BLOODY GOOD:

PROS OF SYNTHETIC BLOOD SUBSTITUTES

By

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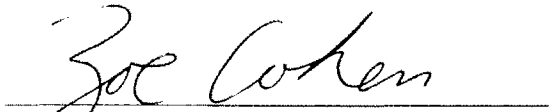

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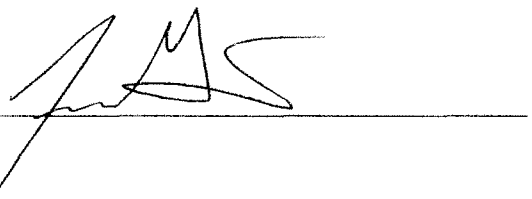
Signed: 

Abstract:

In today's society we unfortunately have and will continue to face the effects of issues such as war, poverty, trauma, and death; all of which have had an impact on the lives of people within these societies, be it religious, medical or social. An important, yet subtle common denominator present within these issues is blood. Blood and its transfusion have had an enormous impact on these issues and disappointingly, today doctors have been facing increasing difficulties with providing blood to those who need it; mainly shortages and donor complications. Today and in past decades researchers and medical doctors have been increasing their focus on the study of blood and its many fascinating features so as to create a relatively ideal blood substitute. The focus of this thesis is to acknowledge the progressions being made in the creation of blood substitutes and to gain an understanding of the positive impacts it would have on society as a whole. With blood substitutes one would not have to worry about donors, infections, or shortages. We delve into a world full of issues related to blood where scientists strive to create a substance that can meet the important goal blood accomplishes; maintaining life.

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Introduction:

Blood is arguably the most vital component of the human body. The delivery man of our bodies, it is able to transport what we need and take away what we don't. To the naked eye it is such a simple substance; red in color and liquid in state. But look a little closer and the complexity of blood reveals itself; a complexity that has been the subject of blood substituting for decades. The idea of creating an ideal blood substitute has been an ongoing issue for a considerable amount of time. It is dated back to the 17th century when Sir Christopher Wren suggested that opium, wines and ales could be used as blood substitutes.[1] Yet the modern age of blood transfusions began in 1901, when Karl Landsteiner discovered human blood group antigens.[2] This sparked an era of desire to advance the practice of substituting blood safely. Despite the struggles, this subject has continued to improve as medical research and technology have advanced.

In medicine today many problems related to blood substitutes have been of ongoing concern. The most common of these is a rapid loss of blood such as in hemorrhage; this is most prevalent when a trauma has occurred, resulting in volume depletion and shock. Be it car accidents or bullet wounds, both can and have been seen in hospitals, in public and on the battlefield. Other issues include the risk of infection when transfusing blood, treating dehydration and volume depletion, and the issue of correct blood types from donors to avoid an attack on the donor blood. Using medicine as its model is the issue of performance enhancing methods through the use of blood substitutes, such as blood doping. It is important to note that the intended advancements in blood substitutes and fluid replacement is not only prevalent in the medical field but also in the social environment, particularly in athletics and religion. The focus of this thesis is to delve into the research that has and is currently being done with blood

substitutes as well as focus on the positive impact it would have in different fields that desperately need it and would greatly benefit from it.

In order to better comprehend blood substitutes, their complexity and their importance, an understanding of natural blood and its components is essential. Blood consists of many components, but the most important are erythrocytes, plasma, plasma proteins, leukocytes and platelets. Each of these components plays a crucial role in the effectiveness of blood and a deficiency in any of them could pose serious problems for the success of blood substitutes. Still, today the main focus of blood substitutes has been on the characteristics of erythrocytes.

Erythrocytes, commonly known as red blood cells (RBCs), comprise about 40-50% of blood and are created by hematopoietic stem cells signaling with erythropoietin. Their structure can be described as that of a biconcave disk with no nucleus (Figure 1); this structure is vital to the red blood cell because it not only allows a greater surface area for oxygen diffusion, but it allows for circulation through tight passage ways, such as capillaries. Blood carries oxygen in two ways; around 3% is dissolved in the plasma while 97% is carried by the more notable hemoglobin located in RBCs.

Understanding hemoglobin is likely the most important factor in improving blood substitutes today. Its structure consists of a heterotetramer comprised of four subunits; two alpha and two beta chains (Figure 2). Its unique feature is that each subunit contains a cofactor known as a heme group (Figure 3) which contains an iron ion in the center surrounded by a heterocyclic ring known as a porphyrin; these hemes are what bind oxygen. Even more notable is the fact that the binding of oxygen is cooperative, meaning that the hemoglobin's affinity for binding oxygen rises as more oxygen is bound.[2] An equally important function of erythrocytes is their ability to

remove carbon dioxide from the body. Carbonic anhydrase, an enzyme that facilitates the creation of bicarbonate from water and carbon dioxide, is vital to the process of carbon dioxide elimination. Bicarbonate acts as a buffer for blood as it maintains a physiological pH to avoid blood from becoming too acidic or basic. Erythrocytes then convert the buffer back into carbon dioxide when it reaches the lungs so that it can be exhaled. One can imagine what would happened to erythrocytes and consequently blood's abilities if there were a lack of or a dysfunctional hemoglobin; there would be a lack of oxygen transport from the lungs to the tissues of which the consequences are endless. Some examples of this would be organ failure, fainting, dizziness, tissue damage and more. Equally deadly would be a dysfunction of an erythrocyte's ability to provide the tools necessary for carbon dioxide removal, a consequence of which would be an abnormal environment too acidic for blood's components to function properly and an obvious accumulation of carbon dioxide in the system.[2]

