Polymeric Synthetic Oxygen Carriers for Transfusion at the Location of Injury

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Abstract

During excessive blood loss, hemorrhagic shock can occur if tissues do not receive adequate amounts of oxygen. As a result, intracellular lactic acid and oxygen radical concentrations increase, leading to widespread inflammation and eventually cell death across the body. The leading cause of death in Americans aged 46 and under is hemorrhage due to physical trauma; as such, research into methods of rapidly controlling hemorrhagic shock is particularly important, especially for military healthcare. Blood transfusion can be an effective hemorrhage treatment, but blood has a relatively short shelf-life of 35 days and supply can often fall below demand due to sourcing difficulties. Perfluorocarbons are small molecules with high oxygen dissolving capabilities, but are not stable during lyophilization and while inert, may have some adverse effects *in vivo*. Thus, polymer-based nanoparticles or emulsions will be employed to encapsulate the perfluorocarbons and create synthetic oxygen carriers. The synthetic oxygen carriers can be transfused during severe hemorrhage to restore the oxygen-carrying capability of the blood. These synthetic oxygen carriers have a high dissolved oxygen capacity, while being stable either before and after lyophilization or over two weeks. Additionally, we demonstrate techniques to reduce the size of the synthetic oxygen carriers, which will be important for *in vivo* use. Murine hemorrhagic shock models show an increase in the partial pressure of oxygen in the blood after transfusion of the emulsion-based synthetic oxygen carriers.

Keywords: hemorrhage, polymer, perfluorocarbon, transfusion, dissolved oxygen

Introduction

Hemorrhagic Shock

The circulatory system is responsible for transporting gasses, nutrients, hormones, immune cells, waste products, and other functional molecules and cells to maintain homeostasis throughout the body. Red blood cells, responsible for carrying oxygen to tissues, comprise around 40% of total blood volume.[1] A healthy adult typically has between 4.5 and 5.5 liters of blood, and can lose an estimated 14% of this blood volume due to hemorrhage without significant change in the ability to transport oxygen.[2] However, during excessive hemorrhage, tissues do not receive sufficient oxygen and are unable to maintain aerobic metabolism, leading to anaerobic metabolism and the subsequent production of lactic acid. Due to the significant risk of shock and death following severe hemorrhage, patients require immediate and rapid treatment. Hemorrhage secondary to physical trauma is the number one cause of death in Americans under the age of 46.[3] However, in military combat settings, hemorrhage is associated with over 90% of all potentially survivable deaths.[4] Of these deaths, a vast majority were pre-medical treatment facility deaths, meaning that those injured were unable to be transported to a medical treatment facility before succumbing to their injuries. In addition to methods to control bleeding, emergency blood transfusion is the standard of care for hemorrhage. Shorter times between injury and emergency transfusion were associated with a decrease in mortality,[5],[6] leading to emergency medical services in the United States adopting blood transfusions as a part of pre-hospital care.[7]

However, whole blood transfusion is associated with certain clinical risks. Unsafe transfusion practices can lead to worse patient outcomes,[8] while blood is also difficult to source, requiring a steady donor supply that must be additionally screened for diseases. O-negative blood is of particularly high demand for emergency use. Red blood cells have a finite shelf life, losing efficacy in transfusions after 21 days,[9] while whole blood has a shelf life of 35 days.[10] As such, a synthetic blood supply represents a significant improvement over whole blood or blood components for transfusion. Safely recreating each of the necessary blood components in a synthetic formulation completely eliminates concerns related to transmitted diseases and donor sourcing difficulties. Additionally, a shelf-stable formulation provides a significant advantage in terms of storage and transport, especially in military settings. Perhaps the most critical component of such a synthetic formulation is the oxygen carrier. Analogous to hemoglobin in red blood cells, the synthetic oxygen carrier must be able to increase the dissolved oxygen capacity in blood to prevent tissue hypoxia following hemorrhage.

