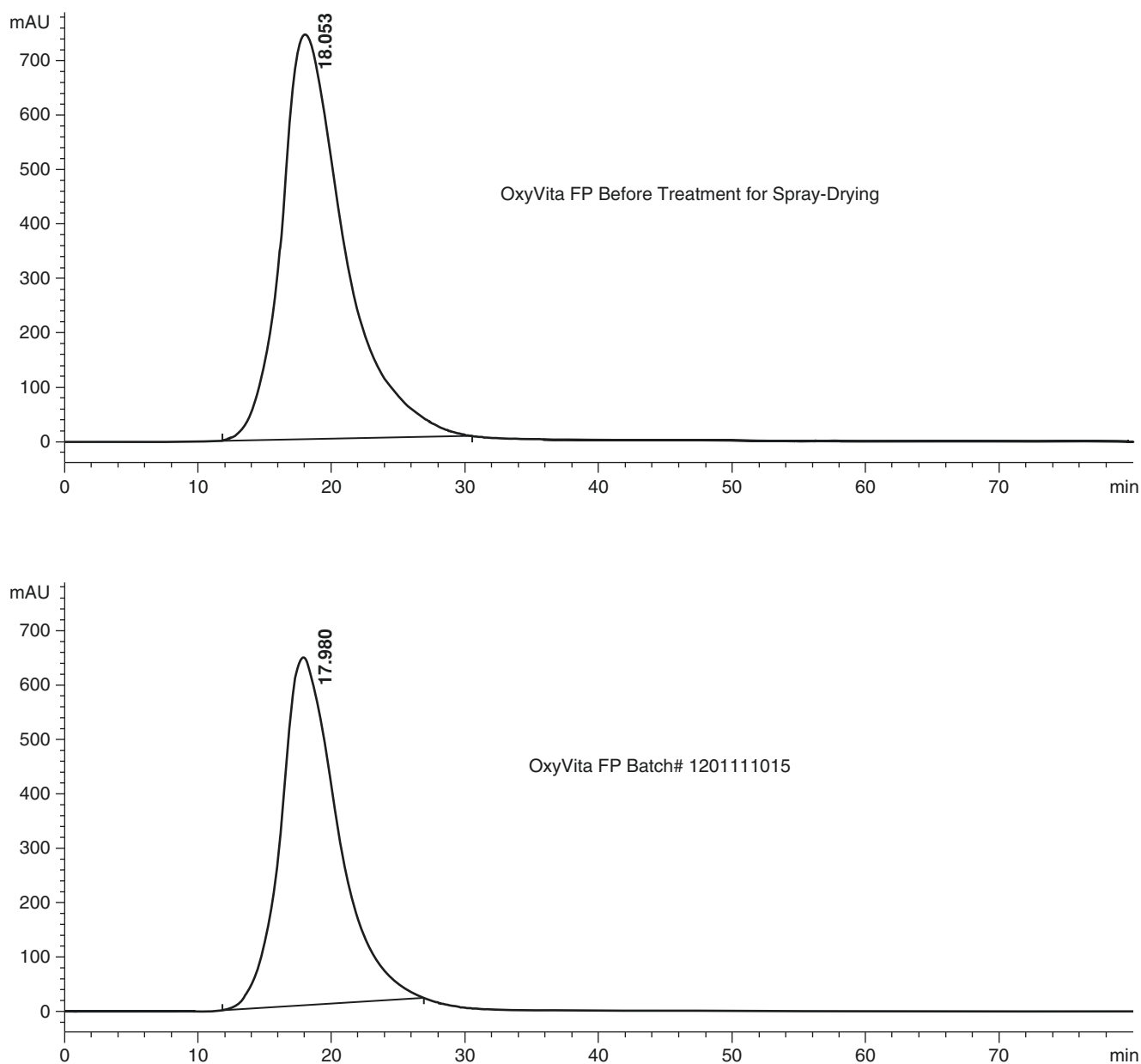
Fig. 25.2 Size Exclusion Chromatography (SEC) analysis: OxyVita liquid and reconstituted powder

Assessment of Effectiveness of OxyVita in the Treatment of Myocardial Infarction and Cardiac Arrest

Ischemic heart disease is the leading cause of death worldwide [17]. According to the statistics provided by the American Heart Association (2019), over 356,000 CAs occur outside the hospital annually, and nearly 90% results in patient mortality [18]. Over 750,000 of CAs occur in the hospital annually with a reported mortality as high as 78% [19, 20].