Plasma is the non-cellular component of blood that comprises about 55% of the total volume. This substance provides a medium for blood cells to be suspended in and is extremely important in pressure maintenance and transportation. The reason is because, despite plasma being mainly water (~90%), the remainder is made up of plasma proteins, glucose, mineral ions, hormones, clotting factors and carbon dioxide; these plasma proteins play a crucial role in creating and maintaining oncotic pressure, which is vital to the transport of fluids across the membranes of the circulatory system. Such proteins include fibrinogen and albumin. This pressure, in balance with hydrostatic pressure, which pushes fluid out of the circulatory system, creates a good environment for transport and maintains an ideal blood pressure. Without this pressure there would be no diffusion of fluids and also no transportation ability which is why plasma also plays an important role in blood substitutes.[2]

Leukocytes (Figure 4), equally important as erythrocytes, are the immune defenses of the body. Commonly known as white blood cells, leukocytes embody an array of different cells and are generally divided into three classes; granulocytes, lymphocytes and monocytes. Granulocytes, which make up 50-60% of leukocytes, can be further divided into three more categories which include neutrophils, eosinophils and basophils. Evident by its name, granulocytes contain granules which contain varying chemicals unique to each cell. Lymphocytes comprise about 30-40% percent of leukocytes and also consist of more subcategories which include B cells and T cells. Also evident by their name, B cells mature in the bone marrow while T cells mature in the thymus. Lastly, monocytes comprise around 7% percent of leukocytes and later evolve into macrophages. All of these components, together, defend the body against infection and foreign matter that could harm the body. They are essential to the body if one were to get sick, which is why they can be essential in blood substitutes; blood with no defense would be bait for infection.[2]

Platelets, also known as thrombocytes, comprise the remainder of blood. Platelets, cell fragments released by megakaryocytes, are important in blood because they act as clotting factors. When exposed to air platelets break down and react with fibrinogen to form fibrin, a thread like substance that traps red blood cells to form a clot (Figure 5). Calcium and vitamin K are also important in forming these clots. The importance of platelets is exemplified by the fact that without them one would bleed to death when cut, an occurrence typified by the disease known as hemophilia. Clotting factors are necessary in the blood and without them there would be serious consequences, which is why blood substitutes need to consider their presence.[2]

Having gained an understanding of the components of blood, the difficulties of creating an ideal blood substitute become very relevant. Research continues in an attempt to create an

ideal blood substitute. An ideal blood substitute would be one that is sufficient in oxygen uptake from the lungs, can deliver oxygen to the tissues efficiently, is non-toxic, has a long circulation time, has no side-effects, is stable at room temperature, can be sterilized and excreted without harm, and is inexpensive to manufacture. These facets would be vital in an endless amount of situations. In today's society, for example, poverty is prevalent and many people cannot afford the luxuries of medical attention because of it, also unemployment rates create a lack of health insured people which also effects the medical attention they can receive. An inexpensive blood substitute would help many who would normally not be able to afford regular transfusions. The characteristic of efficient oxygen binding and uptake would be important in every aspect of surgeries, trauma, and medical treatments following chemotherapy, would and attract those athletes looking to enhance their performance capabilities despite the legal consequences. Blood substitutes must take into account all of the components of blood because each provides such an important attribute that without them, it would be useless.

The reality is that an ideal substitute is far from reach but studies are being done to better understand hemoglobin, RBCs and side-effects in an attempt to close the gap towards reaching an ideal substitute. The importance of this literature based study is that it will elucidate the public to the advancements being made and it will solidify the position scientists are in with regards to these advancements and where we could be in the near future. Yet more importantly, it will exemplify just how important blood substitutes are not just in medicine, but in society in general because blood is essential to life.

There are many types of blood substitutes that have some of the same characteristics as real blood such as volume expanders, oxygen carriers, hemoglobin based substitutes, transfusion, and EPO based blood substitutes. Those that are used for volume expansion fall under the

categories of crystalloids or colloids, which will be discussed further. Those that focus on the oxygen transfer fall under the category of being biometric or abiotic. Biometric blood substitutes are those that mimic the natural way oxygen is transferred to tissues and include hemoglobin-based substitutes while abiotic substitutes are fully synthetic in their creation and can include perfluorocarbons.[3]

Volume Expanders:

The human body is dependent on the transfer of sufficient oxygen to its tissues and organs along with the removal of unwanted breakdown products from the tissue. Just like a system of pipes, blood is only able to travel if it is under a pressure gradient, which is why maintaining an adequate blood pressure is so vital to the body. When there is a loss of fluids in the body the result is a decrease in blood pressure. This can have serious detrimental effects on a person's health. As mentioned, without a pressure force to pump blood through the body, tissues and organs will not receive a sufficient amount of oxygen. The result of this can lead to brain ischemia due to lack of oxygen to the brain, which would have symptoms of fainting or dizziness. It could also lead to failure of the heart to function, causing possible myocardial failure. An example of other organs that could fail includes the kidneys and the liver. Essentially it can be said that the organs of the body would have a higher chance of failing and as a result cause the body to go into hypotensive shock, resulting in possible coma or death. Volume expanders work by increasing the intravascular oncotic pressure, allowing the movement of water from the interstitial space to the intravascular space. This results in an increase in blood volume and therefore an increase in blood pressure. For this reason the application and utilization of volume expanders, in the medical field and society in general, has been and continues to be such an integral part in treating patients with fluid loss by its ability to regain and

help maintain a healthy blood pressure, therefore allowing oxygen and other nutrients to be transported to the body's tissues. A facilitator in the transport of these nutrients is albumin, a globular protein, which also helps transport fatty acids from adipose tissue and helps in regulating osmosis. This protein combines with other globular proteins to form a colloid. Volume expanders are generally categorized into two categories; crystalloids and colloids, the subject of which will be discussed further.

Crystalloids are a solution based on sterile water with added electrolytes in an attempt to mimic the mineral content of plasma; they come in many variations relative to plasma, from hypertonic to isotonic or hypotonic. A common crystalloid that is used abundantly throughout hospitals is that of .9% normal saline -with NaCl- which was designed to obtain the function of crystalloids just mentioned. An alternative to normal saline is Lactated Ringer's solution which better mimics plasma with respect to electrolyte concentration while at the same time maintaining low concentrations of lactate; this contains lactate as well as ions such as Ca^{2+} and K^{+}.

Colloids are similar to crystalloids but contain substances that are not permeable to semipermeable membranes and include the common hetastarches, which are starches that contain amylopectin and aim at attaining a molecular weight suitable enough to promote volume expansion. The main difference between the two is that colloid solutions are better able to maintain colloid osmotic pressure compared to crystalloids; crystalloids may experience hemodilution.[4] Because of this colloids are considerably more expensive than crystalloids and as a result many hospitals commonly use crystalloids to avoid financial burdens. Despite the cost of colloids, the fact that there is a choice between two types of volume expanders that essentially are relatively less expensive than other forms of blood substitution exemplifies the pros of

volume expanders. Without these substances people with a sudden loss in blood volume could essentially be a victim of death. But given their ability to regain blood pressure in a timely fashion the importance of their use is prevalent in the medical field.

Oxygen Carriers:

As aforementioned, blood's most vital role in the cardiovascular system is its ability to transport vital substances to different tissues of the body and rid the body of unwanted substances; the main substances being oxygen and carbon dioxide, respectively. Without oxygen the human body becomes useless, literally; as exemplified by the electron transport chain, oxygen is the final electron acceptor, allowing for the creation of ATP and therefore energy for the body to utilize. This phenomenon, blood's ability to transport oxygen and remove unwanted substances, has and continues to be the focus in the research and development of blood substitutes. As mentioned, red blood cells contain hemoglobin, blood's main oxygen carrier. Hemoglobin's structure and function have been an important focus of researchers in their attempts to mimic hemoglobin in blood substitutes. These kinds of blood substitutes are known as hemoglobin based oxygen carriers (HBOCs). Yet there is another type of blood substitute that has been of much focus also in the years past and present; one known as perfluorocarbon blood substitutes. These substitutes are not hemoglobin based and are completely synthetic, accommodating people that have religious views that restrict or prevent them from receiving blood transfusions. Both types of blood substitutes fall under the category of oxygen carriers because their main objective is to carry and transport oxygen throughout the body, especially in times of trauma where blood is lost quickly and needs to be replenished rapidly.

Hemoglobin Based Oxygen Carriers:

Having gained an understanding of the structure and function of hemoglobin the issues related to its isolation can also be understood. Although the focus of this thesis is on the positives of blood substitutes, it is necessary to mention the challenges of isolating hemoglobin from red blood cells in hopes of creating HBOCs so as to realize the advances being made to resolve those consequences. When hemoglobin is not encapsulated in the protective environment of a red blood cell it becomes easily broken down into monomers and dimers and eliminated by the kidney, causing possible renal failure. But scientists have worked towards trying to extend the life span of isolated hemoglobin, a characteristic that is exemplified by an ideal blood substitute. They have done this by chemically modifying isolated hemoglobin. One way is by creating chemical cross-links between specific hemoglobin polypeptide chains to avoid having the hemoglobin tetramer dissociate, as a result increasing its longevity time in the blood. Another method includes using 3,5-dibromosalicyl fumarate to create a strong covalent bond within the tetramer in order to maintain its structure, allowing for survival times of up to 12 hours. The isolated hemoglobin is also treated with bifunctional cross-linking agents, which are agents that polymerize the hemoglobin molecule through the targeting of certain amino acid groups. Such agents include o-raffinose and glutaraldehyde; these agents lead to the creation of a polyhemoglobin, a molecule composed of four or five hemoglobin that allow for an active time of up to one day. The time that modified hemoglobin can be maintained in the blood is able to be increased to up to 48 hours by becoming conjugated to larger molecules, like dextran or polyethylene glycol. Newer HBOCs have been attached to such antioxidant enzymes as catalase or superoxide dismutase in an attempt to reduce an injury known as ischemia-reperfusion injury. Ischemia-reperfusion injury occurs when a tissue's blood supply has been cut off and then

perfused again; this reperfusion essentially causes more damage to the tissue, likely due to the presence of free radicals, which is why antioxidants would help in this case.[6] Finally, other researchers have worked on creating artificial red blood cells to create a protective environment for the hemoglobin with the use of biodegradable polymer nanocapsules or lipid vesicles.[5] Ultimately HBOCs can be put into separate categories respective of how they are made and modified to meet the idea oxygen carriers. These categories include: cross-linked, conjugate, polymerized, and encapsulated.

