

Assessing the Biomechanical Properties of ErythroMer for Particle Integrity in an *ex vivo* Organ Perfusion System: Team HEM(O-)

Joel Bannerman, Claire Kettula, Jackson Lang,
Emily Passaro, Talya Simcox, Matthew Somerville

Team Mentor: Dr. Allan Doctor

Honor Pledge:

We pledge on our honor that we have not given or received any unauthorized assistance on this assignment.

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Abstract

The development of hemoglobin-based oxygen carriers (HBOCs) is critical for advancing organ perfusion systems. This study evaluates the structural integrity of ErythroMer (EM), a novel oxygen carrying nanoparticle, as a potential blood substitute for *ex vivo* kidney perfusion systems. Shear stress assessments were conducted to simulate physiological forces within complex pump systems. This study was organized through three scientific aims: quantification of EM peak tolerance, assessing shear stability over time, and evaluating the impact of suspension medium on shear tolerance. These aims assessed EM shear stability as a safety metric, ensuring maintenance of particle integrity were it to be used as a perfusion solution within an organ pump system. The results demonstrated that EM maintains structural integrity and resistance to lysis, even under high levels of shear stress. These findings support the potential use of the EM particle in future organ perfusion pump systems and its broader applications in biomedical fields.

1. Introduction

Kidney transplantation is currently the standard treatment for patients with end-stage renal disease (Ibrahim, 2009). A person is added to the transplant waiting list every 8 minutes, yet many wait months or even years for a life-saving organ. Limited accessibility to donor organs poses a critical healthcare challenge, with approximately 17 people in the U.S. dying each day. Despite thousands of patients waiting for a lifesaving donation, usable organs are discarded every day due to an inability to assess organ viability or the poor function of the tissue as a result injury or disease (Kahan, 2009). Specifically, patients in need of a kidney transplant face prolonged wait times due to the shortage of viable donor organs, with many kidneys being discarded due to concerns regarding delayed graft function (DGF), when transplanted tissue does not initially function, and ischemia-reperfusion injury (IRI), when tissue undergoes damage due to a buildup of harmful metabolites during oxygen deprivation (Malinoski et al., 2023).

Organs that become available for transplant will only be considered for transplantation to a patient within a certain geographical radius defined on the scale of hours. Upon notification of an available organ, patients have an extremely limited window of time to get to the hospital and prepare for surgery (Cole et al., 2021); however, donated kidneys may not make it to transplantation due to a variety of factors such as deficient organ function or inability to be transplanted to a patient in time before it starts to degrade. Annually in the U.S., 3280 kidneys from deceased donors are discarded (Lum et. al., 2023). *Ex vivo* organ perfusion addresses this issue by extending the preservation time of donor organs, allowing for longer transportation windows, and increasing the number of viable matches across greater distances. By maintaining organs in a metabolically active state outside the body, this technique reduces ischemic injury and improves overall transplant success rates (Pinezich & Vunjak-Novakovic, 2019).

Ex vivo organ perfusion significantly decreases the amount of time a tissue is not actively undergoing metabolism by continuously supplying oxygen and nutrients, which prevents cellular damage and enhances organ viability. It also enables real-time assessment of organ function, allowing for the selection of higher-quality grafts and reducing post-transplant complications. However, its widespread implementation is hindered by high costs, logistical challenges, and the need for specialized equipment and expertise. One of the key limitations of current perfusion systems is their reliance on red blood cell-based oxygen carriers, which introduce compatibility concerns, limited shelf life, and logistical constraints (Iske et al., 2023). To address these issues, oxygen-carrying nanoparticles have emerged as a potential alternative to improve oxygen delivery in perfusion systems.

Among these emerging solutions for oxygen-carrying nanoparticles is ErythroMer (EM), a synthetic red blood cell substitute. This blood substitute is a dried, bio-inspired artificial blood cell that has the potential to save lives when no donated red blood cells (RBCs) are available due to its ability to be stored as a freeze-dried powder. (Brummet et.al, 2022). EM is currently awaiting approval to begin Phase I clinical trials and presents a promising option for enhancing *ex vivo* organ perfusion by providing a stable and scalable oxygen carrier compared to other blood substitutes on the market (More et al., 2023). EM addresses issues with the current standard of RBCs with its ability to be stored without refrigeration making it ideal for perfusion machines in more geographically remote locations.

This research focuses on evaluating the structural stability of EM in high-shear stress *ex vivo* organ support systems to determine its feasibility for improving organ preservation and transplant outcomes. The structural stability of EM is studied with the intention to subject the substitute to a high-shear stress *ex vivo* organ support pump system. It aims to address the time

and distance constraints surrounding transplantation of organs by improving upon current emerging ex- vivo organ perfusion methods with the use of a synthetic blood particle, EM. By using EM to provide oxygen to explanted organ tissue, the ex-vivo kidney perfusion system has the potential to increase the time that donor organs are viable. EM has not yet been evaluated as an alternative to RBCs in organ perfusion systems, yet its chemical composition suggests it may be highly suitable with its advantages in oxygen delivery and long-term stability. The end goal of this project is to improve access to and quality of donated organs by determining the shear stress threshold of a synthetic blood particle to be used in an *ex vivo* organ perfusion system.

2. Literature Review

In 2023, 46,632 organ transplants were performed in the US from live and deceased donors, representing an 8.7% increase from 2022, and a 12.7% increase from 2021 (Procurement, 2023). Despite these positive trends, patients still wait months or years on transplant waiting lists, many of whom die before receiving an organ transplant. Issues surrounding these long waiting lists stem from factors such organ compatibility, poor tissue function caused by disease or injury, and viability issues related to the transplantation process. Accessibility issues are compounded by transplantation time window constraints and geographical limitations, resulting in healthy and viable organs being discarded, despite the ever-growing need of organs for transplantation. To address this, researchers have begun developing *ex-vivo* organ perfusion systems, including the use of hemoglobin-based oxygen carriers (HBOCs), to deliver oxygen to organs during the time window from donor to recipient, thus extending the lifespan and viability of donated organs.

2.1 History of Transplantation

Autogenous skin transplants, where skin is excised from one part of a person's body and grafted onto a different site on the same person, have been practiced since 600 B.C. (Barker & Markmann, 2013). This advancement led to homogenous transplants, where skin grafts were shared between species. However, these grafts largely failed for unknown reasons until transplantation immunology became a focus in the early 1900s. The first recorded attempted solid-organ transplantation occurred in 1906 when Mathieu Jaboulay, a French surgeon, switched kidneys between a pig and a goat. Unfortunately, both transplants failed and the subjects died (Watson & Dark, 2012). In the 1920s, James B. Murphy established that resistance to

homogenous transplants was due to the lymphatic system, but could not identify why or how to bypass the system (Barker & Markmann, 2013). Another problem interfering with the successful transplantation of organs was the lack of knowledge of the deteriorating effects of limited oxygen supply, or warm ischemic time on organ tissue, as many transplanted organs were retrieved from patients who had been deceased for many hours.

The first successful organ transplant, a kidney between identical twins, took place in 1954, which led to research that would breach the genetic barrier for human kidney transplantation. With the aid of immunosuppression drugs to reduce the likelihood of the body identifying and attacking the transplanted organ as a foreign object, Dr. Murray performed a successful kidney transplant between non-identical twins in 1962 (Barker et al., 2013). This breakthrough, along with better understanding of ischemic damage, marked a critical milestone as its success spurred further advancements and the eventual widespread adoption of organ transplantation, enabling the procedure to become more successful and widespread.

2.2 Organ Transplantation

Modern scientific and clinical advances have enabled the transplantation of a wide variety of tissues and organs from person to person. These include, but are not limited to, the heart, lungs, kidneys, liver, pancreas, intestine, skin, and parts of the eye.

Individuals awaiting a transplant are placed on waiting lists that are maintained by organizations such as the United Network for Organ Sharing (UNOS). A person's rank on the waitlist is based on several factors, such as time spent on the waitlist, lifestyle risk factors, severity of condition, and clinical outlook (United Network of Organ Sharing, *FAQs | Facts about Organ Donation & Transplantation*, n.d.). When an organ becomes available for donation,

it is matched based on the physical characteristics of the donor and the recipient, such as height and weight, geographical location, position on the waitlist, and the results of any blood or human leukocyte antigen (HLA) tests (UC Davis Health Transplant Center, *Matching and Compatibility*, n.d.). Of these factors, size of the organ and immunological compatibility are the most important. After a donor is matched to a recipient, the organ is harvested, preserved, and transported to the location of the recipient for transplantation surgery.

2.2.1 Organ Procurement

Organs are typically donated after a person dies, but certain organs, such as a kidney or part of a liver, can be given from a live donor (Health Direct Australia, 2024). Kidney donations from live donors are generally the more desirable option due to the lower risk of rejection. Living donations are planned events that donor and recipient are both able to prepare for and are therefore associated with uniformly high graft success rates, ranging between 84%-95% survival between 1 and 5 years post-transplant (Cecka, 1995). However, due to concerns from potential living donors about the associated risks of surgery, a majority of donated organs come from deceased donors, as seen in [Figure 1](#) (Kaplow, 2023).

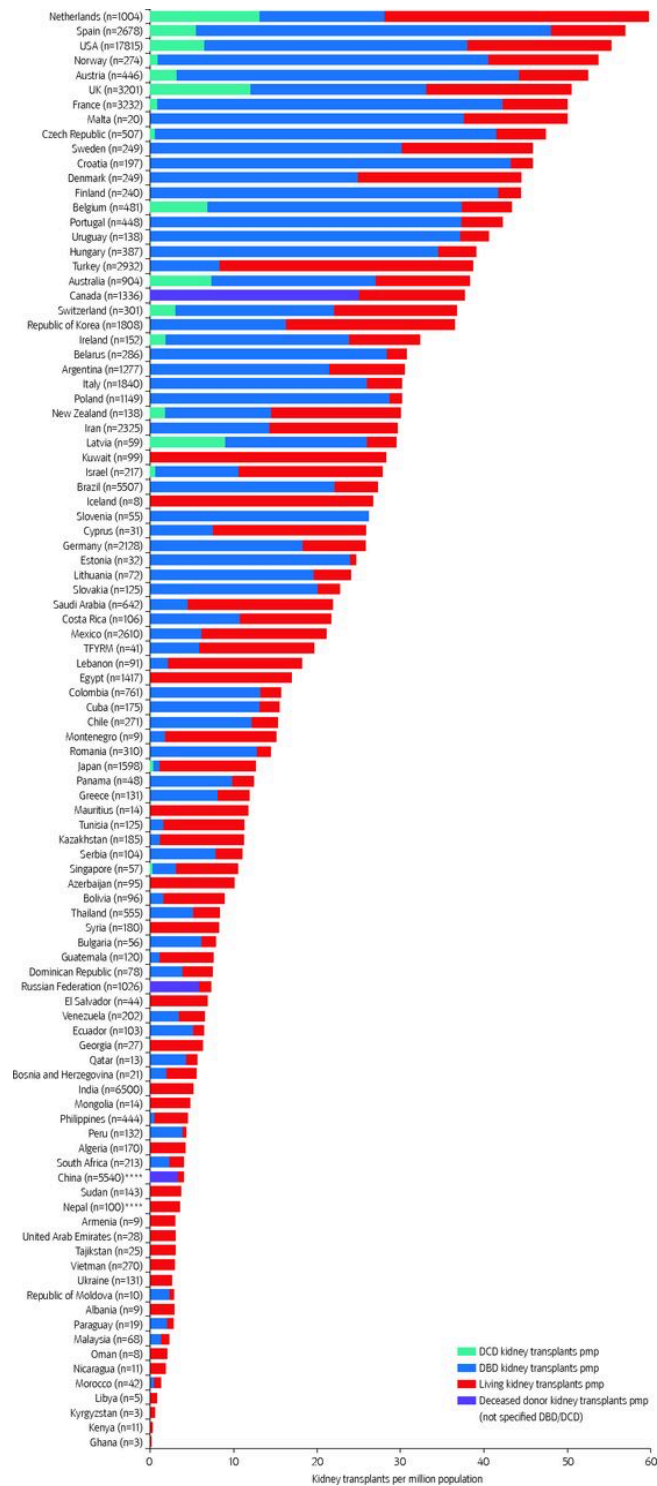


Figure 1. Kidney transplants in 2014 from either live donors (red) or deceased donors (all other colors). In a majority of countries reviewed, donation from deceased donors was the most prevalent donation type (Bailey, 2016).

Organ donations from deceased donors come from one of two scenarios: donation after brain death (DBD) or donation after circulatory death (DCD) (Almeida, 2024). Brain death can result from multiple causes, including stroke, infection, metabolic disorders, poisoning, or traumatic brain injury. Regardless of the cause, brain death almost always results in a system-wide innate and adaptive inflammatory response that can have negative impacts on the outcome of organ transplantation (Watts, 2013). A study looking specifically at the donation of kidneys after brain death showed increased levels of inflammatory and heat shock proteins in renal tissues, indicating a stress response in the tissue that led to DGF of the transplant (Nijboer, 2004). DGF, the medical term for when a transplant does not work right away, is associated with acute tissue rejection and chronic graft dysfunction in renal transplants (Nieuwenhuijs-Moeke, 2020). Despite this, DBD remains responsible for the majority of transplanted organs.

