Iron-Porphyrins: The Best Candidate for Red Blood Cell Substitutes

Sarah Lalancette-Caron
Faculty of Engineering
McGill University
Montreal, Canada
sarah.lalancette-caron@mail.mcgill.ca

***Abstract*—Blood is a scarce medical resource around the world, even in developed countries. Red-blood substitutes in particular are difficult to artificially reproduce due to their complex mechanism of action. Different candidates have been proposed to replace red blood cells, such as synthetic iron-porphyrin systems, perfluorocarbon (PFC)-based emulsions, and hemoglobin (Hb)-based substitutes. According to the evidence presented in this paper, synthetic iron-porphyrin systems is the blood substitute that is the most efficient at oxygen-carrying and delivering, that stays the longest in blood circulation, and that is the least involved in undesired off-target side effects.**

***Index Terms*—synthetic biology, blood substitute, oxygen carriers, nanoparticles, perfluorocarbon (PFC)-based emulsions, synthetic porphyrin systems, hemoglobin substitutes**

I. Introduction

Blood is a scarce medical resource around the world, even in developed countries. Additionally, blood substitutes are difficult to artificially reproduce due to their complex mechanism of action. Red blood cells in particular have a very complex mechanism of bonding and releasing with oxygen. Different candidates have been proposed to replace red blood cells, such as synthetic iron-porphyrin systems, perfluorocarbon (PFC)-based emulsions, and hemoglobin (Hb)-based substitutes. In the case of which blood substitute is the most efficient at oxygen-carrying and delivering, that stays the longest in blood circulation, and that is the least involved in off-target side effects, the strongest case is presently for synthetic iron-porphyrin systems.

II. Background

Blood transfusions are required every day to save lives around the world. However, acquiring blood for patients in need is difficult. There is never enough donors, so blood is considered a rare resource. Even if blood samples are available, they also need to be compatible with the blood type of the patient, since a mismatch is lethal. Even if a match is found a the blood is injected into the patient to save their life, there are still considerable risks that the blood was contaminated with harmful pathogens such as HIV [1]. Blood is also difficult to transport since it needs to be stored in cold temperature [1]. It will also spoil after some time on the shelf. In addition to all of these limitations, there exists varied side-effects to blood transfusion [2]. To counter these limitations, some research teams and private companies have tried to design and manufactures specific components of blood, mainly red blood cells (RBCs), platelets, white blood cells, and plasma, to be used as blood substitutes. For example, a patient in need could receive only the blood components that they require without the need to find a complete blood sample that matches their blood type. There would also be virtually no risk of transmitting harmful pathogens, since the substitutes would be synthetic. They could also be designed to be stored at higher temperature to be more accessible to isolated communities.

The most difficult blood element for researchers to reproduce is the red blood cells. The red blood cells are used to deliver oxygen all over the body as well as carrying out carbon dioxide. However, the mechanism of oxygen delivery is quite complex and then, by definition, difficult to replicate. Red blood cells are covered in particles called hemoglobin (Hb), which will be the ones binding to oxygen when the time comes. The hemoglobin particles are oxygenated when they go through the blood vessels of the lungs. In this location, oxygen is extracted from the air and enters the blood. The oxygen then binds to the hemoglobins attached to red blood cells, and they a transported all over the body. The hemoglobins then release their oxygen molecule, and because they release it at different times, all the cells of the body can acquire their much-needed gas molecules. The red blood cells can then repeat the cycle by reoxygenating their hemoglobins when entering the lungs again. Sometimes, the hemoglobin particles can detach themselves from the red blood cells. When they do so, they are called heme and they are filtered out by the kidneys to be discarded. A simplified drawing explains the process in Fig. 1. [3][4]

There are numerous criteria to consider to design an acceptable red blood cell substitute, but only the three most important will be considered in this paper. To mimic the function of hemoglobin, a synthetic version needs to be able to bind to oxygen long enough to transport it until it is outside the lungs. However, it must be able to release that oxygen after a certain time so that the oxygen can enter the cells that need it. Another constraint is that as soon as it is unoxygenated, and perhaps earlier, it will be filtered by the kidneys and are discarded if not attached to a bigger particle, as hemoglobins are safe from the kidneys when attached to red blood cells. As such, an efficient oxygen-carrying synthetic particle would be able