Based on the limitations of current protocols, specifically the lack of oxygen delivery in the metabolic phase(3rd phase)

of CA, HBOCs can be a solution for this unmet need, as they are potentially able to address the ischemia, reperfusion injury, and organ damage resulting from CA.

OxyVita's characteristics make it an ideal candidate. The high oxygen affinity, as noted by Jahr, et al. allows significant oxygen offload to hypoxic tissue in low doses, such as 2-3 ml/kg [21]. This makes it a low volume injection allowing for an insignificant top load and avoiding fluid overload.

Based on the physico-chemical characteristics of the product and its in-vivo behavior [22], as well as the conducted optimization of its parameters for this application, it is hypothesized that early administration of OxyVita during

the first stages of CA, alongside cardiopulmonary resuscitation, can minimize the occurrence of tissue ischemia while avoiding reperfusion injury. The low P_{50} of OxyVita would allow the release of oxygen under conditions of hypoxia only, enabling oxygen delivery to sites vulnerable to ischemia before damage can occur. Tissue perfusion would be optimized, specifically in the metabolic phase, due to OxyVita's ability to avoid vasoconstriction, while allowing for better oxygenation.

Evidence suggests that OxyVita, like other modified hemoglobins, does not lead to activation of complement, once it is introduced as an oxygen carrier, what has substantial implications when considering reperfusion injury [23]. Currently studies are being conducted using Sandwich ELISA with monoclonal antibody to C3a neoepitope [24]. Without complement activation, one of the major pathways of this pathology is greatly hindered, broadening the usage of this treatment option under hypoxic conditions.

Effectiveness of OxyVita in Treatment of Carbon Monoxide (CO) Poisoning

CO poisoning is one of the leading causes of death due to poisoning in the United States. While fire-related smoke inhalation is responsible for most of the cases, non-fire related CO poisoning is still responsible for up to 50,000 emergency department (ED) visits and 1200 deaths per year

[25]. CO diffuses rapidly across the pulmonary capillary membrane and binds to the heme moiety of hemoglobin (Hb) with approximately 240 times the affinity of oxygen, causing allosteric changes that significantly diminish the ability of Hb to bind and deliver oxygen to the tissues. Poorly treated CO poisoning can lead to rapidly deteriorating bodily conditions that can result in organ failure and tissue death. Hyperbaric oxygen therapy (HBO) has been shown to have the potential of lowering the mortality rate and preventing late neurocognitive deficits. However, a significant impediment is the limited availability of hyperbaric chambers. Thus, the availability of other methods/products to support oxygen delivery to tissues within the body is essential for the effective treatment of CO poisoning. HBOCs can be a solution for this unmet medical need.

Initial proof-of-concept studies with OxyVita treatment in bench simulation of CO poisoning were already conducted and showed promising results. It is theorized that with the different tertiary and quaternary structure of the OxyVita polymer, its ability to exchange between the CO and oxygen ligands might be totally different than that of the RBCs [26].

OxyVita effectiveness in CO poisoning was investigated by evaluating its oxygen binding capacity and ability to exchange between CO and oxygen ligands over time, at different pH, in various combinations with human blood/tetramers, using UV/Vis spectrophotometry.