DCD, although less common, is responsible for a small percentage of donated organs and is associated with its own array of complications. DCD can occur in one of two ways, either controlled or uncontrolled. Controlled DCD occurs after brain death and a planned withdrawal of life support, whereas uncontrolled DCD can occur when an organ is retrieved after a cardiac arrest from which the patient was unable to be resuscitated (Almeida, 2024). DCD is generally associated with higher rates of DGF but overall has post-transplantation survival rates comparable to those from DBD (Smith, 2019). The increased risk of DGF likely comes from an increased vulnerability to cold and warm ischemic time fundamentally linked to DCD.

2.2.2 Organ Quality

Cause of death is not the only parameter linked to the quality of donated tissue. Algorithms such as the Donor Risk Index are typically used to quantify the quality of a donated organ. These algorithms consider factors such as the donor's height, weight, age, cause of death,

and serum sodium content to characterize the quality of the donated organ. Height can impact organ size and matching, serum sodium reflects the donor's hydration and kidney function, and cause of death can impact amounts of warm ischemic time experienced by the tissue. Abnormal values in these factors may signal potential complications, affecting transplant success (Flores & Asrani, 2017). Other factors that may contribute to an organ being deemed unviable may include the presence of malignant growths or damaged tissue (Mohan et al., 2018).

One of the most damaging factors of organ transplantation is IRI which occurs when blood flow is restored to a tissue following a period of prolonged restriction, known as ischemia. An organ that has experienced prolonged warm ischemic time may contribute to the organ being considered unsuitable (Mohan et al., 2018). When donated organs are removed from their blood supply, the lack of oxygen inhibits the electron transport chain and subsequent adenosine triphosphate (ATP) production, causing a shift toward anaerobic metabolism (Knijff, 2022). At this point, acidosis occurs and reactive oxygen species (ROS) are generated within the tissue, causing damage to cell membranes, the cytoskeleton, and even DNA (Nieuwenhuijs-Moeke, 2020). This complex cascade of toxic responses is eventually amplified by reperfusion and can lead to apoptosis and inflammatory or immune responses, resulting in transplantation failures and death (Ponticelli, 2015) (Figure 2).

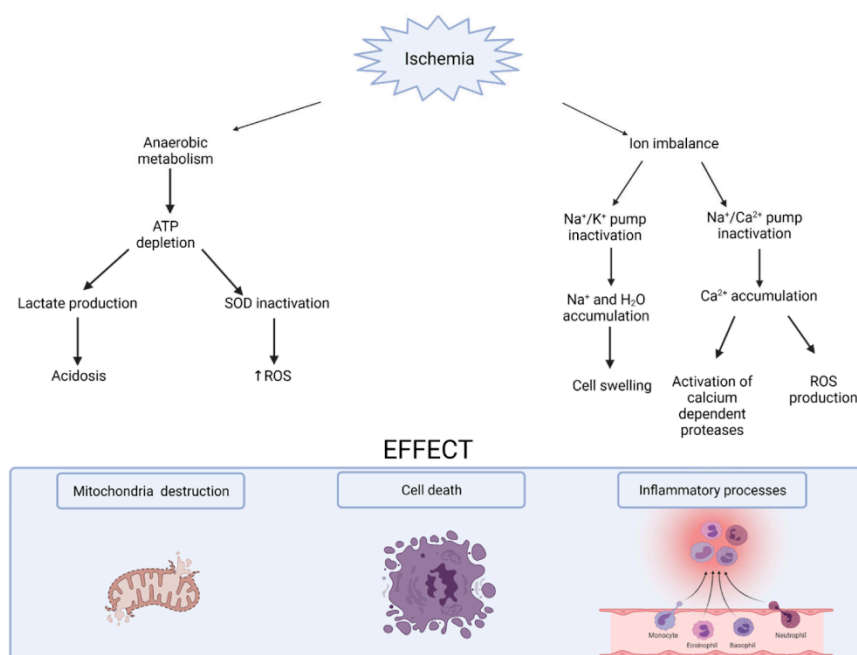


Figure 2. Mechanisms of damage pathways causing IRI and its effect on cell metabolism (Radajewska, 2022).

Ischemia can be categorized into either warm or cold ischemic time. Cold ischemia refers to the period during which donated tissue is kept between 0-8°C, considerably colder than physiological body temperature to slow cell metabolism, and without blood flow to deliver oxygen before being transplanted into the recipient (Jing, 2018). Tissues donated from live donors do not experience cold ischemic time, and therefore cold ischemic injury is only a concern in transplants from deceased donors. Warm ischemia occurs whenever hypothermic conditions are not met, and is generally more detrimental to all donated tissues than cold ischemic time because it leads to faster cellular damage, inflammation, and oxidative stress (Almeida, 2024).

2.3 Demand for Transplanted Organs

The accessibility of donated organs in the U.S. is significantly affected by geographic disparities and time-sensitive constraints due to organ viability. Donated organs are only viable

outside the body for a matter of hours, meaning longer transportation distances and coordination complexities can result in organ wastage or reduced organ viability (Potluri, 2023). While around 100,000 patients wait for kidney transplants, the availability and allocation of organs remain uneven across different geographical areas.

While the supply of donated organs is a major barrier to disease treatment, Kidney transplantation is the preferred treatment over expensive, impermanent treatments such as dialysis (Chaudhry et al., 2022). The growing length of the kidney transplant list is due, in part, to the addition of the patients over the age of 65 caused by the aging population. Epidemiological studies conducted in 2016 showed that 11% of the world's population was over the age of 60, and this number was projected to increase to 22% by 2050 (Kanasi, 2016). As the world population continues to age, there will be an increase in names added to the waitlist, as shown in Figure 3 below. Without a plan to increase the supply of donated organs, the number of patients who die while waiting for an organ transplant will similarly increase.

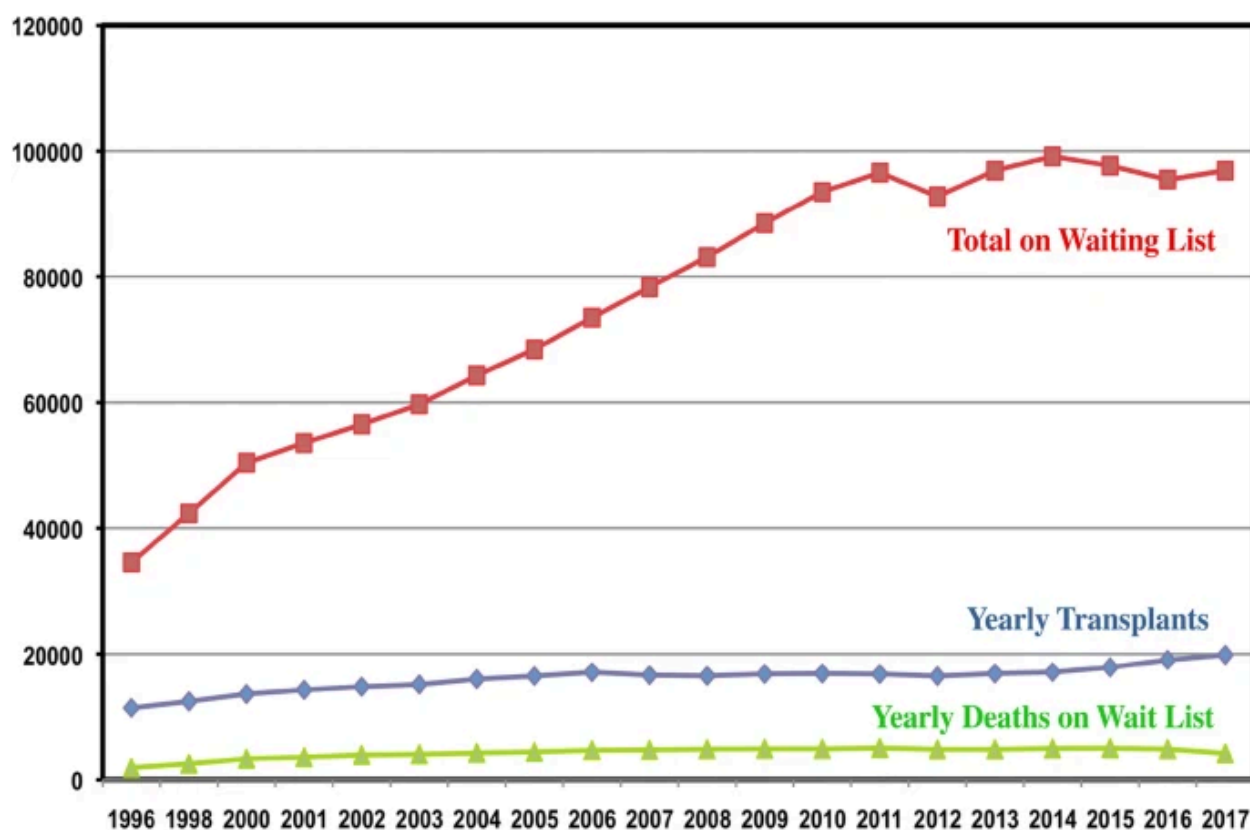


Figure 3. Graph of solid organ transplant waitlist showing growing size of waitlist with the aging population. (Bastani, 2019).

This increased demand cannot be met, and this shortage of available organs has caused an exponential growth of names on the waitlist for organ transplantation. In the United States in 2006, the waitlist had over 95,000 people awaiting life-saving organ transplants. Of these 95,000 people, only 28,140 received a transplant that year, and 6,120 patients died while waiting. (Abouna, 2008). For kidney transplants specifically, between 2003 to 2013 patients listed were identified and followed through 2018. Of these 324,582 patients listed, 18.0% were delisted, mostly from becoming too ill and after delisting, 49.7% died by follow-up (Sokas et al., 2021).

Despite the growing demand for organ transplants, an increasing fraction of donatable organs are being discarded, as seen in [Figure 4](#) below. From 2000-2015, 17.3% of kidneys were discarded, which was a 91.5% increase compared to the previous decade. The cause of this

increase is not necessarily known but may include an increased selectivity of organs by transplantation centers or the increased procurement of lower-quality organs (Mohan et al., 2018). As the demand for organs continues to rise, the development of organ perfusion systems has become increasingly critical to preserving organ viability and improving transplant outcomes.

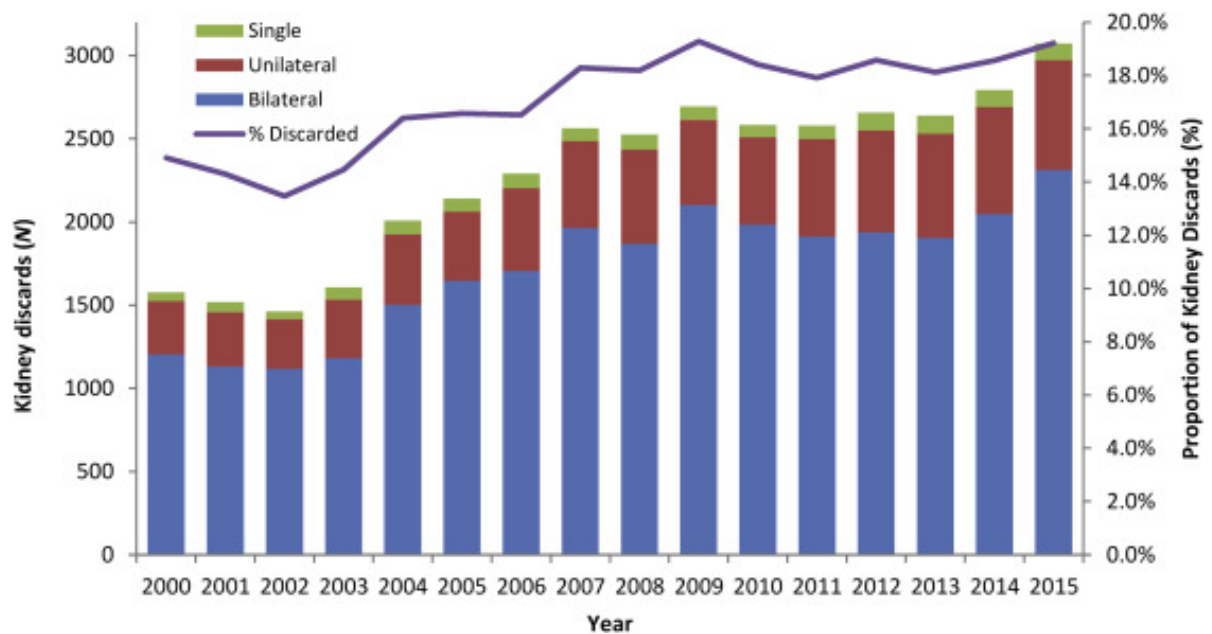


Figure 4. A graph showing the number of discarded kidneys from 2000-2015, including single, unilateral, and bilateral. The general trend of the graph shows that the number of discarded kidneys has almost doubled over that time (Mohan et al., 2018).