Synthetic Oxygen Carriers

To address the limitations of natural blood, several “artificial” blood technologies have been developed. Currently there are two approaches for creating an artificial blood substitute: hemoglobin-based and perfluorocarbon-based oxygen carriers.[11] The former approach stems from hemoglobin, the body’s natural oxygen carrier, and is often isolated from human or bovine sources. When hemoglobin-based oxygen carriers (HBOCs) are administered during a blood transfusion they serve as a direct replacement for endogenous hemoglobin.[12] One study demonstrated that the use of HBOCs showed a similar vascular resistance, oxygen consumption, and metabolic stability in normothermic *ex vivo* kidney perfusion when compared to packed red blood cells.[13] While HBOCs do not contain donor-specific antibodies, require refrigeration, or carry infectious diseases, they have demonstrated some safety issues *in vivo* such as vasoconstriction and oxidative stress.[12] The latter approach, reliant on perfluorocarbons (PFCs), also demonstrates a

promising alternative to natural blood. They are small molecules that not only carry oxygen but are also biologically inert which makes them suitable for administration to the human body (Figure 1). Due to these unique properties, PFCs have been used in numerous medicinal applications such as cancer treatment, cell therapy, imaging, and, naturally, oxygenation of tissues.[14] Although these molecules are capable of dissolving large quantities of oxygen and exhibit many of the same qualities as HBOCs, they lack the stability to make them an effective replacement for natural blood.[15] In efforts to solve these limitations, chemical modifications and encapsulation techniques have been at the forefront of research for both HBOC and PFCs. This project is aimed at encapsulating PFCs in polymeric nanoparticles to increase their stability. Not only will this maintain the benefits outlined above, but it will also enable the lyophilization of the particles which further improves its ease of storage.

Design Criteria

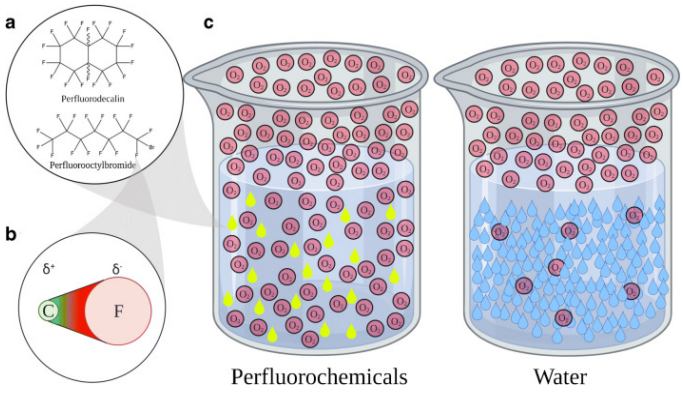

Fig. 1. Perfluorochemicals, including perfluorocarbons, can dissolve more oxygen than water. (A) Perfluoro decalin and perfluorooctyl bromide are two examples of common PFCs. (B) Perfluorocarbons have very polarized carbon-fluorine bonds. (C) At the same pO_2, perfluorocarbons are able dissolve significantly more oxygen than water due to a low molecular density in solution. Adapted from Jägers et al., 2021.

This project compares two different polymer-perfluorocarbon systems: poly(lactic-co-glycolic) acid (PLGA) with perfluorooctyl bromide (PFOB) and soy-lecithin (SL) with perfluoro-15-crown-5-ether (PFCE). Previous work performed with the murine hemorrhagic shock model demonstrated that both systems deliver an adequate amount of oxygen but cause a decrease in mean arterial pressure. To avoid this, and reduce the chance of any other related complications, the nanoparticles should have a decreased size. As this product transitions to a commercial scale, an effective nanoparticle synthesis and loading must be maintained to conserve materials and, ultimately, reduce production costs.

1. The nanoparticles should induce a significant increase in the dissolved oxygen capacity in water
2. The nanoparticles should be stable and maintain efficacy after lyophilization and resuspension or over time
3. The nanoparticles should be able to significantly increase the survival time of mice in a murine hemorrhagic shock model
4. The nanoparticles should be small enough to avoid unwanted interactions *in vivo*

Results

The soy-lecithin-PFCE emulsion has a higher dissolved oxygen capacity than PLGA-PFOB nanoparticles

First, the two oxygen carrier formulations were synthesized using probe sonication with a 1/4-inch tip size (see Methods). While both PFOB and PFCE are known to dissolve high amounts of oxygen,[15] the different formulations may not demonstrate adequate oxygen carrying capabilities. First, the oxygen carrying capabilities of the formulations were tested by measuring the difference in dissolved oxygen of the nanoparticles or emulsion droplets in solutions in unoxygenated (ambient) and oxygenated conditions (Figure 2A). Both formulations show an increase in oxygenated conditions, but the soy-lecithin-PFCE emulsions show a three-fold increase over the PLGA-PFOB nanoparticles. Next, both the size of the PLGA-PFOB nanoparticles and the soy-lecithin-PFCE emulsion droplets were measured using dynamic light scattering (Figure 2B). Both formulations resulted in similar particle sizes, with means between 300 and 400 nm.