As Fig. 25.3 shows, the exchange of CO-O_2 ligands start to occur within the first 5–7 minutes after the addition of the
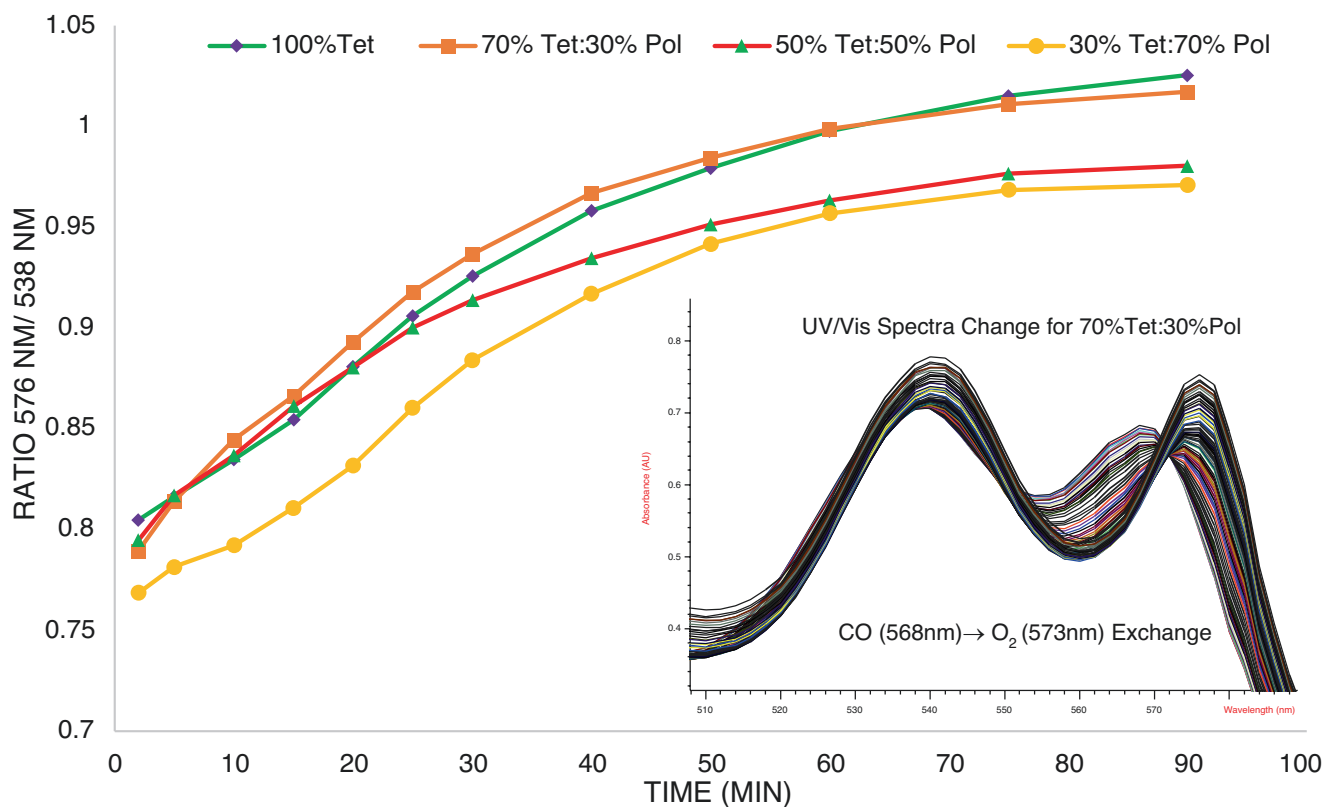
Fig. 25.3 OxyVita and Tetramers: CO and O_2 ligands exchange rate

oxygenated OxyVita to the CO saturated blood/tetramer solution. The solution reaches a good oxygen saturation level at about 15–20 minutes. A combination of 70% blood/tetramers with 30% OxyVita polymer was the most effective for the exchange of CO-O_2. The study indicated also that the rate of ligand exchange is affected by the pH of the solution (which would translate into the presence of acidosis or alkalosis in human body).

Further experiments are clearly necessary.

Organ Storage Solution

Organ transplantation is a clinically effective, life-saving measure. However, there is a large disparity between the number of donors' organ and the number of individuals on the waiting list. In January 2019, 113,000 individuals were on the waiting list. Regretfully, it is estimated that 20 people will die daily while waiting for a transplant [27]. One of the largest limitations of organ transplants extends from the maximum organ preservation times in which organs are viable from time of procurement to transplantation. This time allotment limits the geographical region for effective organ transplantation and time from organ removal to placement [28].

With the improvements in surgical techniques, aseptic and immunosupressive therapies, the main cause of failure in organ transplant procedures lies still in the condition of the transplanted organ. The longer is the procedure of transplant, including procurement, the worse the condition of the organ, as result of ischemia and hypothermia [29]. Improvement of the organ condition for transplant is seen as a space for improvement in the organ transplant field.

The most possible way to achieve this goal seems to be a formulation of an organ storage solution with an oxygen carrying capacity. This would allow the organ to maintain aerobic metabolism outside of the human body and prevent anaerobic metabolism, an eventuallity, which leads to cell dysfunction and death. The application of an organ storage/preservation solution could allow for the extension of storage time outside the body, and therefore an extended time to match, transport, and expand the geographic limitation.The betterment of the organ's condition would result in a greater successs of transplant surgeries.

It would seem to be essential to have an oxygen carreier as a component of the organ storage solution to maintain oxygen delivery. This contrasts previously attempted techniques such as oxygenation of a perfusion solution/and or gaseous oxygen pillow over the transported organ. Excessive amounts of oxygen in the environment of the stored organ contributes to the formation of free oxygen, which creates an avalanche of oxygen radicals (ROS) [30]. These radicals can lead to deterioration of the cell walls, disruption of ion equilibrium, changes of osmolarity, acidosis, and eventually, cell death. Only through delivery of the oxygen into microcirculation, the aerobic metabolism can be maintained and stable organ conditions can be achieved, leading to a higher possibility of survival of the transplanted organ tissue.