2.4 Organ Storage Methods

To reduce the effects of warm ischemic time on tissue quality, static cold storage (SCS) has been the gold standard method of storage for many years, accounting for nearly 95% of all kidney preservations from 1995-2005 (Opelz, 2007). SCS involves storing the tissue on ice after retrieval from the donor and is therefore nonspecific and imprecise (Tingle, 2019). Using SCS methods, the heart and the lungs will remain viable for 4-6 hours, the liver for 8-12 hours, and

the kidneys for 24-36 hours (Middleton, 2023). SCS of organs induces hypothermia to slow metabolism, thereby reducing the buildup of waste products with potential toxicity. The tissue may, in these cases, also be washed and kept in a preservation solution containing electrolytes and other osmotic agents to help maintain the membrane integrity of the cells in donated tissues (Pham, 2010). However, whilst such solutions can significantly increase tissue viability, over time, tissues still degrade due to a lack of oxygen (Finger et al., 2023). Oxygen deprivation is still a factor with cold storage, but its impact is less severe compared to warm ischemia.

Since its invention in 1986, the University of Wisconsin (UW) solution has been the most widely used preservation solution available (tHart, 2002). UW solution was designed to mimic intracellular fluid with high concentrations of potassium and magnesium and a low sodium concentration. Potential disadvantages of this solution, however, include a high viscosity that complicates tissue saturation as well as a considerably high cost per liter (Chen et al., 2019).

The extent of IRI is largely determined by the role and function of the mitochondria as it is the driver of cellular metabolism. Advances in understanding mitochondrial permeability mechanisms has led to the development of targeted interventions aimed at reducing ROS production and mitochondrial damage. These strategies include perfusion fluids that incorporate antioxidants such as MitoQ, a compound that reduces oxidative stress by scavenging ROS within the mitochondria, and SS peptides, which interact with the inner mitochondrial membrane to support mitochondrial function. These interventions have shown promise in mitigating oxidative stress during transplantation. Machine perfusion, which circulates preservation solutions with antioxidants and nutrients through the organ, has emerged as a promising method to improve graft viability. Effective kidney storage strategies addressing IRI hold promise in optimizing

graft viability, especially when using extended-criteria donor organs or high-risk donations (Nieuwenhuijs-Moeke et al., 2020).

2.5 *Ex vivo* Organ Perfusion

Circulating perfusion fluid through donated tissue aims to extend preservation time and reduce the constraints posed by the geographical distance between donor and recipient. Using extracorporeal circulation to combat the deterioration of tissues after death was first introduced in 1812 by Cesar Julien Jean Le Gallois (Boettcher et al., 2003). His idea to inject tissues with blood, either naturally or synthetically formulated, to sustain cellular function has led to the development of extracorporeal membrane oxygenation and *ex vivo* organ perfusion techniques (Jing et al., 2018).

Unlike static preservation, standard *ex vivo* perfusion machines maintain dynamic circulation of specialized perfusate solutions which contain a mixture of nutrients, electrolytes, and oxygen carriers. Propelled by a pump, the perfusate is passed through an oxygenator before reaching the organ's arterial system and is then drained through the venous system. These machines enable the organ to continue functioning at a near-physiological level by providing essential nutrients and removing toxic by-products. Additionally, they allow for real-time monitoring and regulation of key parameters such as oxygenation, temperature, and flow rates, providing clinicians with a more comprehensive picture of tissue viability (Berkane et al., 2024). Perfusion requirements, including pressure, flow rate, and oxygen levels, vary by tissue type but collectively enhance preservation techniques tailored to individual transplants (Kruit et al., 2019). This technology has significantly reduced ischemic time, minimizing the accumulation of toxic metabolites and improving transplant outcomes. This process offers a longer operating

window, reduces the urgency for immediate transplantation, and potentially expands the geographical radius for organ transport, ultimately increasing the pool of viable donor organs while maintaining their quality and function (Yuan, 2010).

Ex vivo organ perfusion has been employed to preserve organs such as the lungs, liver, kidneys, and heart. However, its widespread implementation is constrained by challenges including the structural complexity of different organs and the need for precise metabolic regulation. Given that immune cells undergo metabolic reprogramming to meet bioenergetic and biosynthetic demands, optimizing perfusion conditions to support organ-specific metabolic environments are critical for improving preservation and transplantation outcomes (Iske et al., 2023).

With the growing organ shortage, the use of suboptimal organs for transplantation is becoming more common. These organs may be less ideal due to factors such as donor age, pre-existing conditions, or prior damage (Weissenbacher et al., 2019). Their reduced resilience to preservation stress indicates the limitations of traditional cold storage methods. However, organ preservation using machine perfusion technologies continues to generate results that show improvement in viability, organ utilization, and early graft function. Clinical studies to date report that machine perfusion offers major benefits over conventional cold preservation, including reduced IRI, improved graft survival, enhanced organ function through re-conditioning, and the ability to assess organ viability before transplantation.

2.5.1 Classifications of Machine Perfusion

Machine perfusion can be broken down into several classifications based on the temperature of storage and type of flow in the system. The first machine perfusion strategies that

emerged utilized continuous flow under hypothermia, to slow metabolic processes within the cells while maintaining a steady flow of nutrients to the tissue. However, more recently the field has turned to exploring normothermic conditions and pulsatile flow to better mimic physiological conditions (Sevinc et al., 2019).

Hypothermic machine perfusion (HMP) is especially beneficial for donor organs from older or high-risk donors, providing a cooling environment that decreases the likelihood of graft failure. Hypothermia reduces the cellular metabolism to 5-10%, thereby reducing oxygen consumption and ROS production within the cells, reducing IRI, and improving graft outcomes (Radi et al., 2023). While HMP has been shown to improve outcomes compared to SCS transplants for standard criteria donors, it is an insufficient method of tissue storage in expanded criteria donors (ECD). Those with ECD are over the age of 60, or over 50 matching two of the following three characteristics: history of high blood pressure, serum creatinine >1.5 mg/dL, or death due to a stroke (Argani et al., 2022). The hypothermic conditions are not physiologically similar and have led researchers to wonder whether replicating physiological conditions could further improve transplant outcomes.

In comparison, normothermic machine perfusion (NMP) has emerged as a potential solution, as it maintains donated tissues at 35-37°C in a metabolically active state. In this near-physiological state, cells have the potential to repair and recondition before transplant, improving the quality of the donated tissue (Hamelink et al., 2022). In a study comparing the use of HMP and NMP in the preservation of porcine kidneys, researchers found that NMP performed comparably to HMP in preserving renal function, as illustrated in Figure 5, while also offering increased opportunities for organ assessment and intervention not possible with HMP (Blum et al., 2017). Despite promising early clinical studies in heart, lung, and kidney transplants, the

technology's high costs and complexity present obstacles to the widespread integration of the technology. Nonetheless, ongoing research indicates that normothermic perfusion could significantly improve transplantation outcomes by enabling better organ preservation (Blum et al., 2017).

As previously mentioned, flow is another critical factor used to classify machine perfusion, and can be either continuous or pulsatile. Continuous flow is theorized to be beneficial to donated organs due to the continual vascular shear stress exerted on endothelial cells in the system. Flow stagnation within vascular structures can lead to inactivation of the KLF2-mediated endothelial vasoprotection pathway in cells. Consequently, endothelial cells undergo apoptosis in the absence of flow induced shear stress, thereby leading to failed transplantation outcomes. Pulsatile flow, on the other hand, more accurately mimics physiological conditions and is being explored as an alternative to continuous flow. Initial experiments showed that pulsatile flow better preserved endothelial function by sustaining KLF2 expression, reducing activation and apoptosis, and improving transplant outcomes (Gracia-Sancho, 2010). Further research posits that pulsatile perfusion of kidneys preceding SCS can improve circulation and nutrient absorption and therefore reduces cellular injury experienced by the tissue (Von Horn & Minor, 2018). Understanding the specific requirements of different donated organs can help clinicians determine which method of perfusion and storage will offer the best transplant outcome.

2.5.2 Physiologic Requirements of Machine Kidney Perfusion

Renal transplants from deceased donors are subject to unavoidable effects of IRI, which machine perfusion aims to ameliorate by supporting the metabolic requirements of the tissue. This includes temperature and oxygen parameters, but there is no universal protocol for the

preservation of kidneys *ex vivo* (Zulpaite, 2021). However, there are some guidelines generally followed in the machine perfusion of kidneys. The first is that if NMP is being used to recondition a donated kidney after a period of SCS, the temperature, pressure of perfusion, and flow rate should be raised gradually and in parallel with one another until reaching near-physiological levels, as seen in [Figure 5](#) below (Minor et al., 2020).

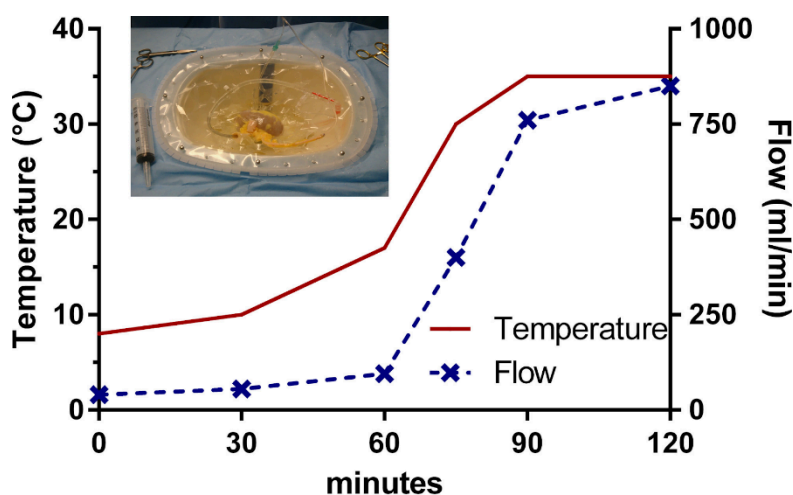


Figure 5. Time course of renal perfusion flow and temperature during NMP following SCS and prior to transplantation.

In the body, the kidneys receive approximately 20% of cardiac output in resting conditions, which is second only to the heart in terms of oxygen consumption (Connor, 2006). This is because renal tissue is extremely vulnerable to hypoxia yet only has an oxygen extraction ratio of about 10-15% in healthy tissue, compared to the 45% extraction ratio in cardiac tissue, necessitating the high cardiac output requirement in kidneys (Edwards and Kurtcuoglu, 2022). Therefore, adequate oxygenation of renal tissue during machine perfusion is a critical consideration, as inadequate oxygenation of tissues will result in damaged tissue and failed graft function.

2.6 Perfusion Solutions

2.6.1 Blood and Machine Organ Perfusion

Whether or not donated tissue is oxygenated during perfusion can, in some cases, have a significant impact on the quality of transplanted organs. In HMP, oxygenation is optional, as the metabolic mechanisms are slowed and the tissue does not actively consume oxygen at the same rate it would at physiological temperatures. For this reason, oxygenation is required for NMP (Gilbo et al., 2017).

Although it is not required, HMP with oxygenation has been shown to improve transplantation success rates in extended-criteria donors when compared to SCS preservation methods. In donated kidneys, graft survival 1-year post-transplant had a success rate of 100% with oxygenated HMP and 93.3% for SCS (Ravaioli et al., 2020). Since oxygen also plays an important role in wound healing, oxygenation of organ transplants could mean the ability for cells to self-improve the quality of the tissue, leading to better transplant outcomes (Leaper et al., 2007).

The use of blood products to perfuse donated organs as a solution to oxygenate the tissue has been explored in NMP for their ability to provide a near-physiological environment, but prolonged perfusion with blood-based solutions has been associated with hemolysis, platelet activation, and deterioration of the function of RBCs (Hosgood et al., 2014). One of the most important functions of RBCs is their ability to transport oxygen, due to the presence of hemoglobin (Hb). While Hb is a necessary protein for an essential process in maintaining life, some of its functions can cause damage to the body. The constant use of the iron binding sites to

transport oxygen can cause a reaction known as ferrous oxidation, or oxidative stress, which can be toxic for nearby cells.

The majority of oxygen in the blood is bound to Hb within RBCs, while a smaller amount is dissolved in the plasma. In tissues, oxygen diffuses down its concentration gradient from high to low concentrations and then gets delivered to the cells. This understanding of oxygen transport underscores the importance of RBC integrity, as mechanical stress during circulation can chronically degrade their structure. However, in the case of donor organs, the degradation occurs over a much more acute time frame, amplifying the need for preserving RBC integrity during the limited window of viability. As a result, oxygen delivery throughout the organ remains a critical area of study to explore how blood flow can potentially increase circulation and thus organ viability.