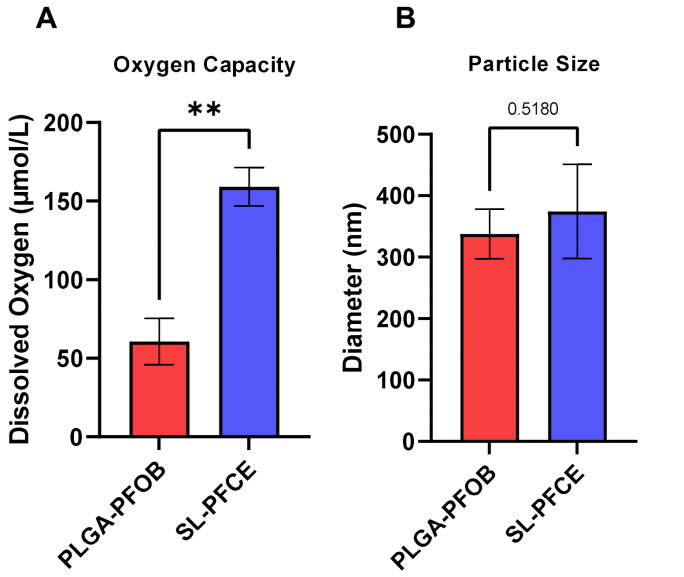

Fig. 2. Oxygen capacity and particle size of the two formulations. (A) Soy-lecithin-PFCE emulsions were able to dissolve significantly more oxygen than PLGA-PFOB nanoparticles in solution. **p < 0.01 (n = 3). (B) Both formulations resulted in similarly sized particles or droplets (n = 3).

Both extrusion and additional microtip sonication resulted in smaller soy-lecithin-PFCE emulsion droplets

We hypothesized that smaller particles would result in better biodistribution *in vivo*, leading to more effect treatments for hemorrhagic shock. As such, we explored methods of reducing particle size after the initial synthesis. The first method analyzed was high-pressure extrusion through a 200 nm diameter filter (see Methods). Each replicate was pushed through the extruder five times. While extrusion did not result in significantly smaller emulsion droplets, a downward trend was observed (Figure 3A). Additionally, a smaller standard deviation was seen after extrusion, indicating some convergence on the size of the particles following extrusion. This uniformity will be a necessary part of future manufacturing attempts on a larger scale. Five passes through the extruder results in smaller droplets than only one pass but using five passes only shows minimal benefit over using three passes (Figure 3B).

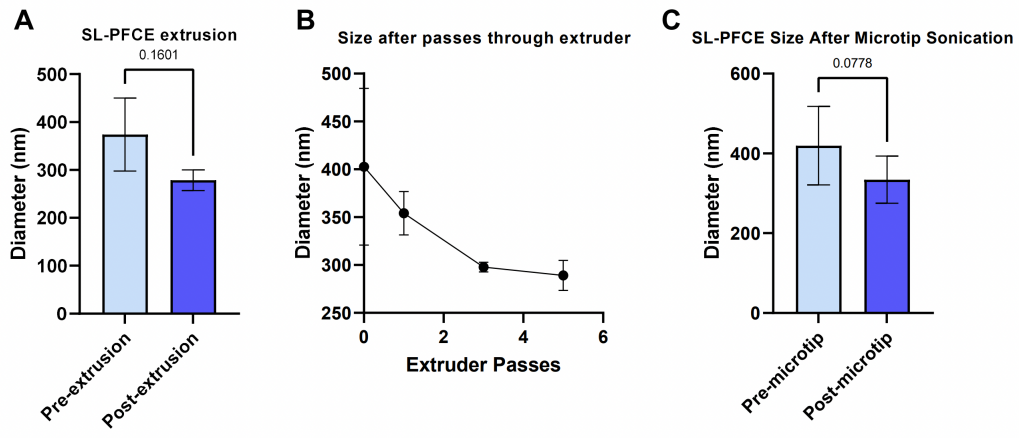

Fig. 3. Methods of reducing the final size of soy-lecithin-PFCE emulsions. (A) Extrusion of the soy-lecithin-PFCE emulsions shows a downward trend in diameter (n = 3). (B) Extrusions were done with multiple passes through the extruder. Shown is emulsion size after different numbers of passes through the extruder. (n = 2). (C) A round of microtip probe sonication following probe sonication with a normal tip shows a downward trend in particle size (n = 3).