Proof of concept preliminary studies conducted at the UWM School of Medicine, in Olsztyn, Poland, evaluated the role of OxyVita as a component of an organ storage solution to preserve renal tissue in a rabbit model (material in preparation for publication).

Further studies are currently in progress on animal kidneys, aiming to assess the organ condition after 24–48 hours of storage in the storage solution with OxyVita, by analyzing serum chemistry and biomarkers, urine output and urine biochemical analyses, sonographic techniques for a real-time assessment of condition of stored organ's tissue and histopathology analysis by sampling during and after the storage procedure.

The Effect of OxyVita Hb on Erythrocytes Sickling in Patients with Sickle Cell Disease (SCD)

Millions of people worldwide are afflicted with SCD, a hereditary life-threatening genetic blood disorder, characterized by a point mutation in β-globin gene (HBB), leading to production of sickle hemoglobin (HbS). HbS polymerizes when deoxygenated [31, 32]. An HBOC which is capable of being used in a top-load application could serve as a mode of treatment when approaching/ or in the crisis state.

Due to the reduced oxygen-carrying capacity of HbS in SCD patients, preventing hypoxia by oxygen therapy has shown to be beneficial. Studies, both in vitro and in vivo, have shown that oxygen by itself may act as a potent antisickling agent, and works by preventing and reversing erythrocytes sickling in SCD [33]. However, the use of oxygen therapy still remains controversial because high levels of oxygen are linked to the suppression of erythropoietin, a key cytokine secreted by the kidneys to promote erythrocyte production. Oxygen therapy is, therefore, only recommended when oxygen levels drop below a critical threshold.

The analysis of Alayash et all [34] points the specific requirements for the characteristics of the oxygen carrier to be able to function effectively as the treatment for SCD. OxyVita fits the pointed requirements, like lack of NO scavenging, low P_{50} and resistance to heme-mediated oxidative reaction. Therefore, it should be a good fit for the treatment of SCD and currently undertaken study aims to determine whether, and the dose at which, OxyVita Hb, is able to delay in vitro HbS polymerization and decrease reversible sickled erythrocyte sickling.

In the current study, the focus is on the polymerization process of HbS itself. Previous studies have shown that the

amount of intracellular deoxygenated HbS polymer increases monotonically with decreasing oxygen saturation [35]. During the early stage of oxygen dependent HbS polymerization, the process of sickled red blood cell formation is reversible. The reversibly sickled erythrocytes (RSC) are able to return to the normal biconcave shape upon oxygen binding and prevent subsequent progressing to irreversibly sickled erythrocytes (ISC). OxyVita, with its small molecular size (MW = 17 MDa) and its oxygen-carrying capacity (P_{50} = 6.4 mmHg) is potentially capable of reaching the microvasculature and delivering oxygen to reverse sickling [36].

Currently planned study aims to: (1) Measure the protective effect of various concentrations of OxyVita on in vitro polymerization. (2) Measure the ability of OxyVita to assist in the reversal of RBC sickling in vitro. (3) Test the hypothesis that OxyVita slows down the creation of irreversible sickled RBCs.

After several circles of deoxygenation and HbS polymerization, a fraction of circulating erythrocytes is permanently deformed into a sickle shape even after vigorous oxygenation [35]; these are defined as irreversibly sickled cells (ISC). ISC are dense, dehydrated, and viscous with a low affinity for oxygen and a very short life span [37]. The short-lived, rigid ISCs make major contributions to the hemolytic events/vaso-occlusive crises in SCD patients. It therefore becomes a therapeutic interest to investigate approaches that may significantly slow down the progress of formation of ISC. The mechanisms proposed for the progress of RSC to ISC are due to the change of erythrocyte membrane properties. The decrease in blood oxygen partial pressure and the polymerized deoxygenated HbS activate a membrane channel called P_{sickle}. Open P_{sickle} channels allow the influx of Ca^{2+} and activation of the Gardos channels. The activation of Gardos channels eventually causes efflux of K^+ and Cl^-, followed by water loss and RBC dehydration. OxyVita Hb as an oxygen carrier, is able to increases the oxygen saturation and partial pressure of the arterial blood and inhibit the activation of P_{sickle} channels and Ca^{2+} influx. A protocol is in the works to test the effect of OxyVita on the ratio of RSC/ISC.