One of the key limitations of natural blood in relation to organ transplantation lies in the rheological properties of the cells and how RBCs respond to increased shear stress. When blood is pumped into transplanted organs, RBCs are subjected to varying shear forces as they move through the vasculature, particularly in areas like capillaries where microvessels are narrow and shear stress is higher. As RBCs age, their membranes become less flexible, causing them to lose the ability to adapt to these mechanical stresses. This degradation can impair their ability to deliver oxygen efficiently, compromising the metabolism of the cell and the quality of the tissue. Moreover, the depletion of nitric oxide (NO) in RBC aging and storage, which regulates vasodilation and blood flow, further exacerbates issues by increasing vascular resistance (Schmid-Schönbein, 1999). Consequently, organs may not receive sufficient oxygen, especially under high-stress conditions, which can hasten organ failure.

RBCs experience significant mechanical stress during their repeated transit throughout the body, which gradually causes their structure to break down. This limits their lifespan to about 3-4 months, after which they are cleared by the spleen and liver as skeletal proteins and membrane lipids degrade. When RBCs are damaged too quickly, organ failure can be caused by reduced oxygen (O_2) delivery to tissues. RBCs also undergo alterations in NO metabolism, linked to conditions such as hypertension, diabetes, and heart failure. This dysfunction in oxygen delivery is strongly associated with critical illness, creating a direct connection between RBC degradation and serious health outcomes (Brummet et al., 2022). Transplanted organs, in particular, rely heavily on uniform perfusion through the microvascular network, but as natural RBCs degrade through physical damage due to aging and shear stresses, this perfusion becomes less efficient, leading to uneven oxygen distribution (Schmid-Schönbein, 1999). The body's inability to maintain consistent oxygen delivery under increasing shear stress highlights a significant limitation in organ viability during and after transplantation. This is particularly concerning as microvessels operate within a lower shear stress range, making the high-shear stress environments typically encountered during perfusion detrimental to the viability of donated tissues. This emphasizes the need for advanced strategies that provide adequate oxygenation while minimizing harmful shear stress.

Blood flow in the microcirculation is dominated by viscous stresses, and because cells within this environment possess viscoelastic properties, they often encounter nonlinear mechanical challenges. Shear stress, a mechanical force generated by blood flow, has a particularly profound effect on RBCs as they traverse through these microvessels. The ability of cells to dynamically adjust their shape and mechanical properties under these stresses influences how blood is delivered to organs, as well as how effectively they can oxygenate. However, as

these stresses increase, especially during critical situations such as organ transplantation or trauma, RBC functionality may become compromised, reducing their capacity to deliver oxygen efficiently and leading to potential organ dysfunction (Schmid-Schönbein, 1999). To address the challenges of oxygen delivery, researchers are investigating alternatives to RBCs, such as HBOCs.

2.6.2 HBOCs

The liquid used to perfuse organs during *ex vivo* preservation can potentially be formulated with blood substitutes, such as perfusing liquids containing HBOCs. The purpose of this liquid is to extend organ viability during transportation and improve the organ's quality before implantation (Cao M et al., 2021).

HBOCs are derived by chemically or genetically modifying free Hb. Approximately 98% of oxygen transported in the body is bound to Hb, and the other 2% is dissolved in plasma (Rhodes et.al, 2021). Many HBOCs are designed to have a lower oxygen affinity than RBCs and can lead to the rapid release of oxygen to tissues, especially when oxygen demand is high. The need for ischemic and hypoxic treatments has now expanded the application of HBOCs beyond their initial use as *in vivo* blood substitutes. Utilizing HBOCs for isolated organ preservation may have several potential advantages, as shown in [Figure 6](#), including maintaining cellular metabolic needs, mitigating hypoxic damage, having a smaller diameter than RBCs for better oxygen delivery, lacking immune reactivity, offering shelf stability for extended storage, and adapting to a wide range of temperatures (Cao et al., 2021).

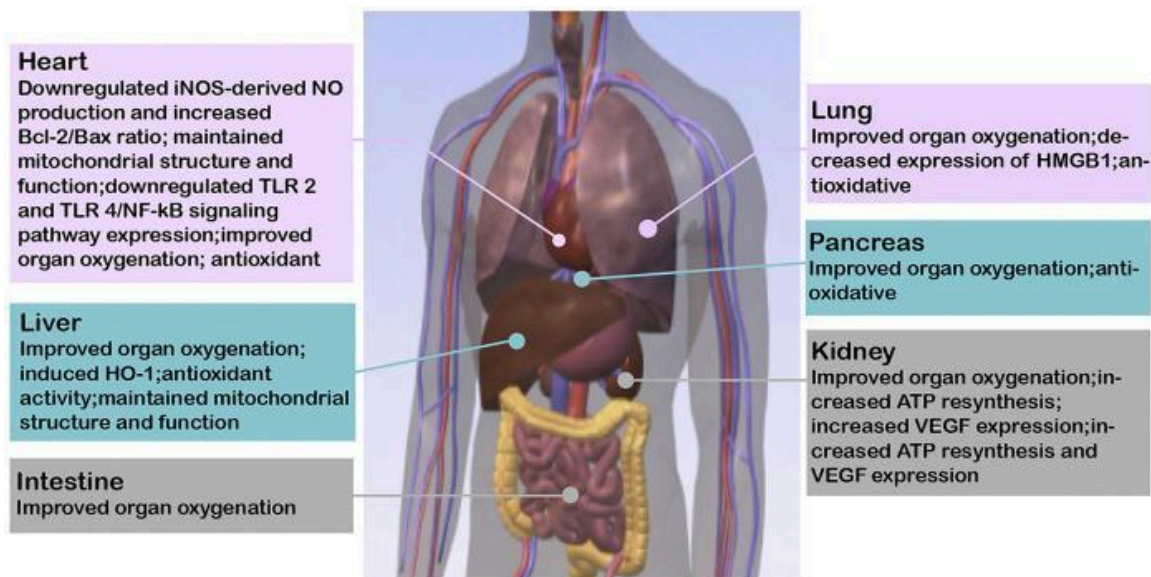


Figure 6. Relevant mechanisms where HBOCs are beneficial for the preservation of various solid organs (Cao et al., 2021).

The effectiveness and safety of HBOCs in the preservation of grafts have been studied in numerous preclinical and clinical studies. Compared with traditional preservation protocols, the addition of HBOCs to traditional preservation protocols provides more oxygen to organs to meet their metabolic needs as shown in [Figure 6](#). Ultimately, these benefits are thought to have potential to prolong the preservation period, reduce the effects of ischemia and reperfusion on grafts, improve graft quality, and even potentially increase the number of organs deemed viable for transplantation (Cao et al., 2021).

2.6.3 EM

To address challenges and shortages associated with the use of RBCs in *ex vivo* organ storage, EM, an HBOC, is being developed as a novel blood substitute. EM offers a more controlled approach to O₂ capture and release while limiting potentially adverse interactions in circulation. It is designed as a deformable, hybrid peptidic-lipid nanoparticle that encapsulates Hb within a biosynthetic artificial cell membrane. This encapsulation helps prevent the harmful

interactions seen with non-Hb encapsulated HBOCs, such as blood vessel constriction known as NO scavenging, radical producing reactions such as Haber-Weiss and Fenton reactions, while also improving oxygen (O₂) delivery. EM allows for extended shelf life by encapsulating Hb within a stable, biosynthetic, hybrid peptidic-lipid nanoparticle, which protects the Hb from degradation and prevents the oxidation and instability commonly seen in HBOCs. This unique encapsulation helps maintain the structural integrity and functionality of the Hb, allowing EM to remain effective for longer periods without the need for refrigeration, making it an ideal candidate for use in emergency situations such as mass traumas or as a stock for blood banks during crises or disasters (Mittal et al., 2022).

The ongoing development of EM is driven by the need to better understand how blood substitutes function under the varying pressures and shear stresses present in the circulatory system. This becomes particularly important during exercise, strenuous activity, or trauma. Under these conditions, the heart's pumping rate and the oxygen demands on the body increase, which places additional stress on blood cells. During these moments of heightened metabolic activity, tissue oxygenation must increase accordingly, making it essential that blood substitutes can handle these physiological demands (Dominelli et al., 2021). EM's bio-inspired design, which replicates key elements of RBC physiology, allows for an efficient and flexible response to these changes. However, to ensure EM can withstand the increased pressures and shear forces experienced during machine perfusion, future improvements to its structural integrity may be necessary. EM is a promising oxygen delivery system that could be used to meet the metabolic demands of normothermally preserved *ex-vivo* organs in order to improve the access to and quality of donated kidneys.

3. Materials and Methods

The goal of our thesis was to quantify EM particle shear stability and thus address the question as to whether EM integrity would be maintained within a perfusion pump system. As EM shell stability ensures Hb encapsulation, this is a highly important safety consideration to inform the use of EM as a perfusion solution. Hemoglobin payload loss would effectively render EM unable to effectively deliver O₂ (free Hb without allosteric effector has a very high O₂ affinity), whilst also resulting in highly damaging radical formation through free Hb oxidative biochemistry, which would likely reduce rather than enhance organ viability in transport from donor to recipient. Our approach was organized into three specific scientific aims:

1. Quantification of EM peak shear tolerance - To identify the shear force at which greater than 5% of the payload within EM formulation was released, indicating significant particle failure.
2. Time course assessment of EM stability at maximal perfusion pump shear stress - To identify whether EM could withstand the prolonged shearing typical of that required by organ perfusion solutions (i.e., ensuring structural integrity of the particles over time).
3. Impact of EM suspension medium on shear tolerance - To address whether the plasma protein albumin impacted the shear stability of EM particles.

3.1 Aim 1 - Quantification of EM Peak Shear Tolerance

EM, a lipid nanoparticle-based HBOC was formulated by Kalocyte Inc. (Baltimore, MD, USA) and provided to Team HEM(O-) in phosphate-buffered saline (PBS, Quality Biologicals). Due to the proprietary nature of EM, we are unable to disclose its formulation. However, it can

be confirmed that each of the batches tested were of the same formulation (same lipid composition) and composed using the same methodology.

3.1.1 ZetaView Particle Characterization

Prior to experimentation and pre and post shearing, EM batch formulations were fully characterized (particle count, particle size distribution, and zeta potential, i.e. particle surface charge), using a Zeta View Nanoparticle Tracking Analysis Instrument (Particle Metrix, Germany). To allow batch to batch comparison, Team HEMO normalized particle count to an expected *in vivo* final circulating particle concentration (1×10^{14} particles/mL), with the addition of PBS (Quality Biologicals, USA).

Zeta-view particle characterization utilizes a combination of dynamic light scattering (DLS) and electrophoretic mobility to determine the size, concentration, and surface charge (zeta potential) of particles within a solution. DLS tracks the Brownian motion of the particles as they scatter laser light (Bachurski et al., 2019). In addition, zeta potential, the electrokinetic potential surrounding a molecule, is also measured to quantify the particles' stability and surface charge. Finally, by analyzing the light scattering of the molecules via optical microscopy, ZetaView can determine the nanoparticle concentration.

A polystyrene standard (diluted 2000k times in deionized (DI) water) was used to calibrate the Zeta View's auto alignment and focus optimization to allow for accurate determination of size and zeta potential. Calibration was performed daily before each experiment. All EM samples were diluted to $\sim 1 \times 10^{14}$ particles/mL with PBS.

3.1.2 Shearing Protocol

A rheometer (M302e; Anton Parr) was used as a shearing device to subject EM to precise and controlled shearing. The controlled variable for the three aims is shear stress (τ) in pascals (Pa). The double-gap geometry was selected to allow for larger sample volumes that were necessary for our analysis of particle stability. EM was suspended in PBS to a particle density ($\sim 1 \times 10^{14}$ particles/mL), which was selected to represent the physiologic particle load expected for *in vivo* EM use as a blood substitute. Rheometer was loaded by connecting polytetrafluoroethylene syringe tubing (Sigma-Aldrich) between an injection pump with a 10 mL syringe (Harvard Apparatus 11 Plus Syringe Pump) and the rheometer. The injection pump was set to load the EM sample at a rate of 2 mL/min (total load ~ 10 mL).

To quantify peak shear tolerance, samples were sheared for 10 minutes (37°C) at constant shear rates ranging from 0 to 190 Pa. Given that the expected shear tolerance of our collaborator's organ perfusion pump system was 10 Pa (Rassmus, n.d.), Team HEM(O-) selected a range of shear forces from the expected pump maximal shear stress to x20 higher than expected perfusion shear stress (i.e. 10 to 190 Pa). EM (~ 10 mL of $\sim 1 \times 10^{14}$ particles/mL) was loaded into the rheometer utilizing a syringe pump. For each experiment, a control sample was collected that did not undergo shearing (Figure 7A). Following shearing, samples were removed from the rheometer and assayed for total Hb and free Hb (non-EM encapsulated) as follows. Sheared (and unsheared control) samples were mixed with either (1) the detergent n-octyl beta-D-glucopyranoside (NODG) (Cas Number: 29836-26-8), which dissolves in the lipid shell of EM to release all Hb, allowing the determination of total Hb within the sample or (2) DI water (which maintains EM particle integrity) allowing the pelleting of intact EM and determination of Hb released from damaged particles (Figure 7B). To pellet intact EM, (un-sheared and sheared)

that were diluted in either NODG or (DI) water were ultracentrifuged (Eppendorf microtubes) (60,000 g, 1 hour, 4°C), leaving released free Hb from EM particles in the supernatant solution above ([Figure 7C](#)).