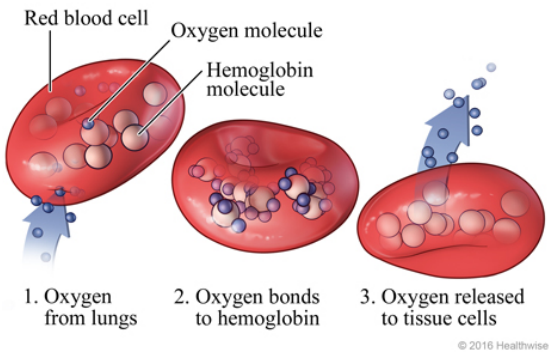

Fig. 1. The red blood cell covered in hemoglobin being oxygenated (1) transported (2), and deoxygenated (3). [3]

to hold on to oxygen and release it at the right time. An ideal particle would also stay in the body as long as possible, without being filtered by the kidneys too early. They would also have limited off-target side effects, meaning they would interact as little as possible with molecules other than oxygen, including immune cells which attack any object considered foreign to the body. These are the most important criteria for a red blood cell substitute to become a candidate for use in humans.

Even with all these constraints to keep in mind, different designs have been developed such as Hb-based oxygen carriers (HBOCs), perfluorocarbon (PFC)-based emulsions, and iron (Fe^{2+})-containing porphyrins [2]. In this paper, I will demonstrate that because it is the most efficient at oxygen-carrying and delivering, the least involved in off-target side effects, and the least rejected, the strongest case is presently for Perfluorocarbon (PFC)-based emulsions to be the best red blood cell substitute that exists.

A. Hemoglobin-Based Oxygen Carriers

The first solution that I will explore is the hemoglobin-based oxygen carriers (HBOCs). They were the first candidate to be explored in a research setting by Chang in 1964 [1]. They work by using natural hemoglobin from humans. If they were directly injected into the blood, the oxygen molecules would still chemically bound to hemoglobin even if they would not be attached to a red blood cell. However, if freely swimming in blood, they would be filtered by the kidneys very quickly and they would not have enough time to accomplish their oxygenating mission. To solve this, the hemoglobin can be encapsulated in plastic nanocapsules made of liposome (fat molecules) covered in polyethylene glycol (PEG) or polylactic acid (PLA). These materials are biocompatible, meaning they are not toxic to the body, and over time, they will break into smaller pieces and will be discarded by the kidneys. However, the particles will have had time to release their oxygen before being thrown away. Fig. 2 shows an exemple of a HBOC encapsulated in liposome and topped with polyethylene glycol (PEG) to increase reactivity. [5]

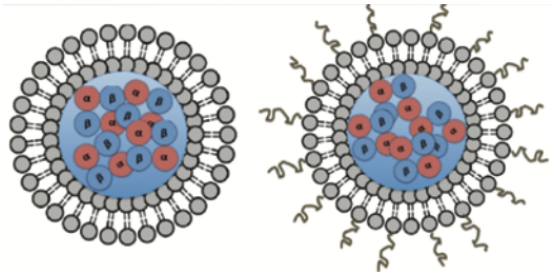

Fig. 2. Left: Hemoglobin encapsulated in a liposome capsule. Right: PEG was added to the outside of the capsule. [2]

B. Perfluorocarbon-Based Emulsions

A second possible way to recreate the oxygen-carrying mechanism of hemoglobin is with perfluorocarbon (PFC)-based emulsions [2]. This solution was explored after HBOCs because researchers had found that the latter were lacking in stability and circulation lifetime and that they were too toxic to humans. This new PFC solution consists of an emulsion of particles of perfluorocarbon (carbon and fluorine) particles. An emulsion simply means that the particles would be floating in blood but not be soluble in it, just as oil does not fully mix with water when the two are combined. In the case of PFC emulsions, oxygen is loosely bound to PFC molecules (see Fig.3) with forces called Van der Waals forces. In short, the carbon and fluorine atoms in the compound create sites that acquire an electromagnetic charge (positive or negative). As such, oxygen is attracted to the molecule's negative sites, but it cannot bind to it chemically as it would to hemoglobin. To better visualize this process, one can imagine a fan in a crowd at concert being attracted to their favourite band on the stage but unable to reach it due to other fans standing in the way.