All over the world advances are trying to be made in order to enhance the stability of these HBOCs so as to release them into the medical field and consequently to the public in need. Many clinical trials have been performed and others are being performed currently. Companies and labs such as Northfield Laboratories have developed a polymerized HBOC known as Polyheme, a first generation cross-linked human polyhemoglobin and essentially a modified glutaraldehyde hemoglobin taken from human blood and placed into a separate solution. The process by which they prepare this involves steps such as separation, filtration, and chemical modification.[7] These modifications allow for reduced side effects such as vasoconstriction, kidney and liver dysfunction.

Another company on the forefront of clinical trials is one called Biopure, a company whose product, Hemopure, also a polymerized HBOC, has been approved for use in the Republic of South Africa and has reached a clinical phase III trial in the U.S. in hopes of obtaining positive responses from the FDA.[5] Hemopure is described as a cross-linked bovine polyhemoglobin; one that suspends in plasma, is less viscous and smaller, and can more readily release oxygen, a factor extremely vital to the development of oxygen carrier substitutes.[6] Other

polymerized HBOCs include that of Hemosol, created by Hemolink[5], and Hemozyme, produced by Synzyme[6].

There have also been laboratories that have been working on cross-linked HBOCs. One such company is Baxter, who produced a substitute known as HemAssist using recombinant technology.[7] This process involves the isolation of the gene responsible for hemoglobin; the gene is copied and modified in order to enhance the structure and functionality of the hemoglobin so as to possibly reduce side effects.[7] It is then inserted into bacteria, such as E. Coli, so that more hemoglobin can be produced and later purified.[7] Baxter, in working with the company Optra, has also worked on a substitute known as Somatogen, a human hemoglobin that is cross-linked by a single polypeptide with two alpha chains and then joined by a shorter polypeptide to two beta chains.[7]

Conjugated HBOCs have also been in development and companies such as Sangart have been trying to improve upon them. Sangart has produced a hemoglobin modified with polyethylene glycol (PEG) known as Hemospan. This product is characterized by high oxygen transport capability and a lower concentration of hemoglobin. Hemospan hemoglobin is taken from outdated human blood and modified with PEG in order to avoid toxicity from free hemoglobin, allowing for its long half-life; characteristic of PEG modified hemoglobins.

Encapsulated hemoglobin has been made to create an environment for the hemoglobin to avoid its degradation. These kinds of HBOCs work well at avoiding hypertension and are good binders and releasers of oxygen and nitrogen oxide. HBOCs have created significant headway into the development of a more ideal blood substitute, and as typified by the aforementioned research companies and laboratories, scientists around the world are putting forth the effort to

create such an ideal blood substitute using this method. But, again, there is the subject of the purely synthetic blood substitute unique to perfluorocarbons, which are being created in an attempt to avoid using real hemoglobin.

Perfluorcarbons:

An ongoing issue in today's medical field, with regards to blood substitutes, has been directed towards those people who hold religious views against receiving blood transfusions; for example Jehovah's Witnesses. Not only does this subject raise medical issues for doctors wanting to save a life, but also ethical issues given that they must respect their patients' rights. It also raises issues for the patient because many times a patient has decided he or she would rather live than die as a result of their religion's prohibition of blood substitutes. Having made this decision they risk being shunned or banned by their religious enterprise. Perfluorocarbons (PFCs), molecules similar to hydrocarbons except that fluorine atoms replace the hydrogen atoms, are an answer to this problem.[6] Perfluorocarbons must actually be prepared as emulsions because they are not miscible with aqueous solutions given their hydrophobicity; this has to be done in order for them to act as a blood substitute.[5] Having said this, PFCs are a stepping stone in the field of blood substitutes, one that can open an avenue for those who may require it, be it because of religious views, preference, or simply necessity.

To reiterate, oxygen is carried in the blood through hemoglobin and by being dissolved in the plasma; while HBOCs focus on the hemoglobin aspect of blood, PFCs focus on the dissolved oxygen aspect of it. In liquid PFCs, oxygen is actually very soluble and therefore is easily dissolved in it. This is exemplified by the fact that salt water and blood plasma, by volume, are able to dissolve about 3 percent oxygen while 20 percent oxygen is dissolved in whole blood.

PFCs, on the other hand, are able to dissolve up to 40 percent oxygen and able to dissolve carbon dioxide even more readily, considering it is almost two times as soluble, all while remaining inert.[8] These facets were utilized in 1966 when Leland C. Clark, of the University of Cincinnati was able to submerge a mouse for days in inert liquid PFCs; the mouse would breath the liquid and actually came out showing no signs of negative side effects[8] (Figure 6) One of the highlights of PFCs is that they are completely synthetic, in other words the issue of having to isolate and strengthen hemoglobin in order to attain a method for carrying oxygen is unnecessary. Also, given the fact that they dissolve oxygen, one can control the amount of it needed or required in the emulsion, a valuable tool when encountering different types of trauma that call for differing approaches. Yet as was done with HBOCs, in order to see the advances being made in PFCs today and in the past, one must take into consideration some of the setbacks they exhibit. With the case of PFCs, the possibility of accumulation, due to viscosity or excessively large particles, was a big issue and continues to be addressed today with new discoveries and techniques, a glimpse of which will be disclosed further on.