3.1.3 Drabkin's Assay

To quantify heme (and thus [Hb]; i.e., total [Hb] or [Hb] released from EM) we utilized the Drabkin's assay (Green, 1959). Hb exists in different oxygenation and oxidation states (oxygenated, deoxygenated, carboxyhemoglobin, and methemoglobin), each with distinct absorbance spectra and absorbance maxima at different wavelengths ([Figure 8A](#)). RBCs or HBOCs, such as EM, contain Hb in various proportions of these different Hb oxygenation/oxidation states. The Drabkin's reagent (Sigma D-5941) converts all Hb states (oxygenated, deoxygenated, carboxyhemoglobin, and methemoglobin) to cyanmethemoglobin, which has a single absorbance peak maximum at 540 nm, thus allowing the quantification of total [heme] in a single measurement ([Figure 8B](#)).

Following ultracentrifugation, supernatant samples were diluted in Drabkin's reagent, adjusted differently for total vs free Hb to obtain an optimal absorbance for heme quantification (i.e., between 0.1-1 AU). The following dilutions were prepared: W1 (supernatant water: Drabkin's, 1:1, no shear), W2 (supernatant water: Drabkin's, 1:1, sheared supernatant), D1 (supernatant detergent: Drabkin's, 1:1, unsheared), and D2 (supernatant detergent: Drabkin's, 1:1, sheared). Drabkin's samples were vortexed, incubated (30 minutes, room temperature) in amber Eppendorf tubes ([Figure 7D-F](#)), and subsequently plated in triplicate (300 µL; 96-well plate), with absorbance read at 540 nm (Biotek Neo2, USA) ([Figure 7G-H](#)). Appropriate blank samples were assayed for NODG or DI water with Drabkin's reagent, and the blank absorbances

subtracted from sample absorbance, following which free Hb was calculated for each sample (as in Section 3.1.4) included WB (water blank) and DB (Drabkin's blank). Blank absorbances were subtracted from sample absorbance, following which free Hb was calculated for each sample. (as in Section 3.1.5) A full graphical representation of the procedure can be found in Figure 9.

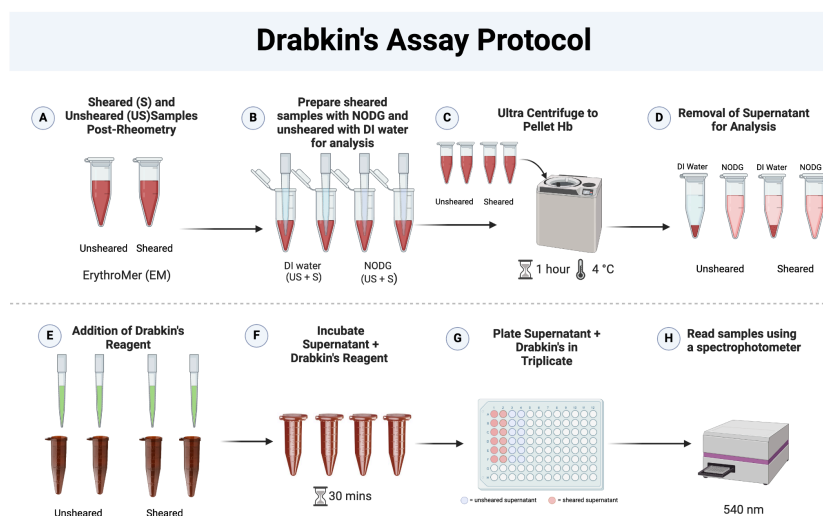
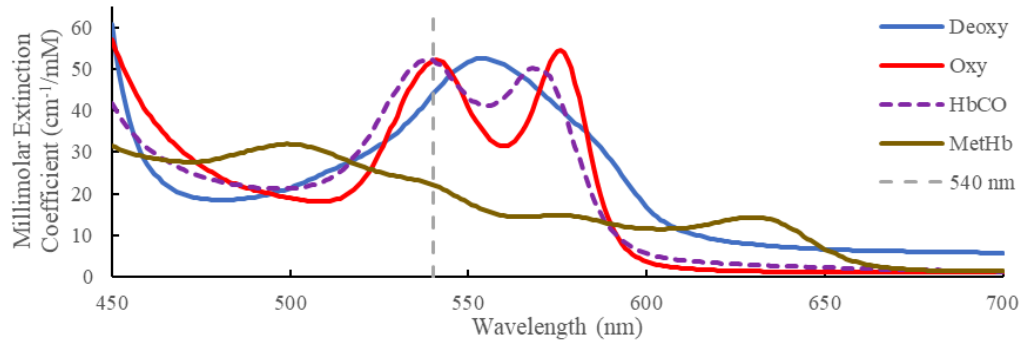


Figure 7. Graphical Abstract of Drabkin's Assay. Experimental workflow illustrating key steps in free Hb analysis. Image generated with BioRender.

A.



B.

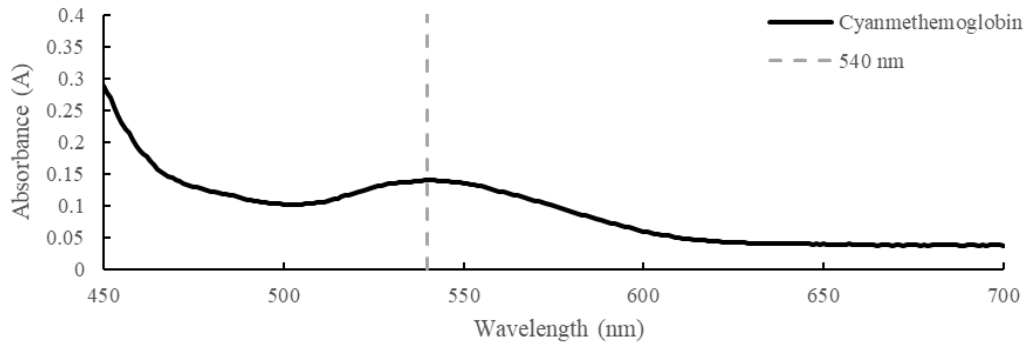


Figure 8. Comparison Between Spectra of Hb in its Various Oxygenation and Oxidation States With Cyanmethemoglobin Between 450 and 700 nm. (A.) The absorbance characteristics of oxyhemoglobin (Oxy), deoxyhemoglobin (Deoxy), carboxyhemoglobin (HbCO), and methemoglobin (MetHb), measured across a spectrum of wavelengths. The distinct absorbance peaks reflect the unique light absorption properties of each type. (B.) The absorbance readings of cyanmethemoglobin measured at different wavelengths. The absorbance peak at 540 nm is used for accurate quantification of concentration in blood samples.

3.1.4 Free Heme Calculation

In each sample (sheared and unsheared), total heme and free heme released from EM was quantified using the following equation:

$$\text{Heme (mM)} = \frac{(A)(DF)}{(\epsilon)(l)} \quad (1)$$

A sample absorbance at 540,

DF dilution factor

ϵ extinction coefficient = $11\text{mM}^{-1}\text{cm}^{-1}$,

l Pathlength (a pathlength correction of 0.8 was used as we utilized a 96 well plate rather than a 1cm cuvette)

From this heme, [Hb] was calculated as follows

$$Hb (mM) = \frac{Heme (mM)}{4} \quad (2)$$

From Hb calculation, the % EM lysis was calculated as follows:

$$\%EM \text{ Lysis} = \frac{\text{Free [Hb] from DI water sample}}{\text{Total [Hb] from NODG sample}} \times 100 \quad (3)$$

3.2 Aim 2 - Time Course Assessment of EM Shear Stability at Maximal Perfusion Pump Shear Stress

EM ($\sim 1 \times 10^{14}$ particles/mL) was sheared for varying lengths of time at the expected shear force of our collaborators organ perfusion pump system (10 Pa; Rassmus, n.d.). This experiment was designed to assess whether EM could withstand prolonged shearing in the pump system itself over durations one might expect an organ to be transported. We selected 10, 30, 60, 120, and 240 minutes at 37°C, based on feasibility within the testing time frame. The methodology followed for free heme, [Hb], and % lysis was identical to that described in Section 3.1.

Throughout the process of data collection, multiple batches of EM particles were used to compile the data set. While the samples were prepared separately, they were formulated from the same procedure yielding insignificant differences between concentrations of particles and their lipid makeup. Despite the anticipated insignificance of variance between the samples, the formulations yielded varying free Hb prior to any subjugation to shear stress. To account for this, the percentage of free Hb released was normalized to an unsheared control sample of the same

formulation. This normalization accounts for particles that may have been damaged due to storage or time, freeing Hb into the batch prior to shearing, and allowing the varying formulations to be compared with each other.

3.3 Aim 3 - Determination of the Effect of EM Suspension Medium on Shear Tolerance

For Aim 1 and 2, the impact of shear stress on EM particle integrity was tested in a simple buffer (i.e., PBS). However, in a vascular system, oncotic pressure (i.e., colloid osmotic pressure), which is the pressure exerted by blood proteins in the plasma that draws fluid back into the capillary, is an essential consideration. This could also influence the movement of fluid into and out of EM, which could impact shear stability. Subsequently, we tested two different EM suspension media (i.e., PBS vs 5% albumin). Albumin was selected because it is the major plasma protein contributing ~80% of plasma oncotic pressure and often used as a perfusion solution.

To assess the effect of the suspension medium on EM shear tolerance, EM ($\sim 1 \times 10^{14}$ particles/mL) was divided into two groups: one suspended in 5% Human Serum Albumin (HSA, Sigma-Aldrich) and the other in PBS. Both groups were subjected to shear stress (10 Pa, 37°C) for varying durations (10, 30, and 60 minutes) to evaluate whether the suspension medium influenced EM particle stability. The methodology followed was identical to that described in Section 3.1.

3.4 Statistical Analysis

3.4.1 Aim 1- Quantification of EM Peak Shear Tolerance

To quantify peak shear tolerance of EM, % free Hb data was plotted and fit with a linear regression.

3.4.2 Aim 2 - Time Course Assessment of EM Shear Stability at Maximal Perfusion Pump Shear Stress

To analyze the data collected for this aim, the data was tested for normality and variance to confirm that the assumptions required for the use of a one-way analysis of variance (ANOVA) test was usable. Bartlett's test of Variance and the Shapiro-Wilk Test for Normality was conducted using MATLAB R2023b (MathWorks, USA), followed by the one-way ANOVA after the assumptions were met. Additionally, data was plotted and averaged data was fit with a logarithmic regression. The goal of this test was to assess the statistical significance between the time samples across the 10 Pa of shear stress. The function *varestn* and a subset of the *normalitytest.m* normality test package (Öner, M. et. al., 2017) for the Shapiro-Wilk test were used to establish the variance and normality respectively within the data for assessing the assumptions to conduct the ANOVA test. The *anova1* function was subsequently used to conduct the ANOVA test, with *multcompare* executing a Bonferroni Test for post hoc assessment. The value $p < 0.05$ was chosen to establish significance.

3.4.3 Aim 3 - Impact of EM Suspension Medium on Shear Tolerance

To evaluate if suspending EM in albumin caused significant differences in shear tolerance (Aim 3), we utilized a two-way ANOVA. Residuals of models were tested to ensure they met

linear regression assumptions of normality and homogeneity of variance between groups using Shapiro-Wilk & Bartlett's tests, respectively. For the two-way ANOVA, if the group-time interaction was not statistically significant, then the main effects were reported. A Bonferroni correction for multiple comparisons was utilized to control for type 1 errors. All graphs were made using Excel (Microsoft Office 2016). Statistical analyses were conducted on MATLAB r2023 (MathWorks, USA) where a subset of the *normalitytest.m* normality test package and *varestn* (Öner, M. et. al., 2017) were used to test assumption of variance and normality respectively. The *anova1* function was subsequently used to conduct the ANOVA test, with *multcompare* executing a Bonferroni Test for post hoc assessment. Significance was set at $p=0.05$ for all tests.