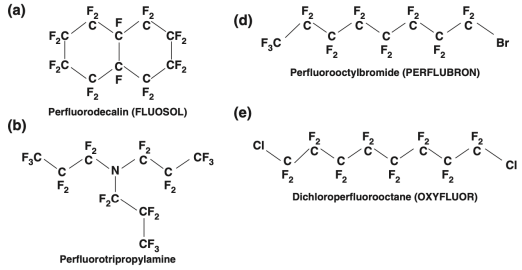

Fig. 3. The chemical structure of different examples of perfluorocarbon (PFC) compounds that have been studied for oxygen-carrying applications [2]

C. Synthetic Porphyrin System

The last solution that I will discuss is the synthetic porphyrin system. It was explored in research for the same reason that perfluorocarbon emulsions were. The goal of this system is to imitate natural hemoglobin molecules with both shape and content. First, like for HBOCs, a liposome vesicle (or bubble) is created and is the equivalent of the red blood cell. Then, molecules that are the equivalent of hemoglobin are attached to its surface. These molecules are iron-porphyrins (a circular

molecule around an iron atom) to which other bulky molecules are attached in a "picket fence" formation around the ring (see Fig.4a). This way, the oxygen is attracted to the pocket inside the ring but is loose enough to be eventually released. Iron-porphyrins work the same as in hemoglobin, but instead of being attached to a cell, they will be on a liposome vesicle (see Fig.4b). [6]

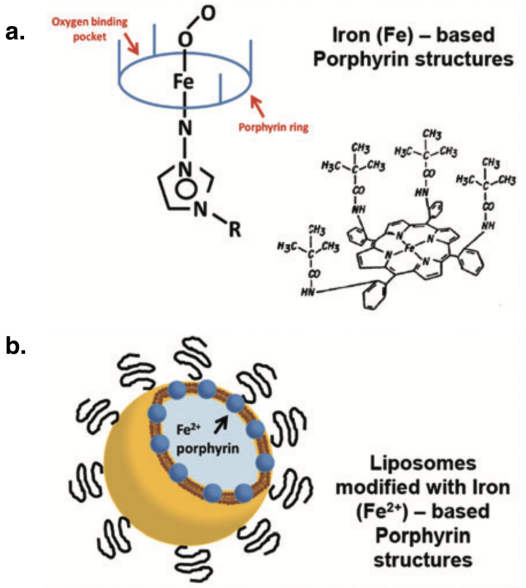

Fig. 4. a: A representation of the structure of an iron-based porphyrin molecule with its oxygen-attracting ring in blue on the left. b: iron-based porphyrin molecules (in black) are attached to a liposome (in yellow). [2]

III. Analysis

A. Oxygen Transport Efficiency

First, how well the different proposed solutions can transport oxygen and release it at the right time? Normal red blood cells can carry a hemoglobin concentration of 140-150 g/L.

- In the case of HBOC's, the most optimistic study found that can only carry a hemoglobin concentration of about 70g/L [2]. This cuts by half the quantity of oxygen they can carry and deliver to the body compared to whole blood as even if they have the same efficiency as red blood cell hemoglobin to bind to and release oxygen, because less hemoglobin can bind to HBOCs, less oxygen can be transported overall.
- In the case of PFC-based emulsions, the oxygen-binding process is different. In short, because oxygen do not completely bind to the PFC molecules (with Van der Waals forces), the chemical laws that regulate the bonding of oxygen to it are related to the pressure of the oxygen gas, as opposed to oxygen's chemical reactivity. Therefore, the graphs representing how quickly it binds to oxygen will be different: they will form a line instead of a sigmoid curve, as for hemoglobin in both HBOCs and red blood cells. Fig.5 displays and compare the binding profile of HBOCs and PFC emulsions. Because of the linear binding profile of PFC emulsions, or because they do not chemically bind to Oxygen but rather attract it, "oxygen off-loading capacity is better for PFCs compared to Hb" [2, p.11]. Even if Perfluoroncarbons never reach a stable carrying concentration like HBOCs and whole blood, they still end up being more performant because they are better at releasing the oxygen over the entirety of their transport around the body. On model (perfluorodecalin, shown in Fig.3a) was extensively tested on animal models and was even approved by the FDA in 1989 to be used on humans, but was unfortunately removed from the market for only carrying 0.4 mL oxygen/100 mL compared to 20.1 mL/100 mL for whole blood [2]. However, many other perfluorocarbons could be used and approved in the future, perfluobron being my personal favourite.
- In the case of iron-porphyrins, their oxygen-carrying capacity were tested by [5] in 1994 on dogs and the results were promising: 15.7 to 22.3 mL/min of oxygen were delivered to the tissue (about 11-16% of the speed at which whole blood can do it) [5]. New versions of iron-porphyrins have recently been developed by Kitagishi and Kano, who published the results of their extensive research in 2021 [6]. Their first design (hemoCD1) was evaluated as having oxygen-binding and release abilities "comparable to those observed for Hb in RBC under the same conditions" [6, p.167] in animal models, which in my opinion is very encouraging and shows potential. Their most advanced model, G2-hemoCD1, has been conjugated with PEG to better control its affinity with oxygen and was found to have an oxygen affinity of about $P_{1/2}^{O2}$ = 20 torr at 37°C, which is the same as human hemoglobin.