The second method analyzed is an additional microtip sonication step during synthesis. After the initial probe sonication with a 1/4-inch tip, the probe sonication is repeated with a 1/8-inch microtip. Additional microtip sonication again shows a downward trend in soy-lecithin-PFCE particle size (Figure 3C).

Lyophilized PLGA-PFOB nanoparticles and soy-lecithin-PFCE emulsions did not change in size

To maintain a longer shelf-life of the nanoparticles, lyophilization and resuspension of the PLGA-PFOB nanoparticles were explored. Nanoparticles were resuspended in deionized water at least one week after lyophilization and dynamic light scattering measurements were taken (Figure 4A). Nanoparticles maintained similar sizes before and after lyophilization. Next, the soy-lecithin-PFCE emulsion was analyzed for its shelf-life potential as well. Instead of lyophilization, we took measurements emulsions at various time points within a two-week period. The emulsions maintained both a consistent droplet size (Figure 4B) and a high dissolved oxygen capacity (Figure 4C) over the two-week period.

Discussion

In conclusion, this study confirmed that the oxygen delivery capacity of the PFCE emulsions is significantly higher than that of the PFOB nanoparticles while maintaining a similar size. Furthermore, we demonstrated the use of two effective methods for reducing the size of PFCE emulsions: microtip sonication and extrusion. Finally, the experiments illustrated that both perfluorocarbon encapsulation systems, PLGA-PFOB and soy-lecithin-PFCE, are relatively stable over time as they both maintain their size despite lyophilization and long-term storage, respectively. The PFCE emulsion even maintains its oxygen delivery capacity over time.

Nonetheless, there are several future directions for this project. First off, there are numerous synthesis parameters that can be altered to decrease the size of the emulsion. These include sonication amplitude, duty cycle, polymer/perfluorocarbon ratio, perfluorocarbon type, etc. Additionally, the stability study of the PFCE emulsions should be extended to at least 1 month to compare against whole blood. Finally, we must evaluate these treatments in the murine hemorrhagic shock models by assessing survival times and plasma pO_2.

However, the implications of the research are numerous. In terms of our original design criteria, we were able to demonstrate that (1) the nanoparticles/emulsions induce a significant increase in the dissolved oxygen capacity in water and (2) the nanoparticles/emulsions are stable and maintain efficacy after lyophilization and resuspension or over time. Although we were unable to verify the safety and efficacy of the treatments *in vivo* as outlined in our criteria, we demonstrated the use of two methods to reduce the diameter of emulsion droplets. Together, researchers can utilize this data as a justification for the use of the soy-lecithin and PFCE encapsulation system. Along with further experimentation, these studies will provide a foundation for the use of SL-PFCE *in vivo* as a safe, effective alternative for whole blood. Ultimately, this will translate into a more accessible treatment option for patients experiencing hemorrhagic shock.

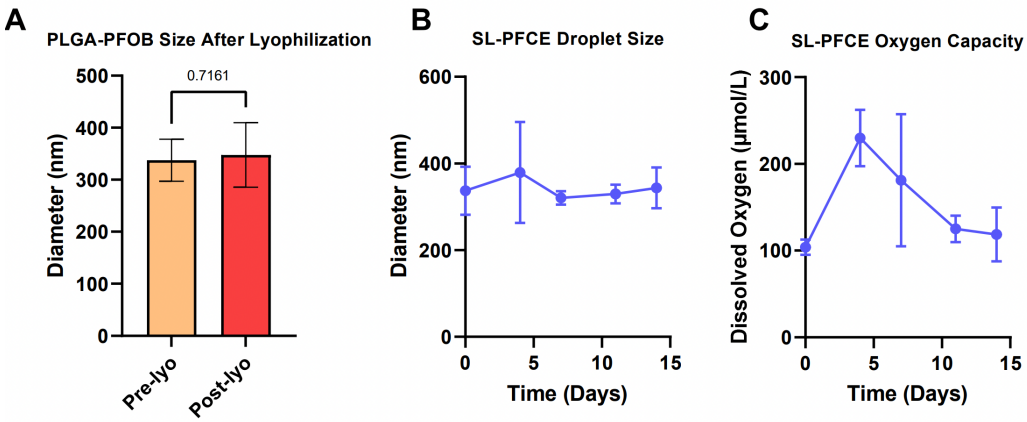

Fig. 4. Monitoring the stability of synthesized PLGA-PFOB nanoparticles and soy-lecithin-PFCE emulsions over time. (A) There are no significant changes in PLGA-PFOB nanoparticle diameter with lyophilization (n = 3). (B) The diameter of the soy-lecithin-PFCE emulsion droplets measured over a period of 14 days does not change in diameter (n = 3). (C) The soy-lecithin-PFCE emulsions maintain oxygen dissolving capabilities over a period of 14 days (n = 3).