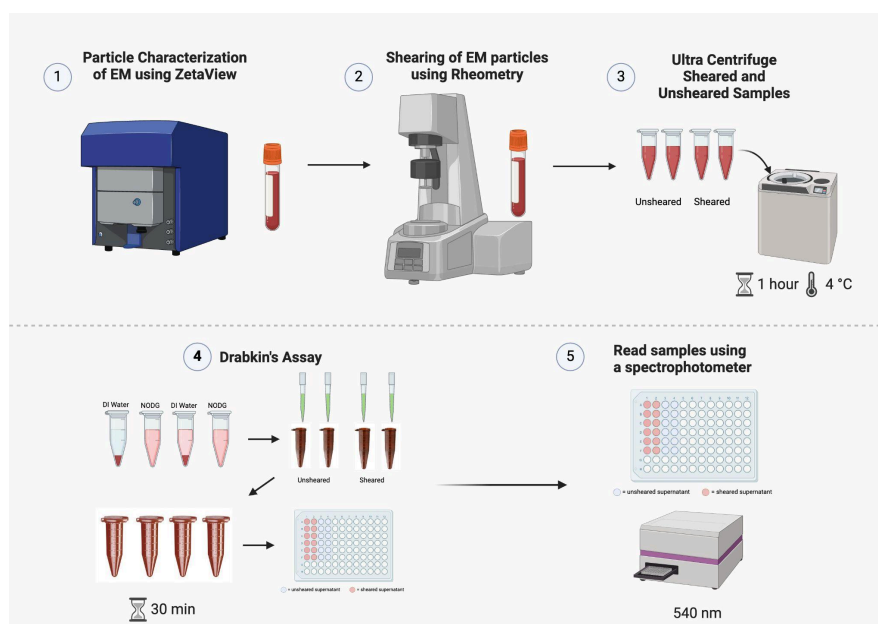


Figure 9. Graphical Abstract of Three Aims. Experimental workflow illustrating key steps: Particle characterization using ZetaView, rheological analysis with a rheometer (M302e; Anton Paar), ultracentrifugation for sample separation, and quantification through Drabkin's assay. Followed by absorbance measurements using spectrophotometry.

4. Data and Results

4.1 Aim 1 - Quantification of EM Peak Shear Tolerance

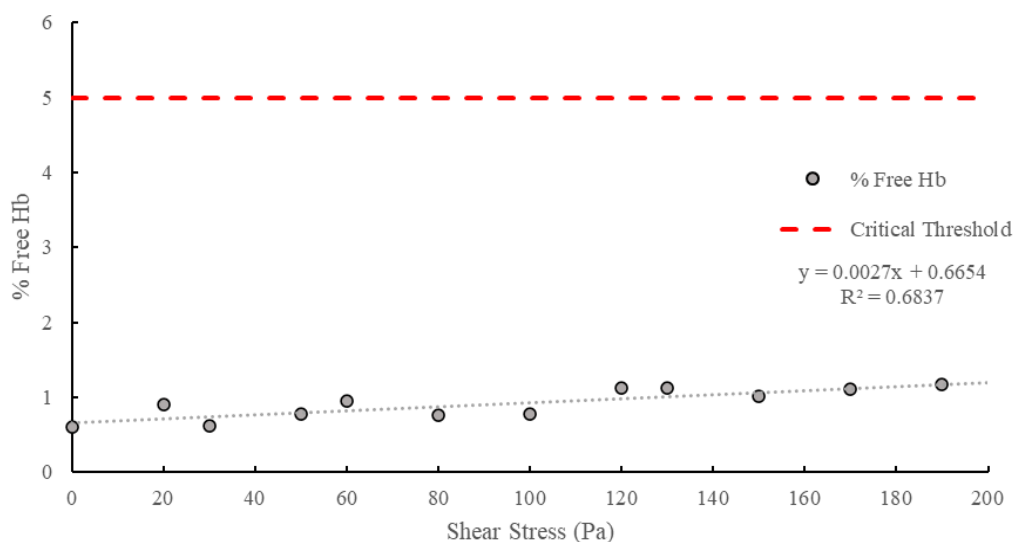


Figure 10. Percentage of Hb Payload Released from EM Following Short Interval Shearing. Percent free Hb of an EM particle solution as a function of shear stress following subjugation to shear force for 10 minutes. Hb release was far lower than the 5 percent free Hb critical threshold established. (n=1)

We hypothesized that the EM particle membrane would fail above shear tolerance, thereby releasing Hb payload into the suspending fluid. For this experiment, the failure criteria was established at 5% free Hb. Following subjugation to constant shear forces ranging from 0 to 190 Pa for 10 minutes at 37 °C in a double gap geometry (M302e, Anton Paar), a portion of the EM Hb payload was released (defined as free Hb). We observed a linear relationship between shear stress and free Hb between 0 and 190 Pa. Maximal Hb payload loss after 10 minutes of shear stress subjugation over this shear stress range was < 1.2%, which fell well under our 5% threshold.

4.2 Aim 2 - Time Course Assessment of EM Shear Stability at Maximal Perfusion Pump Shear Stress

Multiple EM batch formulations were used to compile the data for this project. Although all EM batches were formulated from the same components and with the same procedure, small but significant differences in stock EM free [Hb] was observed between batches and even within the same batch, prior to any subjugation to shear stress. To account for this, the percentage of free Hb released as a result of shearing was normalized to an unsheared control sample of the same formulation. This normalization accounts for particles that may have been damaged due to storage or time, freeing Hb into the batch prior to shearing, and allowing the varying formulations to be compared with one another.

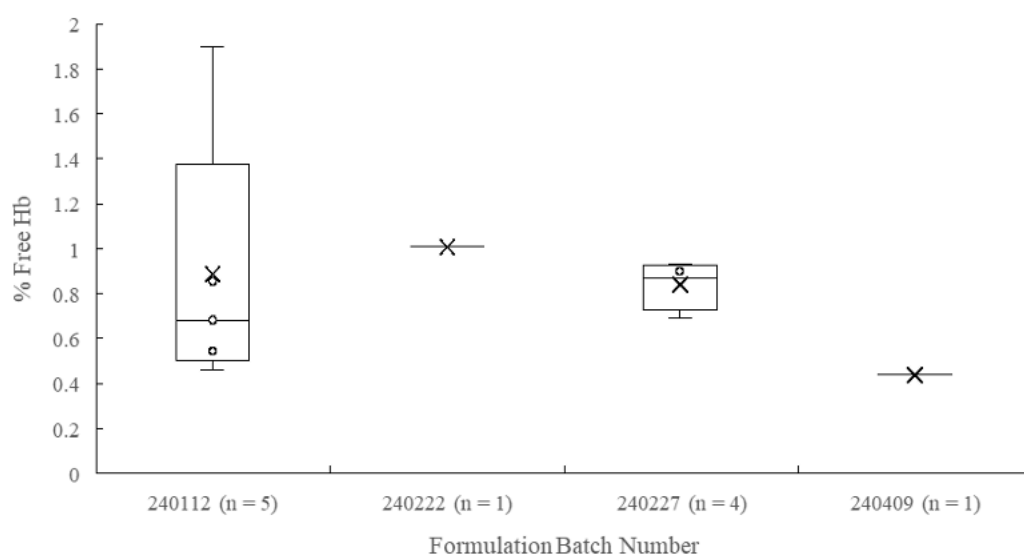


Figure 11. Free Hb in each EM formulation prior to shearing. Variation between percentage of free Hb within and amongst different formulation batches.

To assess the ability of EM particles to withstand extended shear forces which would be expected within a perfusion pump system during donor organ transport, particles were subjected

to a constant shear force of 10 Pa (Figure 12) at varying time points up to 240 minutes. An initial increase in free Hb was observed up to 30 minutes, which appeared to plateau at approximately a change of 4% free Hb. Loss of EM particle integrity (i.e. release of Hb from EM) followed a logarithmic trend.

The preliminary statistical assessments confirmed that the assumptions for conducting a one-way ANOVA test were met. The Shapiro-Wilk test indicated that the data was normally distributed ($p = 0.3232$), and the Barlett's test of assessing variance showed no significant difference in variance across groups ($p = 0.0649$). The results of the one-way ANOVA revealed a statistically significant difference between time points ($p = 0.0043$). To determine which time points differed, a Bonferroni test was conducted, revealing that the 10-minute time point was significantly different from the 120- and 240-minute time points ($p = .0120$ and $p = 0.0030$). No other time points held significant differences ($p > 0.05$).

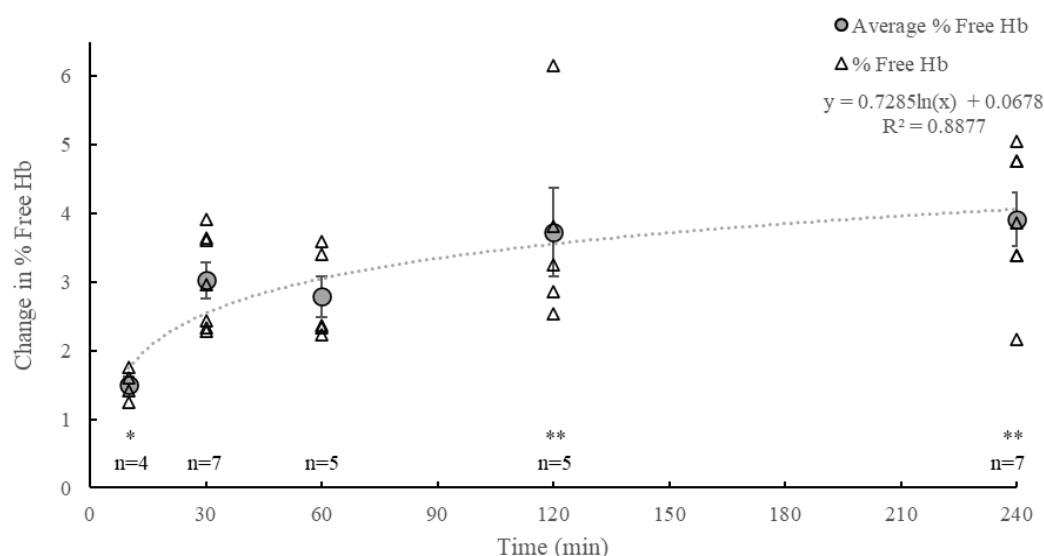


Figure 12. Time Course of Hb Released From EM at Constant Shear Stress (10 Pa). Change in percent free Hb of EM particle solutions as a function of time following subjugation to 10 Pa of shear stress. Hb is quickly lysed out of the particle causing a quick release in the first 30 minutes followed by a plateau. Statistical significance observed

between the 10 minute time point (*) and the 120 and 240 minute time samples (**), $p = 0.0120$ and $p = 0.0030$ respectively. No statistical significance observed between any other groups ($p > 0.05$).

4.3 Aim 3 - Determination of the Effect of EM Suspension Medium on Shear Tolerance

To explore methods for reducing particle failure rates, the polymer albumin was added at a 5% concentration to reduce drag during shearing. Again, the percentage of free Hb released was normalized to an unsheared control sample of the same formulation for both albumin-positive and negative samples. The percentage of free Hb released was compared between samples at the same time point with and without albumin (Figure 13).

The preliminary statistical assessments confirmed that the assumptions for conducting a two-way ANOVA test were met. The Shapiro-Wilk test indicated that the data was normally distributed ($p = 0.1060$), and the Barlett's test of assessing variance showed no significant difference in variance across groups ($p = 0.6201$). The results of the two-way ANOVA revealed a statistically significant difference between the albumin and no albumin samples ($p = 0.0001$), and between time points ($p = 0.0142$), but did not confirm significance between the two variables ($p = 0.0817$). To determine which time points differed, a Bonferroni test was conducted, revealing that the 10 minute time point was significantly different from the 30 minute time point ($p = 0.0152$). No other time points held significant differences ($p > 0.05$).

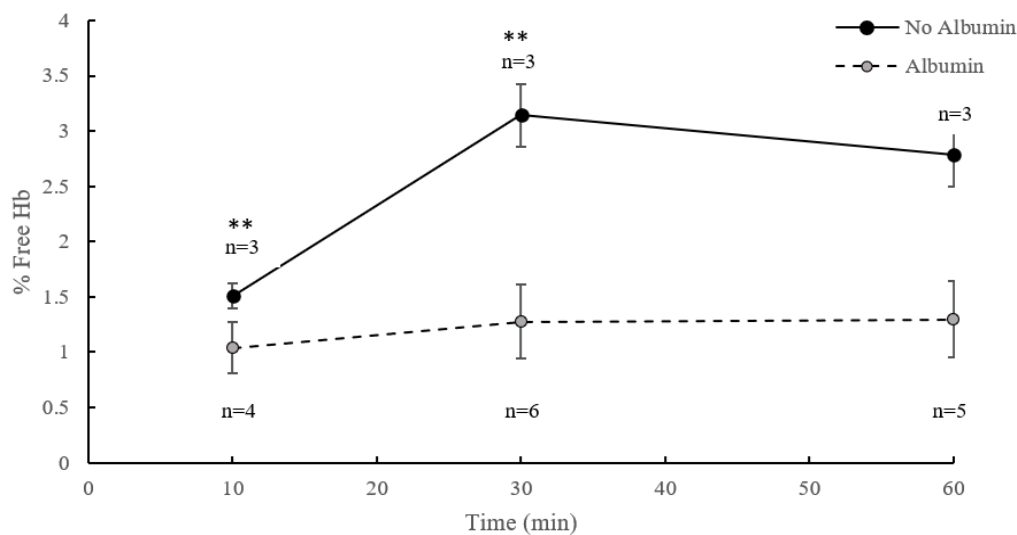


Figure 13. Effect of Buffer Solution on EM Shear Stability. Percent free Hb as a function of time demonstrates the effect of the presence of 5% albumin in the EM particle solution after 60 minutes of 10 Pa of shear stress. The difference between the presence of 5% albumin and no albumin were statistically significant ($p = 0.0001$). A statistically significant difference was observed between the 10 and 30 minute time points (**, $p = 0.0152$).