Given the fact that PFCs are completely synthetic it is understood that many types can be created and are being tested, but most are characterized by three characteristics; oxygen carrying capacity, how long the emulsifying agent is able to circulate stable PFCs, and the rate of excretion from the body.[5] One must remember that PFCs, in order to carry oxygen, must be part of an emulsion; this emulsifier plays a critical role in the PFCs' effectiveness. The reason is because it allows the PFC to be circulated throughout the blood without producing embolisms; a fault now disclosed.[8] An example of the progressions being made to avoid this was discovered by Robert P. Geyer at Harvard School of Public Health; Geyer discovered a certain polymer, belonging to the polyoxyethylene-polyoxypropylene family, named Pluronics. This polymer not

only acted as an emulsifying surfactant, but it also mimicked the functions of blood proteins by establishing an oncotic pressure, therefore establishing itself as a plasma expander also.[8] Other emulsifying agents have been treated with a fluorinated aliphatic chain in order to allow the PFCs, while in the organic phase, to permeate into smaller particles; this is due in part because of the chains high affinity for liquid PFCs.[8] To clarify, smaller particles allow for passage through capillaries, and therefore avoid a risk of accumulation that could lead to an embolism. This typifies yet another advantage of PFCs; their ability to be manipulated and treated in a way that changes their size and viscosity in order to accommodate the size of the blood vessel. When PFCs circulate in the blood they are absorbed by phagocytic cells coming from the reticuloendothelial system and are later re-dissolved in the bloodstream and removed through the lungs, unaltered.[5]

In order to show the promise that PFCs have displayed in past and present years, examples of their uses in experiments is necessary. In the aforementioned experiment that Clark performed he also demonstrated that such PFC liquids could actually help avoid the effects of decompression in the mice he was working with.[8] In one set of experiments he replaced up to 90 percent of the blood of dogs with PFC emulsions and was able to demonstrate that the emulsions were in fact providing oxygen; these dogs showed no adverse side effects and actually went on to live for years after.[8] It has also been suggested that the best types of PFCs prepared contain particles with diameters less than 0.2 micrometers in size, which puts them in the category of colloidal suspensions as opposed to emulsions, a suspension that is able to dwell in the bloodstream for up to 7 days.[8] Geyer, mentioned earlier, was able to successfully replace the blood of rats with a PFC emulsion; these rats began producing erythrocytes and blood proteins with the PFC in use and were able to regenerate all of their erythrocytes within a week after the

PFC was removed.[8] Another experiment performed at the University of Pennsylvania Medical School by Henry A. Sloviter showed that isolated rat brains retained their electrical activity when perfused with PFCs, in fact, some showed that they retained activity better than those perfused with erythrocytes.[8] He even showed that a portion of the blood of mice and frogs could be replaced by PFC emulsions without any negative side effects.[8] At the Medical College of Virginia, Richmond, William H. Rosenblum measured several brain parameters of mice of whom 75 percent of their blood was replaced by PFCs and was able to show that the PFC emulsion did not stress the brain.[8] It can essentially be noted that there has been many studies performed in the days past and present on PFCs; and despite the products' flaws one must take into account the positive effects they have had while in testing.

Certain companies have been working hard to try and get some of these PFC products FDA approved, unfortunately not many have succeeded in doing so. One of these rare few, Fluosol DA, was actually approved for use during heart surgery and managed to reduce cardiac trauma and pain during the procedures.[6] Another company, Alliance Pharmaceuticals, has developed a product known as Oxygent which is in stage II/III clinical trials in the U.S.[6] Despite the lack of FDA approved PFC products the hard work that is being done in order to better understand their use and to expand the possibilities of this product should be acknowledged. There has been and continues to be a notable rise in the progression of PFCs and with the advent of new technology one can only predict that these types of blood substitutes will be better developed so as to reduce any negative side effects. To focus on their pros would be to focus on the facts and the possibilities of this product. For example the fact that they could be made abundantly and at a low cost is a significant pro, especially given the state of our economy and the extreme need for blood substitutes. In addition, with the manipulation of the size and

viscosity of PFCs doctors could possibly utilize this in order to bypass tumors or to perfuse tissues that need it; the possibilities are immense.