Materials and Methods

Nanoparticle and emulsion synthesis

The nanoparticle and emulsions were synthesized by mixing appropriate volumes of the corresponding perfluorocarbon (PFCE and PFOB) and polymers (PLGA and soy-lecithin). Then, the solutions were laid to rest on ice for approximately 10-15 minutes before being probe sonicated with a 1/4-inch tip (Qsonica, Newtown, CT). For the micro-tip sonication samples, an additional sonication was performed with a 1/8-inch tip. Each sonication was performed with 20 second on / 15 sec off duty cycles for a total sonication time of 3 minutes. Samples that were lyophilized were frozen in a -80°C freezer for ten minutes before lyophilization.

Dissolved oxygen concentration in solution

Dissolved oxygen in solution was measured with a Foxy FOSPOR-R O_2 sensor, which measures changes in fluorescence quenching by oxygen (Ocean Optics, Orlando, FL). For each sample, an ambient and oxygenated condition was performed. The latter condition was prepared by saturating the nanoparticle or emulsion solution with oxygen gas for 10 minutes. In contrast, no modification was made to preparation of the ambient condition.

To measure the oxygen delivery capacity of each condition, the oxygen sensor was first used to measure the dissolved oxygen of 3 mL of DI water in a cuvette. After approximately 2 minutes, 100 µL of the oxygenated condition was injected and thoroughly mixed into the cuvette (Figure 5). Then, the dissolved oxygen concentration at approximately 5 time points both before and after the addition of the solution was averaged. Next, the oxygen delivery capacity of this condition was calculated by taking the difference between these averages. This process was repeated for the ambient condition. Finally, the oxygen delivery capacity of the nanoparticle or emulsion was calculated by taking the difference between the oxygen delivery capacity of the ambient and oxygenated conditions.

Size of the nanoparticles and emulsion droplets

Nanoparticle or emulsion droplet diameters and polydispersity indices (PDI) were measured with dynamic light scattering (Malvern Panalytical, Westborough, MA). Samples were thoroughly shaken to ensure homogeneity and diluted 100X before being measured. The instrument performed three distinct diameter and PDI measurements from which the average and standard deviation were calculated.

Emulsion extrusion

The emulsions were extruded using a high-pressure extrusion system. First, 5 mL of emulsion samples were filtered using a 0.45 µm syringe filter. Then, the sample was loaded into the extruder with a 0.2 µm pore-size membrane. Then, the pressure was increased to approximately 150-200 psi at which point the emulsion began extruding from the solution at a steady, constant rate. This process was repeated for a total of 5 passes through the extruder with emulsion droplet size measurements being made at 1, 3, and 5 passes.

End Matter

Author Contributions and Notes

J.K.A. and C.R.S. designed/performed research and analyzed data. J.K.A. and C.R.S. wrote the paper. The authors declare no conflict of interest.

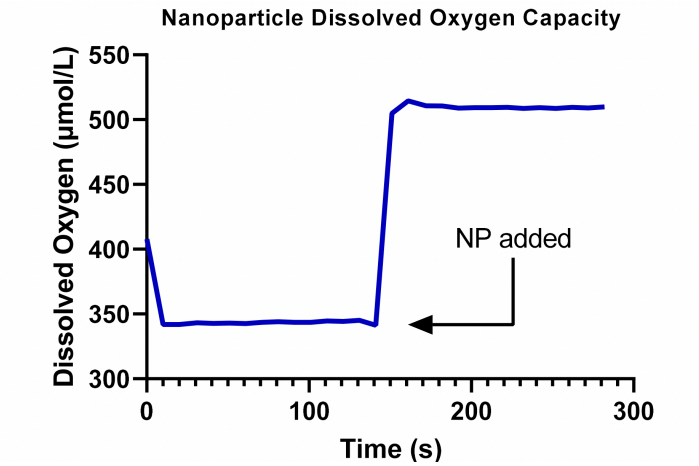

Fig. 5. Example trace of dissolved oxygen over time, with nanoparticle titrated into the cuvette at approximately 140 seconds.