ROS Induced Oxidative Stress: Blood Transfusion Versus Blood Substitutes

Overproduction of reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, and hydrogen peroxide, results in oxidative stress, which leads to cellular damage. RBCs have an inherent ability to combat oxidative stress through enzymatic (peroxiredoxin-2, catalase, superoxide dismutase, and glutathione peroxidase) and non-enzymatic (glutathione, vitamins C and E, and urate) antioxidant pathways. Previous studies provided evidence that donated

RBCs, during blood processing and storage, will face the environmental challenges like lower temperature, differing pH and residual plasma, and imbalance between oxidants and antioxidants [38]. RBCs begin to accumulate oxidative lesions due to damages to its lipid and protein structures. Such alterations of RBC components may contribute to cell lysis with releasing vesicular microparticles including free hemoglobin, heme, and iron, which may contribute to the generation of additional ROS. It has been suggested that since these microparticles are abundant in iron, the free radicals are generated through iron dependent mechanisms - the Haber-Weiss and Fenton reactions [39].

Further, in diseases requiring chronic transfusions (like β-Thalassemia, myelodysplastic syndrome), significant iron loading leads to free iron exceeding the binding capacity of the plasma transferrin, and therefore, results in an increase in non-transferrin bound iron (NTBI). NTBI promotes the production of free hydroxyl radicals and the accumulation of transfusion-associated iron in tissues causing organ damages [40]. In addition to causing parenchymal organ damage by ROS via upregulation of biological cellular processes that increase cellular apoptosis, iron overload by repeated blood transfusions also causes dysregulation of the hematopoietic system, most probably by the suppressive effect on hematopoiesis. This includes an increase in apoptosis of hematopoietic progenitors, shortened life spans of mature RBCs, and inhibited differentiation of hematopoietic stem cells [41]. To alleviate this problem, iron chelating agents such as deferoxamine are introduced in iron overloaded environments. Deferoxamine has been shown to partially reduce iron overload and injury and block certain ROS-related signaling pathways. It is believed that the iron chelating agent serves as an antioxidant by eliminating free-iron species [42].

In an effort directed towards a safe and efficacious replacement for blood transfusion/RBCs transfusion, the ROS generation during the storage of HBOCs has to be taken into consideration. This assessment will also serve as a safety characteristic for clinical use of the blood substitutes. Moreover, the research should be extended to investigation of the benefits of usage of oxygen carrier treatment in repeated blood transfusion with the addition of antioxidants, such as deferoxamine as an adjunct therapy.

Previous investigations also showed that OxyVita has a greater tendency to resist molecular unfolding when compared to blood with tetramer hemoglobins, and therefore, OxyVita does not readily release free heme-iron into the circulatory system [8]. In the current study, we compare the level of ROS formation in the stored OxyVita product with the one of stored RBCs, the ROS levels after the introduction of ROS formation promoting agents, as well as after the addition of antioxidants, to mitigate the ROS damage, in order to conclude on the possibility of limiting/controlling the ROS formation process.

Summary

OxyVita, as a new generation of hemoglobin-based oxygen carriers, has had the benefit of avoiding the shortcomings of previous generations of products. The polymer has proven both by in-vitro and in-vivo animal studies, to have promises in many different therapeutic areas – many of which have not been considered to be a potential area for blood use. The characteristics of the product, as well as the ability to customize the molecule as necessary (size, P_{50}, etc), have granted a unique product that can fit many different applications.

Over time, the product has been shaped by the teams of researchers that have worked with it, independently, as well as collaboratively, with its creators. The future of this product is still being decided, as well as the direction that it will ultimately take. With potential for use in both human and animal markets, spanning from blood transfusion alternative to other unmet and previously unconsidered applications, there seems to be a multitude of avenues for the future. It is the hope of the teams that have worked with it that the product will progress to take a position to support and improve the healthcare industry in the coming years.

Key Points

- OxyVita is a new generation oxygen carrier, a polymeric hemoglobin manufactured utilizing zero-link polymerization technology, with very defined and specifically designed characteristics.
- Extensive in-vitro and in-vivo testing of OxyVita has brought significant insights into the physico-chemical characteristics of the product and its mechanisms of action, which translates into the product's functional attributes and potential physiologic applications.
- Research on the product continues to assess the full scope of the product's safety profile in applications as a human and animal therapeutic.
- The product's aim is to be a safe and effective alternative for the RBC/blood transfusion in the future.
- Other product applications are being considered for conditions currently treated with blood transfusions as well as for these in which this type of treatment is not considered as a primary mode of therapeutic intervention.

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