Autologous Transfusions/EPO:

With blood transfusions today many people face issues regarding infection, mismatched donor blood, and of course, shortages of blood. Autologous transfusions are an answer to these problems. Such autologous transfusions can be considered "self-donations". Because one is donating to him or herself they avoid the possibility of receiving the incorrect donor blood and they also avoid the risk of infection due to suppression of the immune system in order to accept another donor's blood. More importantly these kinds of transfusions allow for an actual supply of blood ready at hand; the fact that one is relying on his or her own blood renders the issue of a shortage of donor blood moot. There are actually three main types of autologous blood transfusions; predoposit transfusion, normovolemic hemodilution, and interoperative and postoperative blood salvage.[9]

Predoposit transfusion is arguably the most common form of autologous transfusion, though this is a matter of opinion. This kind of transfusion entails collecting blood from the patient about 3-5 weeks prior to surgery all the way up until 48-72 hours before the procedure; the blood is stored with anticoagulants until the time of the procedure.[9] This type of transfusion has many advantages; it not only reduces and almost eliminates the chances of contracting a virus or enduring an allergic or febrile reaction, it also reduces the chances of postoperative infection and recurring cancer given the fact that the suppression of immunological cells is avoided.[9] Normovolumic hemodilution is performed before surgery and is generally performed

with patients whose procedure will involve a big loss of blood.[9] In this process blood is removed from the patient while being replaced with colloids or crystalloids at the same time so as to maintain adequate blood volume. Once the procedure has begun and the loss of blood has ceased, the patient's own blood is returned and the surgery moves forward.[9] This kind of transfusion provides advantages similar to that of predeposit transfusion and it also reduces pain and stress for the patient because it is performed while they are under anesthesia. Also, because this blood is stored for a short time at room temperature less clotting factors are activated and there is decreased cell deterioration.[9] In addition, this transfusion is less expensive because the occurrence of cross matching and testing is not necessary and less blood is also wasted.[9] The third type of transfusion is known as salvage autologous transfusion and is rather interesting because it can occur during surgery. This process involves the collection of lost blood during surgery, blood which is put into a centrifuge and paired with anticoagulants such as citrate or heparin; filtering also occurs so as to remove unwanted debris or clots.[9] Because of centrifuging the lost red blood cells become concentrated, saline washed, and then reinfused into the patient. It is no secret that the advantages of this kind of transfusion includes the lack of wasting blood and the fact that colloids and crystalloids do not need to be used in order to keep volume expansion up.[9]

Autologous transfusions are critical in today's society for the aforementioned reasons. With the existence of this type of transfusion doctors avoid the hassles that normal donor transfusions bring with them; certain tests, infections, immunosuppression, and the worry of not having adequate quantities of blood. It has even escaped the medical field and has entered the athletic field; many athletes have begun to "blood dope", an illegal action in U.S.A. sports. Blood doping is an autologous transfusion, only the patient is a healthy male or female hoping to

increase the amount of red blood cells in his or her body and therefore increase his or her oxygen carrying capacity, as a result allowing for a better athletic performance. Just as in an autologous transfusion, the athlete removes his or her blood, stores it and freezes it for the foreseeable future while the body begins to create more red blood cells in the meantime. When the time comes for him or her to perform the athlete returns the older blood to his body and officially has an excess amount of oxygen carrying soldiers ready to help him or her win. This is obviously the only benefit to blood doping, given the moral objections that surround the action. Many athletes go so far as to use others blood; the only advantage of this would be that they do not have to endure the process of having to take out their own blood and they avoid the time needed to replenish the lost blood cells. Despite these advantages the action, as mentioned, is highly objected and athletes who are caught tend to be disqualified and possibly banned from their sport.

Having brought up blood doping with the use of autologous transfusions, another subject of blood substitute comes to mind, that of erythropoietin. This hormone is produced in the kidneys and is responsible for the production of red blood cells. With relation to blood doping this hormone has been of much conversation. By injecting oneself with the hormone their body is actually able to produce more red blood cells and therefore they exhibit a higher oxygen carrying capacity. One can see how in athletics this would be just as immoral as autologous transfusions. Yet aside from the field of athletics EPOs do have their positive aspects in the medical fields, ethical and moral ones. Imagine a patient with leukemia who has to undergo chemotherapy; one can imagine that red blood cells would be attacked and destroyed and the patient's RBC count would go considerably down. EPOs in this case would help a patient with this by allowing for a production of RBCs in order to replace those destroyed. People who are highly anemic would also benefit from EPOs given the fact that they are faced with a lower red blood cell count.

Having said this one can see the similarities that EPOs and autologous transfusions have between each other and also the relative differences. Yet both are extremely useful for those patients who are in dire need of blood. EPOs focus more on those who don't need it as quickly while transfusions focus on those who need it immediately.

Conclusion:

It is well known that the subject of creating and developing the ideal blood substitute is unfortunately far from possible; even if one were to create an ideal blood substitute, the fact that the physiology of every human being on this planet is different would mean it would have to accommodate and be ideal for everyone, not just the general public. Yet despite this, many aspects of an ideal blood substitute are continuing to be focused and improved upon. Although there has not been an immediate rise in the development of an amazing blood substitute, there has been a prevalent increase in effectiveness. Scientists like Geyer or Clark influence other scientists to produce the best product, be it a volume expander, an HBOC or a PFC. The most important thing to note is that doctors know what they want and researchers know what they need to do. Everyday advances are being made, discoveries of a better emulsifier or cross-linking agent are made and these little things can add up to successful results.

People tend to believe that in today's 21st century society developing a blood substitute would be an easy task to fulfill. But understanding now the complexities that entwine the substance of blood and its many functions will hopefully elucidate the public with the hard work that is necessary to produce such a substance and this will, hopefully, also result in a newfound respect for the scientists working in the field. With the advent of new technology on the rise everyday more and more advances can be made in the medical field and subsequently in the field

of blood substitutes also. So although an ideal blood substitute is far reaching, one that is capable of performing at the most important aspects that blood does is within reach, possibly even within our lifetime.