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References

[1] R. Sharma and S. Sharma, "Physiology, Blood Volume," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2025. Accessed: May 02, 2025. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK526077/

[2] A. B. Johnson and B. Burns, "Hemorrhage," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2025. Accessed: May 02, 2025. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK542273/

[3] A. P. Cap *et al.*, "Whole Blood Transfusion," *Mil. Med.*, vol. 183, no. suppl_2, pp. 44–51, Sep. 2018, doi: 10.1093/milmed/usy120.

[4] B. J. Eastridge *et al.*, "Death on the battlefield (2001–2011): Implications for the future of combat casualty care," *J. Trauma Acute Care Surg.*, vol. 73, no. 6, p. S431, Dec. 2012, doi: 10.1097/TA.0b013e3182755dcc.

[5] C. M. Torres *et al.*, "Timing to First Whole Blood Transfusion and Survival Following Severe Hemorrhage in Trauma Patients," *JAMA Surg.*, vol. 159, no. 4, pp. 374–381, Apr. 2024, doi: 10.1001/jamasurg.2023.7178.

[6] M. A. Braverman *et al.*, "Prehospital whole blood reduces early mortality in patients with hemorrhagic shock," *Transfusion (Paris)*, vol. 61, no. S1, Jul. 2021, doi: 10.1111/trf.16528.

[7] D. S. Kauvar, R. Lefering, and C. E. Wade, "Impact of Hemorrhage on Trauma Outcome: An Overview of Epidemiology, Clinical Presentations, and Therapeutic Considerations," *J. Trauma Inj. Infect. Crit. Care*, vol. 60, no. 6, pp. S3–S11, Jun. 2006, doi: 10.1097/01.ta.0000199961.02677.19.

[8] M. F. Murphy, S. J. Stanworth, and M. Yazer, "Transfusion practice and safety: current status and possibilities for improvement," *Vox*

Sang., vol. 100, no. 1, pp. 46–59, Jan. 2011, doi: 10.1111/j.1423-0410.2010.01366.x.

[9] M. S. Y. Ng *et al.*, “Transfusion of packed red blood cells at the end of shelf life is associated with increased risk of mortality - a pooled patient data analysis of 16 observational trials,” *Haematologica*, vol. 103, no. 9, pp. 1542–1548, Sep. 2018, doi: 10.3324/haematol.2018.191932.

[10] S. Huish, L. Green, E. Curnow, M. Wiltshire, and R. Cardigan, “Effect of storage of plasma in the presence of red blood cells and platelets: re-evaluating the shelf life of whole blood,” *Transfusion (Paris)*, vol. 59, no. 11, pp. 3468–3477, Nov. 2019, doi: 10.1111/trf.15549.

[11] D. R. Spahn, “Artificial oxygen carriers: a new future?,” *Crit. Care*, vol. 22, no. 1, p. 46, Feb. 2018, doi: 10.1186/s13054-018-1949-5.

[12] F. Khan, K. Singh, and M. T. Friedman, “Artificial Blood: The History and Current Perspectives of Blood Substitutes,” *Discoveries*, vol. 8, no. 1, p. e104, Mar. 2020, doi: 10.15190/d.2020.1.

[13] M. M. Aburawi *et al.*, “Synthetic Hemoglobin-Based Oxygen Carriers are an Acceptable Alternative for Packed Red Blood Cells in Normothermic Kidney Perfusion,” *Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transpl. Surg.*, vol. 19, no. 10, p. 2814, Apr. 2019, doi: 10.1111/ajt.15375.

[14] N. Kakaei, R. Amirian, M. Azadi, G. Mohammadi, and Z. Izadi, “Perfluorocarbons: A perspective of theranostic applications and challenges,” *Front. Bioeng. Biotechnol.*, vol. 11, p. 1115254, Aug. 2023, doi: 10.3389/fbioe.2023.1115254.

[15] J. Jägers, A. Wrobeln, and K. B. Ferenz, “Perfluorocarbon-based oxygen carriers: from physics to physiology,” *Pflüg. Arch. - Eur. J. Physiol.*, vol. 473, no. 2, pp. 139–150, Feb. 2021, doi: 10.1007/s00424-020-02482-2