As of now, the iron-porphyrin model G2-hemoCD1 seems the most efficient at carrying oxygen, since it behaves very similarly to human hemoglobin *in vivo*. In Fig.5, its curve would be close to the one for whole blood, which surpases both HBOCs and perflubron.

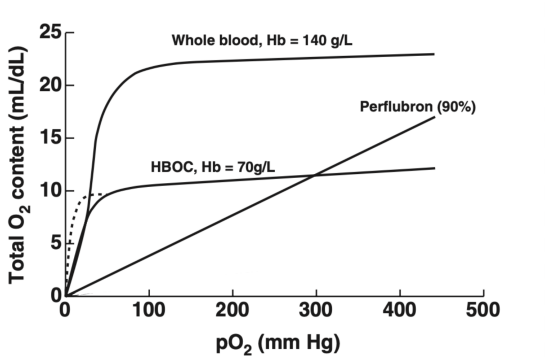

Fig. 5. Perflubron, a perfluorocarbon, has a linear binding profile compared to whole blood and HBOCs. [2]

B. Longest Circulation Time

Secondly, how long will these artificial oxygen carriers stay in the body to accomplish their goal before being eliminated through the kidneys? The key is to be big enough, but not made from a toxic material.

- HBOCs were found to be very quickly eliminated by the body, as liposomes are quickly broken down in the blood and the hemoglobin is rapidly filtered by the body. The earliest models would stay less than an hour in total in the body, with the newest ones staying for 60 hours at most [7]. Multiple doses of this solution would be required to have the same effect as blood.
- For PFC emulsions, the best results that were obtained by [8] showed that the particles would leave the body in about 10 hours.
- In the case of iron-porphyrins, 97% of the particles of Kitagishi and Kano's model (G2-hemoCD) would circulate in the body after 5 hours, which suggest an incredibly long circulation time [9]. This is because the authors used phospholipid vesicles with PEG molecules attached to them, which make the particles hardly filtered by the kidney but with the advantage over the other two solutions that they also hardly accumulate in organs.

Clearly, the iron-porphyrin model G2-hemoCD1 surpasses HBOCs in terms of circulation time. Multiple doses would not be required for full efficacy (as for HBOCs). Also, even if it is not as efficient as PFC emulsions (which stay in the body for 10 hours), I think the other criteria of oxygen-carrying efficiency as well as off-target reactions make up for that fact.

C. Off-Target Reactions

The last criterion to be evaluated is the off-target reactions the particles can encounter.

- In the case of HBOCs, they were found to have "NO-scavenging properties", which is harmful to the body [4]. They are also suspected to react with platelets in the blood and form clots, since patients in clinical trials have experienced strokes [2]. They also cause an immune reaction proportional to the amount injected into the body, which could cause uncomfortable and/or dangerous effects on the patient (pain, nausea, vomitting, fever, etc) due to the immune cells attacking the HBOCs [2].
- For perfluorocarbons, the Oxycyte model that was commercialized and FDA approved in 1989 caused concerns on the immune system and were suspected to cause hemorrhagic side effects [10]. More studies are currently being performed in Russia and Mexico [2].
- As for iron-porphyrins, the immune response they cause is currently unknown [2]. However, because they were found to have a very long circulation time, this means they were not destroyed by the immune cells, which I think mean that the immune reaction they cause is mild or absent. Additional research is ongoing to evaluate its NO-scavenging properties. About its CO-scavenging properties, they seem to be quite useful. Like hemoglobin, hemoCD1 was found to selectively bind to CO before being excreted from the body, which could be quite useful in areas other than blood substitutes:

  > Upon the injection of a solution of oxy-hemoCD1 in PBS into rat vein, the injected hemoCD1 was immediately excreted in the urine, as denoted in the previous section. Because of its extremely high CO-binding affinity, hemoCD1 binds endogenous CO during circulation in the blood stream and is excreted in the urine as the CO-bound form. HemoCD1 is also highly capable of capturing the CN- [cyanide] ion. HemoCD1 characteristics have inspired new research areas regarding CO as well as cyanide, including (1) removal of endogenous CO from animals for elucidation of its biological roles, (2) development of highly sensitive and convenient method for the detection of CO contained in biological samples, and (3) development of the CO/cyanide antidotes. [6, p.170]

Apparently, according to the criterion of off-target reactions, iron-porphyrins are the ones resembling hemoglobin the most. They are capable of removing CO from the body, and if injected at an appropriate quantity, it could help the treatment of CO and/or cyanide poisoning. HBOCs seem more harmful because of their evident NO-scavenging properties and perfluorocarbons also seem unsafe to the immune system.