Figures:

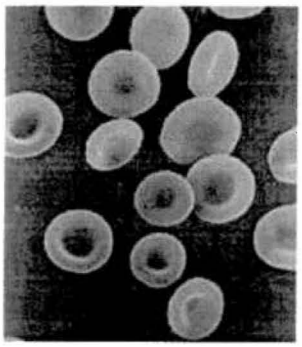

Figure 1: RBC

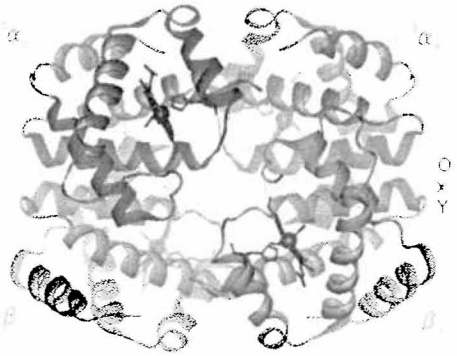

Figure 2: Hemoglobin Heterotetramer

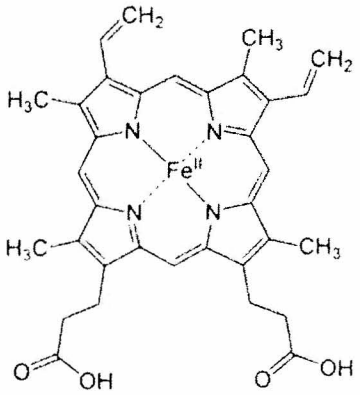

Figure 3: Heme

Figure 4: Leukocytes

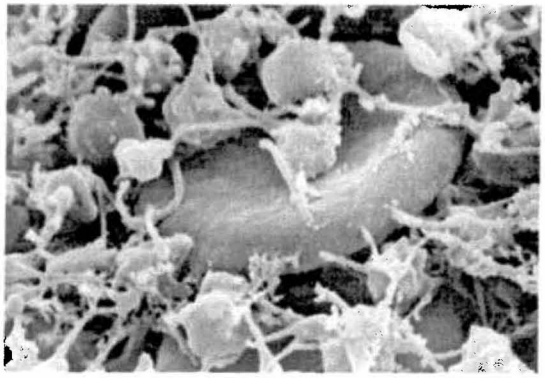

Figure 5: Platelets Trapping RBC

Figure 6: Clark's Submerged Mouse

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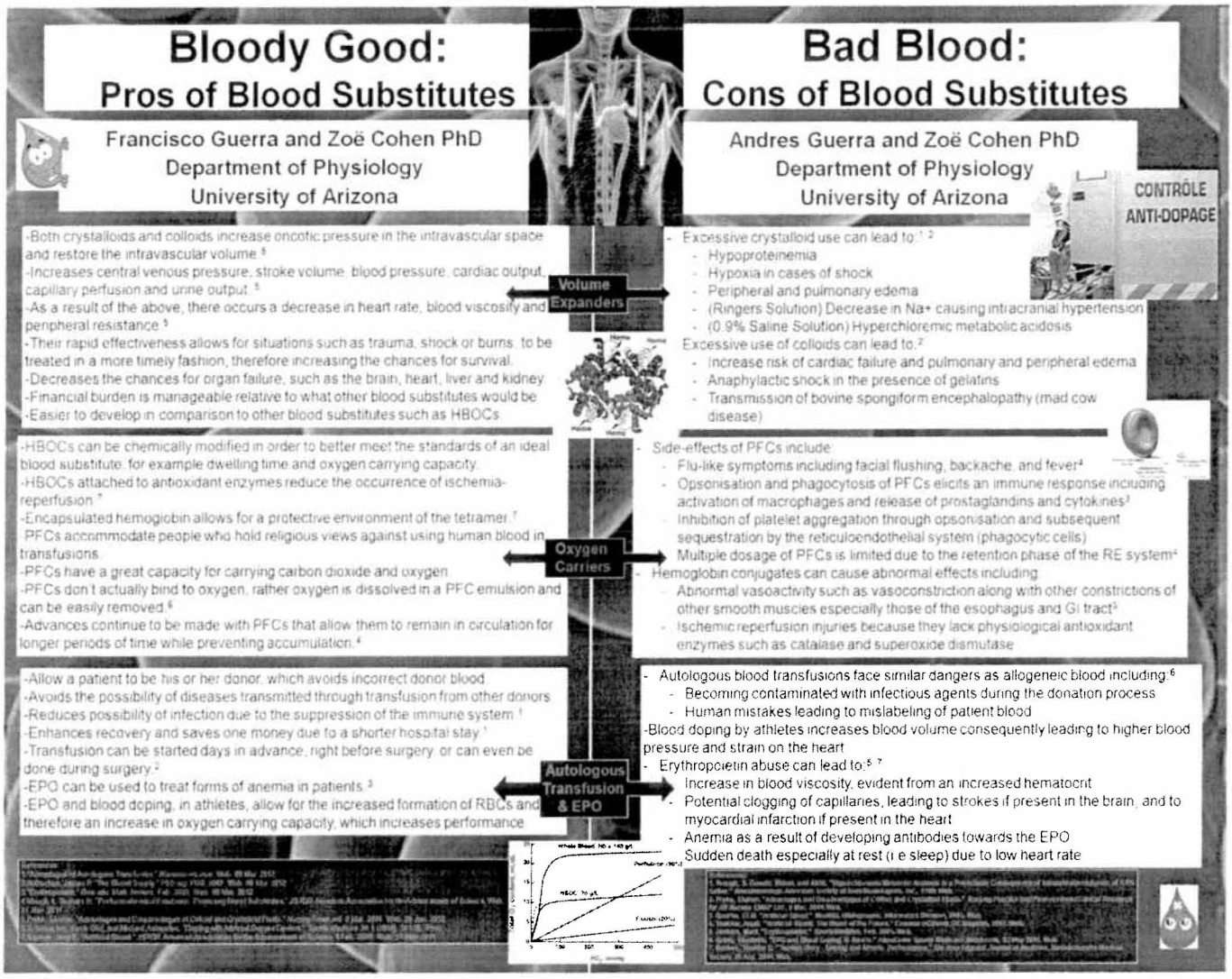
Appendix:


Bloody Good:
Pros of Blood Substitutes
Francisco Guerra and Zoë Cohen PhD
Department of Physiology
University of Arizona
Bad Blood:
Cons of Blood Substitutes
Andres Guerra and Zoë Cohen PhD
Department of Physiology
University of Arizona
CONTRÔLE
ANTI-DOPAGE
-Both crystalloids and colloids increase oncotic pressure in the intravascular space and restore the intravascular volume.[5]
-Increases central venous pressure, stroke volume, blood pressure, cardiac output, capillary perfusion and urine output.[5]
-As a result of the above, there occurs a decrease in heart rate, blood viscosity and peripheral resistance.[5]
-Their rapid effectiveness allows for situations such as trauma, shock or burns, to be treated in a more timely fashion, therefore increasing the chances for survival.
-Decreases the chances for organ failure, such as the brain, heart, liver and kidney.
-Financial burden is manageable relative to what other blood substitutes would be.
-Easier to develop in comparison to other blood substitutes such as HBOCs.
Volume Expanders
- Excessive crystalloid use can lead to:[1,2]
- Hypoproteinemia
- Hypoxia in cases of shock
- Peripheral and pulmonary edema
- (Ringers Solution) Decrease in Na+ causing intracranial hypertension
- (0.9% Saline Solution) Hyperchloremic metabolic acidosis
Excessive use of colloids can lead to:[2]
- Increase risk of cardiac failure and pulmonary and peripheral edema
- Anaphylactic shock in the presence of gelatins
- Transmission of bovine spongiform encephalopathy (mad cow disease)
-HBOCs can be chemically modified in order to better meet the standards of an ideal blood substitute, for example dwelling time and oxygen carrying capacity.
-HBOCs attached to antioxidant enzymes reduce the occurrence of ischemia-reperfusion.[7]
-Encapsulated hemoglobin allows for a protective environment of the tetramer.[7]
-PFCs accommodate people who hold religious views against using human blood in transfusions.
-PFCs have a great capacity for carrying carbon dioxide and oxygen.
-PFCs don't actually bind to oxygen, rather oxygen is dissolved in a PFC emulsion and can be easily removed.[6]
-Advances continue to be made with PFCs that allow them to remain in circulation for longer periods of time while preventing accumulation.[4]
Oxygen Carriers
- Side-effects of PFCs include:
- Flu-like symptoms including facial flushing, backache, and fever[4]
- Opsonisation and phagocytosis of PFCs elicits an immune response including activation of macrophages and release of prostaglandins and cytokines[3]
- Inhibition of platelet aggregation through opsonisation and subsequent sequestration by the reticuloendothelial system (phagocytic cells)
- Multiple dosage of PFCs is limited due to the retention phase of the RE system[4]
- Hemoglobin conjugates can cause abnormal effects including:
- Abnormal vasoactivity such as vasoconstriction along with other constrictions of other smooth muscles especially those of the esophagus and GI tract[3]
- Ischemic reperfusion injuries because they lack physiological antioxidant enzymes such as catalase and superoxide dismutase
-Allow a patient to be his or her donor, which avoids incorrect donor blood.
-Avoids the possibility of diseases transmitted through transfusion from other donors.
-Reduces possibility of infection due to the suppression of the immune system.[1]
-Enhances recovery and saves one money due to a shorter hospital stay.[1]
-Transfusion can be started days in advance, right before surgery, or can even be done during surgery.[2]
-EPO can be used to treat forms of anemia in patients.[3]
-EPO and blood doping, in athletes, allow for the increased formation of RBCs and therefore an increase in oxygen carrying capacity, which increases performance.
Autologous Transfusion & EPO
- Autologous blood transfusions face similar dangers as allogeneic blood including:[6]
- Becoming contaminated with infectious agents during the donation process
- Human mistakes leading to mislabeling of patient blood
-Blood doping by athletes increases blood volume consequently leading to higher blood pressure and strain on the heart
- Erythropoietin abuse can lead to:[5,7]
- Increase in blood viscosity, evident from an increased hematocrit
- Potential clogging of capillaries, leading to strokes if present in the brain, and to myocardial infarction if present in the heart
- Anemia as a result of developing antibodies towards the EPO
- Sudden death especially at rest (i.e. sleep) due to low heart rate