5. Discussion

EM has been optimized as a bridging blood substitute, to allow administration in austere environments where blood is not an option. Its goal is to provide sufficient O₂ delivery to allow patient transport to a location where blood transfusion can be administered. To adapt EM from a blood substitute to a perfusion solution, first we had to ensure that the lipid-based nanoparticle was robust enough to withstand the shear stress of a perfusion pump system. Our project therefore focused on quantifying EM lipid shell stability, essential to ensure retention of Hb payload (the oxygen carrier) within the particles. This was important for two main reasons. Firstly, free Hb is a Fenton reagent displaying strong pro-oxidative qualities (and having many detrimental effects when released in the circulation – which are likely enhanced in an organ perfusion system), and secondly, hemoglobin payload loss would effectively render EM unable to effectively deliver O₂ (free Hb without allosteric effector has a very high O₂ affinity), which would likely reduce rather than enhance organ viability in transport from donor to recipient.

To quantify EM particle stability and thus address the question as to whether EM particle integrity would be maintained within a perfusion pump system, we utilized a rheometer to precisely and accurately assess particle shear stability, addressing the following three scientific aims:

1. Quantification of EM shear tolerance
2. Time course assessment of EM stability at maximal perfusion pump shear stress
3. Impact of EM suspension medium on shear tolerance

5.1 Quantification of EM Shear Tolerance

EM shear tolerance was assessed to establish boundaries of EM stability, thus ensuring the maintenance of particle integrity within a perfusion pump system. Our goal was to identify the shear force at which a pre-defined failure point of the EM particles was observed. For our testing standard, failure was defined as the point at which the percentage of free Hb exceeded 5%. Hemolysis is considered negligible below 5% by the American Society for Testing and Materials (Luna-Vázquez-Gómez et al., 2021). We consequently adopted this standard as the maximal amount of hemolysis that EM should not exceed. A study by Lawrence et al. have found that the shear pressure at which the tissue is perfused should not exceed 7 Pa in order to avoid graft injury on the endothelial cells that line the renal arteries (Lawrence, 2009). Thus, pressures greater than 7 Pa were tested to identify the stability of EM when perfused.

To assess the shear tolerance of EM, particles in the buffer were sheared from 0 to 190 Pa for 10 minutes at 37C. Our findings, as illustrated in [Figure 10](#), demonstrate that less than 1.2% of the Hb payload was released from EM, with a moderate linear relationship over this wide shear range. Even after 10 minutes of EM shearing at 190 Pa, almost 20x the maximum force EM would encounter in the perfusion pump system, the percentage of free Hb was less than 1.2% which was 3.8% below the threshold. As we did not identify an inflection point in Hb release (i.e., linear response) and Hb payload release remained below our predefined 5% failure point, we conclude that the shear tolerance of the particles is greater than 190 Pa given the tested parameters of time and temperature. We utilized the linear relationship between shear forces and the percent free Hb to extrapolate the shear force at a 5% failure point. From our data, the failure point of 5% was never reached and stayed below 2% free Hb. Future tests could be conducted to validate the critical failure point of EM. Considering that the expected maximal shear stress of

the perfusion pump system of our collaborators was stated to be 10 Pa, we are confident that the present EM formulation is robust enough to withstand the shear force of the proposed perfusion pump system.

The primary function of EM with regard to organ perfusion would be to provide O₂ delivery. EM has been designed with the incorporation of a proprietary lipid (KC1003) into its shell. KC1003 is designed to regulate allosteric effector binding as a function of pH. By capturing the allosteric effector at high pH (i.e., lung) and releasing it at a low pH (i.e., tissue), this recapitulates the Bohr effect within the RBC, enhancing EM O₂ flux from lung to tissue and thus O₂ delivery. Another advantage of EM as a perfusion HBOC is the ability to tune O₂ delivery through manipulation of the Hb to allosteric effector ratio. This could be optimized to minimize the concentration of Hb necessary for kidney perfusion, thereby minimizing potential side effects of Hb release. Additionally, O₂ delivery dynamics could be manipulated to match O₂ requirements of different organs during machine perfusion.

The physiologic consequence of shear-induced hemolysis would be the release of free Hb into a perfusate solution. Human hemoglobin has a naturally high O₂ affinity that is reduced by the presence of allosteric effectors within the RBC. As highlighted in the previous paragraph, the same is true for EM. However, release of Hb from EM would result in reduced O₂ delivery, releasing high affinity with free Hb into the perfusate which could no longer load and unload O₂ (Horobin, 2017). Thus, EM particle stability within the perfusion system is essential to ensure effective O₂ delivery to the kidney.

EM was deliberately designed to encapsulate Hb in a lipid nanoparticle, thereby ensuring Hb was not free in circulation. Particle failure, however, would result in the release of Hb, which

could undergo Haber-Weiss and Fenton reactions, leading to reactive oxygen species (ROS) formation and oxidative stress. Notably, within RBCs, autoxidation naturally occurs to 3% of Hb during circulation, creating hydroxyl radicals which contribute to ROS formation (Kuhn et al., 2017). In addition to this damaging biochemistry, *in vivo* release of Hb into the bloodstream is known to result in NO scavenging, increased endothelial cell permeability, protein oxidation, inflammation (Ross, 2024), and fluid extravasation (Dull, 2004). These consequences would be detrimental to a harvested organ on a machine perfusion system. NO scavenging leads to vasoconstriction which causes increased vascular resistance and decreased perfusion. Decreased blood flow can result in kidney tissue hypoxia or damage. Increased endothelial cell permeability is indicative of a loss of vascular integrity of tight junction proteins which can result in a leakage of plasma out of the vasculature to cause inflammation in the tissue. Ultimately, the breakdown of the endothelial barrier from the release of free Hb into the bloodstream can have destructive effects on the kidney tissue and decrease the kidney's long-term survival outside of the human body.

5.2 Time Course Assessment of EM Shear Stability at Maximal Perfusion Pump Shear Stress

To test EM stability under a more clinically relevant time frame, EM was sheared over an extended period (to reflect organ transport times) at the reported maximal shear force (10 Pa) of our collaborators *ex vivo* perfusion pump system ([Figure 12](#)). As we were testing at 37C, we identified the median perfusion time for a normothermic *ex-vivo* kidney perfusion (NEVKP), which is reportedly ~171 minutes (Mazilescu, 2022). A NEVKP is a NMP but specifically for kidneys. Other studies report that 300 minutes of NEVKP reduces IRI (Kawamura et. al., 2024). Team HEMO consequently selected a timeframe of 0 to 240 minutes to assess EM particle shear stability at the maximal shear conditions of the perfusion pump.

Through the one-way ANOVA ($p = 0.0043$), the time points reflected significant differences in the change in percent of free Hb as time increased and shear pressure remained constant. The time course indicates that the longer EM is in the NEVKP system, the greater the change in percent free Hb. Through a Bonferroni test, it was found that time points 120 and 240 minutes significantly differed from the 10 minute time point ($p = 0.0120$ and $p = 0.0030$, respectively). This could be due to the extreme values of the outliers at both time-points. Amongst the same time-point, there is a vast difference in change in the percentage of free Hb, which can be explained by different points being different batches of EM. All EM samples tested have the same chemical composition, but they are from different batches. Even with the variability within the batches, EM was below the 5% threshold of percent free Hb at 240 minutes.

Given these results approaching the 5% free Hb threshold, we investigated potential additives to enhance the EM formulation and increase EM's resistance to shear forces and minimize free Hb levels in the sample. When EM was sheared over time, we observed two distinct kinetic phases of Hb release from EM: a rapid initial release within the first 30 minutes, followed by a plateau. This suggests that some of the particles in each batch of EM might be more vulnerable to shear stress than others, i.e., rapid lysis of the weaker particles. Additionally, since lipid shells can be reformed under mechanical stress with the lipid molecules flipping or exchanging into the bilayers and among the EM shells it could further increase the free Hb concentration and decrease encapsulation integrity. Rapid lysis of EM particles limits their functional lifespan and could potentially overwhelm the kidney causing oxidative stress or an inflammatory response. The logarithmic trend indicates a threshold for weaker EM particles leaving behind more stable particles capable of withstanding prolonged shear stress.

The variability in the sample values in [Figure 12](#) suggests that within each batch, some particles are weaker and more prone to breakage at 10 Pa shear stress. The loading of Hb into EM particles could vary due to particle size and surface area as larger particles would be able to contain more Hb, but could experience increased shear forces. Thus, encapsulation of Hb is crucial for oxygen delivery and the size of EM particles could highlight which particles are more likely to hemolyze.

5.3 Determination of the Effect of EM Suspension Medium on Shear Tolerance

To examine shear stability of EM, two different EM suspension medias were tested: PBS and 5% albumin. Shear stress (τ) was constant and controlled with the rheometer used as a shearing device. The albumin media was created to demonstrate a more physiologically relevant situation and possibly decrease the shear forces EM encounters in the rheometer. Albumin is a plasma protein that influences colloid oncotic pressure which can impact the shear stress on the EM particle. Without albumin, EM could have decreased shell stability and induced cell content leakage. Albumin is considered to add protection against the lysis of RBCs and has been proven to protect RBCs against amyloid peptide-induced lysis 8 times more effectively than apolipoprotein E (Galeazzi et. al. 2002). Adding albumin into the perfusate sheared with EM could then theoretically decrease the percent free Hb due to added protection. Perfusate composition was shown to prolong NEVKP in Pool et.al. and all four perfusive solutions tested on porcine kidneys contained significant amounts of albumin (Pool et.al., 2021). Adding albumin into the EM solution was thus hypothesized to decrease NO scavenging activity and inflammation through maintaining EM particle integrity.

Given that albumin has been shown to help prevent further lysis of EM, [Figure 13](#) highlights the effect of EM with a 5% albumin media to demonstrate the importance of the perfusion solution composition into the NEVKP. The suspension media with the 5% albumin had a statistically significant effect on the lysis of EM ($p=0.0001$) and was able to effectively decrease the amount of free Hb as time increased. The pairwise comparisons between time points displays the effects of an increase in time on the percentage of free Hb. Lysis increases as time increases; however, albumin was able to successfully minimize the effects of time sheared. This means instead of the percent free Hb increasing as time progressed, albumin had the effect of making EM not time dependent in regards to percent free Hb.

EM exhibited stable and consistent levels of free Hb. EM is able to withstand shear forces without significant degradation of the structure to ensure minimal hemolysis and effective oxygenation. The highest value recorded for the EM without albumin was 3.1% free Hb compared to the highest value for EM with 5% albumin which was 1.3% free Hb. This was a 1.9% difference that would most likely increase given the results from [Figure 13](#) displaying how free Hb logarithmically grew until it plateaued. The increasing divergence in the percent free Hb indicates that the EM particle by itself undergoes cumulative damage, but the albumin is able to mitigate the effect of cumulative stress on the EM particle. Since albumin protects shell stability and helps maintain normal colloid osmotic pressure, an EM and albumin perfusate represent a promising clinical application in a NEVKP to prolong the *ex-vivo* lifespan of a kidney.

It is important to note that the EM and 5% albumin solution compared to the EM and PBS solution had different viscosities. Assuming both solutions are Newtonian fluids, the 5% albumin solution would have a higher viscosity, thus the shear rate to achieve the same shear

stress would be lower. This could explain why the particles are more stable in albumin and the importance of the viscosity of the perfusate solution.

5.4 Limitations

The study encountered variance in the Drabkin's reagent assay measurements during the initial stages of experimentation. Variance occurred due to inconsistency in pipetting techniques between group members as group members had slightly different techniques and practice with pipetting. To address this issue, we divided components of the lab between group members such as designating one individual to prepare the rheometer, one analyzing the particle dynamics of EM, and one for performing the Drabkin's assay. This effectively reduced the measurement variance and allowed us to improve the reproducibility of our results and consistency across measurements. A practice experiment was conducted before we recorded actual results to ensure understanding of all laboratory procedures and techniques. By refining laboratory techniques, researcher error and learning bias was minimized. The final data in the results came from experiments when the protocols were able to be accurately performed to limit incorrect conclusions. As team members were trained and became familiar with the protocol and proper techniques, variance decreased significantly, and reliability in our results increased.

In regards to the quantification of EM shear tolerance, the peak shear tolerance of EM was not identified. By identifying the peak shear tolerance of EM, different perfusate formulations could be created and tested to examine which perfusate would be most effective at decreasing the effects of shear stress on EM particles. When conducting the time course assessment of EM, longer timepoints could have been used, such as going to 360 minutes of shearing of EM. For the purposes of this study, it was not feasible to shear for such a long

amount of time as the team had to commute to Baltimore from College Park to conduct these tests and were unable to stay for that long to finish the rest of the experiment. Additionally, due to time constraints, different perfusate compositions of EM were not able to be tested and compared to an EM solution. Ultimately, time had an impact on how long the experiments were and the amount of experiments that could be conducted.