IV. Conclusion

According to the criteria of oxygen-carrying power, circulation time, and off-target reactions, iron-porphyrins seems like the most promising red blood cell substitute candidate as of today. Kitagishi and Kano's model (G2-hemoCD) was found to be comparably efficient at carrying and releasing oxygen as whole blood [6]. Additionally, it was found to have a high circulation time due to not being filtered by the kidneys and not accumulate in organs. It is also suspected that the immune reaction that it could cause in humans is minimal. Its CO-filtering properties also seem quite advantageous. However, more extensive research needs to be performed in clinical trials to assess its safety in humans. Also, other criteria would need to be considered for an ideal red blood cell substitute to improve other aspects of its use: manufacturing and storage requirements are a few examples.

V. Recommendations

More information needs to be collected on the manufacturing and storage methods of these different solutions, as this could have a great impact on their usage in isolated or vulnerable communities which would benefit the most from blood substitutes. Additionally, more extensive research needs to be done on iron-porphyrin systems in preclinical trials, as they are the synthetic option that is the most similar to natural hemoglobin.

References

[1] Thomas Ming Swi Chang. "Blood substitutes based on nanobiotechnology". In: *Trends in Biotechnology* 24.8 (2006), pp. 372–377. ISSN: 01677799. DOI: 10.1016/j.tibtech.2006.06.005. URL: http://dx.doi.org/10.1016/j.tibtech.2006.06.005.

[2] Anirban Sen Gupta. "Bio-inspired nanomedicine strategies for artificial blood components". In: *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* 9.6 (2017). ISSN: 19395116. DOI: 10.1002/wnan.1464. URL: http://dx.doi.org/10.1002/wnan.1464.

[3] G. o. Alberta. *Hemoglobin*. URL: https://myhealth.alberta.ca/Health/Pages/conditions.aspx?hwid=tp10337. (accessed 10-03, 2021).

[4] James P. Collman et al. "Functional Analogues of Cytochrome c Oxidase, Myoglobin, and Hemoglobin". In: *Chemical Reviews* 104.2 (2004), pp. 561–588. ISSN: 00092665. DOI: 10.1021/cr0206059. URL: http://dx.doi.org/10.1021/cr0206059.

[5] T. Kakizaki et al. "Lipidheme-microsphere (LH-M) a new type of totally synthetic oxygen carrier and its oxygen carrying ability". In: *Proceedings of the 5th International Symposium on Blood Substitutes, March 17, 1993 - March 20, 1993*. Vol. 22. Artificial Cells, Blood Substitutes, and Immobilization Biotechnology. Publ by Marcel Dekker Inc, pp. 933–938. ISBN: 10731199.

[6] Hiroaki Kitagishi and Koji Kano. "Synthetic heme protein models that function in aqueous solution". In: *Chemical Communications* 57.2 (2021), pp. 148–173. ISSN: 13597345. DOI: 10.1039/d0cc07044k. URL: http://dx.doi.org/10.1039/d0cc07044k.

[7] Z Tao and PP Ghoroghchian. "Microparticle, nanoparticle, and stem cell-based oxygen carriers as advanced blood substitutes". In: *Trends in Biotechnology* 32.9 (2014), pp. 466–473. DOI: 10.1016/j.tibtech.2014.05.001.. URL: http://dx.doi.org/10.1016/j.tibtech.2014.05.001.

[8] In: *Trends in Biotechnology* 32.9 (2014), pp. 466–473. DOI: 10.1016/j.tibtech.2014.05.001.. URL: http://dx.doi.org/10.1016/j.tibtech.2014.05.001.

[9] Keiichi Karasugi, Hiroaki Kitagishi, and Koji Kano. "Modification of a Dioxygen Carrier, HemoCD, with PEGylated Dendrons for Extension of Circulation Time in the Bloodstream". In: *Bioconjugate Chemistry* 23.12 (2012), pp. 2365–2376. ISSN: 1043-1802. DOI: 10.1021/bc300303z. URL: https://doi.org/10.1021/bc300303z.

[10] E Maevsky et al. "Clinical results of Perftoran application: present and future". In: *Artificial Cells, Blood Substitutes, and Biotechnology* 33.1 (2005), pp. 37–46. DOI: 10.1081/bio-200046654. URL: http://dx.doi.org/10.1081/bio-200046654.