5.5 Future Directions

Future directions of this study include additional testing of the peak shear tolerance of the EM particles to identify their failure point. After identifying the EM particle's failure point, addressing how the particles would behave at a slightly higher shear stress and for a prolonged period of time would help further prove the likelihood of the EM particles being an effective good oxygen carrier to perfuse the NEVKP system. Increased kidney perfusion duration has been shown to increase the viability of the donated kidney (Almeida et al., 2024), and by comparing the length of time and the shear rate it could allow insights on the optimal amount of time EM should be perfused. Additionally, investigating particle and batch heterogeneity could explain why certain particles release free Hb and degrade too early. Through statistical analysis of the differences between batches, heterogeneity could be analyzed. Before perfusing EM in a kidney, future studies should include assessing how albumin affects key markers like glomerular filtration rate and renal resistance, specifically within the context of NEVKP, as albumin is an important factor in plasma colloid osmotic pressure (Aldecoa et. al., 2020). Since albumin is an important determinant for plasma colloid osmotic pressure, it could influence renal hemodynamics the fluid dynamic flow through the renal capillaries and affect glomerular filtration and thus the survival of the perfused kidney. Future research should thus test varying albumin concentrations for longer periods of time and assess the effect it has on EM. Researchers

have measured the success of perfusion solutions by testing the vascular resistance, flow rate, pH, weight, and histological assessment of the muscle in different perfusates (Ng, 2024). EM could be incorporated into these types of studies to assess organ viability.

To our knowledge, current perfusion solutions do not contain any mechanism to detoxify free Hb. *In vivo* haptoglobin, a human plasma Hb-binding protein, plays a crucial role in binding free Hb, essentially filtering it out and thereby reducing oxidative damage (Schaer et. al., 2014). However, when haptoglobin is depleted, hemopexin, a rescue pathway, binds free heme eliminating the potential for toxicity in the vascular system. Reduced hemopexin levels are associated with disorders with free heme, suggesting that hemopexin levels are crucial to decrease tissue damage to the kidneys. With high levels of hemolysis being an important factor to consider for kidney health, free hemoglobin is extremely relevant to the perfused kidney, which would not be protected by the haptoglobin/hemopexin system. We speculate that the addition of a heme scavenging system to organ perfusion solutions (especially kidney organ perfusion), should be a future area of focus.

After initial testing is complete, further investigation into the behavior of the EM particles in the NEVKP system could provide insights into their long-term stability in the NEVKP system. Accounting for the specific set-up from the NEVKP would be important in testing the biomechanical forces the EM particles would face along the tube walls and through the actual kidney. Thus, testing EM particles in an NEVKP system itself with a functioning kidney is a possible future experiment. After perfusion, the functional parameters of the kidney can be assessed to determine the kidney's stability through a series of tests such as a renal blood flow analysis, oxygen consumption of the kidney, and urine output (Stone et.al., 2016). Specifically, cytokine secretion was measured to assess inflammation during perfusion as well as

immune cell efflux was analyzed to determine the possibility of acute rejection of the kidney and overall renal function.

Future studies could follow protocols found in a study by Juriasingani et al. which analyzed a blood substitute in a perfusion system (Juriasingani et. al., 2021). Different blood substitutes, such as Hemopure, could be compared to EM to analyze the efficacy of EM as a blood perfusate and the advantages it could have over the current RBCs used. Ultimately, the goal would be to test EM perfusion in a NEVKP system and compare it against currently perfused solutions and RBCs to see which is the most viable and effective option. After different perfusates are compared in an NEVKP against EM, tissue oxygenation and inflammation can be measured through histological staining.

While EM currently lacks the capability to scavenge ROS and reduce the accumulation of metHb, future studies could modify EM to include antioxidant properties. This would enable the kidneys to experience significantly less damage and increased oxygenation. As previously mentioned, haptoglobin could possibly be chemically linked to EM and co-delivered during perfusion. A method for doing this might be to PEGylate the haptoglobin and chemically link it to EM (Khan et. al., 2020). This will allow EM to retain its oxygen therapeutic agent properties as well as theoretically further decrease renal damage and free Hb. To decrease metHb, a blood substitute membrane containing polyethylene glycol–polylactic acid and RBC enzymes was found to increase the half-life of major enzymes, such as reductase (Moradi et. al., 2016). Since reductase was shown to reduce the accumulation of metHb, EM's membrane could be modified to contain enzymes like reductase.

As shown through the variance in [Figure 12](#), smaller EM particles within each batch have been thought to have been lysed with shearing. To account for this, future experiments should analyze the size distribution of the EM particles on the ZetaView pre and post shearing. This approach will help determine whether the observed differences are due to differences in particle size or from an inherent chemical component of EM. Separation of EM particle sizes can occur through field-flow fractionation which separates particles based on size as small as a couple of nanometers (Giordani et. al., 2023). This analysis would be best for the purposes of studying EM free Hb in response to stress because it helps maintain the structure of the original particles as much as possible. By separating the particles by size, performance of EM particles can be improved since the particles will be experiencing the same amount of force. The smaller, weaker particles will be removed and thus will be less likely to release free Hb from the shear forces. Field-flow fractionation can be integrated into the NEVKP system to only select particles that are most resistant to shear stress, potentially enhancing the preservation and outcomes of donated kidneys.

Improvements on experiments that were conducted and additional experiments that could be conducted all reflect how EM is a viable alternative to RBCs and may even improve the longevity of a kidney *ex-vivo* in a NEVKP. Once an optimal perfusate solution of EM is created, it can be tested on porcine kidneys to examine if EM can hold kidney function for longer than current perfusate solutions. Given the experiments conducted in this study and the chemical make-up of EM, with the right perfusate solution, EM will be able to withstand the shear forces it encounters in the NEVKP and ultimately enhance the longevity of the kidney *ex-vivo*.

6. Conclusion

In this study, we identified the shear tolerance of EM for short durations as well as its resistance to shear stress over an extended time in order to investigate the threshold force an EM particle is able to withstand. From the first experiment where EM was tested with shear forces up to 190 Pa, the percent of free Hb was below 1.2% which demonstrates the particles' integrity to withstand the high forces they would encounter in the NEVKP. In addition, we tested varying batches of EM all at a 10 Pa shear rate but with increasing time in the rheometer to demonstrate the potential efficacy of the EM particle in a NEVKP. This resulted in a logarithmic trend of particle-free Hb release suggesting that EM could sustain delivering oxygen throughout the kidney. Yet, when sheared for longer periods than 10 minutes, the EM underwent greater shear-induced hemolysis than RBCs; however, adding albumin reduced the effect from shear stress on EM particles so that the percent of free Hb decreased a statistically significant amount from that of the EM without albumin.

Through our research, EM was shown to be able to sustain shear forces with minimal hemolysis when albumin was added and with further testing, EM could sustain and be used in a NEVKP system. In powder form, EM has a shelf life of several years; however, there is no antioxidant system, so Hb encounters oxidation over the time of shear stress. If EM degrades too quickly, it would have no use as a perfusion solution from the Hb rapid oxidation and inability to carry O₂. Thus, through experimentation of EM's capabilities to withstand shear stress, EM viability in a NEVKP was determined.

7. Equity Impact Statement

In the United States, nearly one-third of Americans will need a lifesaving blood transfusion in their lifetime. As a result, about 4.5 million Americans receive a blood transfusion every year and are slowly starting to outpace the donors (Riley W., 2007). Furthermore, more than 125,000 people in the United States await organ transplants, yet less than 35,000 patients receive donor organs. Seventeen people die everyday waiting on the transplant list (*US Blood Supply Facts*, 2022). As patients await a potential blood transfusion or organ transplant, they must continue to undergo medical care, incurring additional healthcare costs that can put significant financial strain on individuals who are unable to afford lengthy hospital stays (Tullius, 2018). Additionally, the costs of a kidney transplantation is significantly cheaper than a patient staying on dialysis. With a HLA-compatible living donor transplantation, the cost per quality-adjusted life year was about \$39,939 compared to dialysis which was about \$72,476 (Axelrod et al., 2018). A close immune match kidney nearly decreases the healthcare costs by half that of long-term dialysis. With the kidney perfusion system and EM, kidney transplantation can be more accessible to those who cannot afford methods such as dialysis. EM in a NEVKP has the potential to impact so many lives positively as a transplant not only saves in overall healthcare costs, but it also increases a patient's quality of life, adding more than one full quality-adjusted life year, which is a measure of a year of a person's life in perfect health, compared to dialysis. With transplantation, the patient's quality-adjusted life years has the potential to be between 5.20-6.34 compared to dialysis at 4.03 (Axelrod et al., 2018). Transplantations not only extend the life of the recipient but also improve how well that life is lived. With more organs viable and available for transplantation due to NEVKP and EM, it can reduce costs and help improve patient outcomes.

Another issue that comes with organ transplantation is that the organs that are being preserved do not have the ability to be transported long distances. For example, a kidney can only be viable outside of a body for about 24 to 36 hours (LifeSource, 2021), therefore, the kidney could only be transported a certain distance that is within that time frame. With the organ perfusion system, a kidney would theoretically be able to last longer *ex vivo*. Through EM, the organ would be able to travel longer distances to more disadvantaged communities with limited access to donated organs and be matched with someone who is a close immune match, thus increasing the chances the kidney is able to be successfully transplanted.

Glossary

ANOVA Analysis of Variance: A statistical method used to compare means among groups to determine if there are significant differences between them.

DBD Donation after Brain Death: Referring to when an organ is donated after neurological death.

DCD Donation after Circulatory Death: Referring to when an organ is donated after cardiac death.

Deoxy Deoxygenated Hemoglobin: The form of hemoglobin unbound to oxygen.

DGF Delayed Graft Function: A term used to describe when a transplanted organ fails to function immediately.

DI water Deionized Water: Water which has been purified, removing mineral ions through ion exchange.

DLS Dynamic Light Scattering: A technique used to measure the size distribution of small particles in suspension or polymers in solution by analyzing the fluctuations in scattered light caused by their brownian motion.

ECD Expanded Criteria Donors: Categorization of organs donated from “higher risk” donors. This includes donors over the age of 60, or over 50 matching two of the following three characteristics: history of high blood pressure, serum creatinine >1.5 mg/dL, or death due to a stroke

EM ErythroMer: A dried, bio-inspired artificial blood cell.

Hb Hemoglobin: The protein responsible for the majority of oxygen transport in blood.

HbCO Carboxyhemoglobin: A compound between hemoglobin and carbon monoxide, preventing the binding of oxygen.

HBOCs Hemoglobin-Based Oxygen Carriers: A polymer meant to mimic the oxygen transport functionality of red blood cells.

HLA Human Leukocyte Antigen: A cell surface immune protein used to distinguish foreign material.

HMP Hypothermic Machine Perfusion: Preservation technique that uses a pump to circulate a cold solution, containing nutrients and electrolytes through the organ, often an alternative to static cold storage.

HSA Human Serum Albumin: The most abundant protein in human blood plasma.

IRI Ischemia-Reperfusion Injury: The tissue damage that occurs as a result of cessation and subsequent restoration of blood flow to tissue.

Ischemia is a condition that occurs when blood flow to a part of the body is reduced, leading to a shortage of oxygen and nutrients that your tissues need.

MetHb Methemoglobin: Hemoglobin in which the iron found in the heme group is oxidized to the ferric state, preventing oxygen binding.

NEVKP Normothermic *ex-vivo* kidney perfusion: A system to improve the length kidneys can be held for, and to test their function prior to a transplant.

NMP Normothermic Machine Perfusion: A technique that aims to preserve organs *ex vivo* at 37°C to replicate physiological conditions.

NO Nitric Oxide: A free radical that expands the bloodstream, increasing the flow of blood and transport of oxygen.

NODG n-Octyl B-D-thioglucopyranoside: A non-ionic detergent.

O₂ Oxygen: Important molecule that binds to hemoglobin.

Oxy Oxygenated Hemoglobin: Hemoglobin which has been bound to oxygen

PBS Phosphate-Buffered Saline: A water-based solution used for dilution due to its physiological mimicking conditions.

RBCs Red Blood Cells: The most common part of the cellular portion of blood, containing hemoglobin and work to assist in the oxygenation of the body, and certain immune system responses.

ROS Reactive Oxygen Species: Unstable molecules that are produced from diatomic oxygen, water, and hydrogen peroxide, are also known as oxygen radicals or free radicals.

SCS Static Cold Storage: Standard method of organ preservation where the organ is placed in ice and preservation solutions.

Shear Stress is a biomechanical force that occurs when blood flows through a blood vessel, causing arteries to adapt to responses to chronic and acute changes in blood flow.

UNOS United Network for Organ Sharing: A nonprofit organization that manages the U.S. organ transplant system.

UW Solution University of Wisconsin Solution: A perfusing solution that maintains nutrient levels in cells to preserve organ tissue in the absence of blood flow.

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