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An *in vitro* comparison of Red Blood Cell haemolysis in neocyte-enriched and leucocyte-poor blood

by

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Declaration

I, Burnet Adriaan Meyer, declare that the contents of this thesis represent my own unaided work, and that the thesis/dissertation has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed:_____**Date:**__**03/02/2025**_____

Burnet Adriaan Meyer

Abstract

Introduction

Red blood cell concentrates (RBCC) have a shelf life of 42 days when stored at 1-6 ºC. This may lead to shortages and wastage as biological changes develop due to storage lesions leading to the unit having to be discarded. Pooled neocytes could potentially extend the shelf life of the RBCC unit. Patients with chronic anaemia depend on regular blood transfusions which could lead to transfusion complications. This study was conducted in an attempt to improve the longevity of stored red blood cells (RBC), through isolating neocytes and comparing the rate of haemolysis, biochemical changes and viability with filtered blood.

Methodology

Thirty filtered units were processed from fresh whole blood. Two transfer bags were attached to the filtered blood cell unit by using a sterile heat sealer. The neocytes and the additives were extracted from the filtered blood unit. These were centrifuged and the two transfer bags were placed on separate scales. The additive was separated into one satellite bag while the concentrated neocytes were separated into the second satellite bag. The Saline Adenine Glucose Mannitol nutrients were added to the neocyte-enriched bag and the transfer tube between the two bags was sealed. The nutrient transfer bag was then discarded and the neocyte bag was the final required product. Both unit types were stored for 42 days and every 14 days samples were analysed for RBC count, mean corpuscular volume, mean corpuscular haemoglobin concentration, haemoglobin, sodium and plasma haemolysis using standardised techniques.

Results

Although the red cell count was decreased in the filtered blood unit compared to the neocyte-enriched unit, this difference was not significant ($p = 0,27$). In both unit types the mean corpuscular volume increased ($p = 0,87$), while the mean corpuscular haemoglobin concentration decreased over all time points ($p = 0,44$). Sodium levels decreased over the storage period ($p = 0,14$) while the percentage plasma haemolysis increased steadily in both units ($p = 0,65$). The haemoglobin remained stable for both neocyte-enriched and filtered blood.

Conclusion

The outcome of this study demonstrated that neocyte-enriched blood did not have a survival advantage when compared to pre-stored leucocyte reduced RBC using the conventional manual collection method. These results are similar to previous studies which utilised multiple methods to collect a high neocyte yield. Feasibility was highlighted

as the main challenge as many of these methods have proven to be too expensive and too laborious.

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Abbreviations and Acronyms

Abbreviations & Acronyms	Definition
2,3-DPG	2,3- Diphosphoglycerate
ANOVA	Analysis of Variance
ARDS	Acute Respiratory Distress Syndrome
ATP	Adenosine Triphosphate
CPUT	Cape Peninsula University of Technology
DBA	Diamond Blackfan Anaemia
EDTA	Ethylenediaminetetraacetic acid
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
HWS-REC	Health and Wellness Sciences Research Ethics Committee
HREC	Health Research Council
K^+	Potassium
LDH	Lactate Dehydrogenase
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
Na^+	Sodium
NAT	Nucleic Acid Technology
NTBI	Non-Transferrin-Bound Iron
RBC	Red Blood Cell
RBCC	Red Blood Cell Concentrate
RBCs	Red Blood Cells
RCF	Relative Centrifugal Force
RD	Returning Donors
ROS	Reactive Oxygen Species
SAGM	Saline Adenine Glucose Mannitol
SANAS	South African National Accreditation System
SCD	Sickle Cell Disease
SD	Standard Deviation
SLS	Sodium Lauryl Sulphate
SSA	Sub-Saharan Africa

SST	Serum Separating Tube
TRALI	Transfusion Related Acute Lung Injury
TTD	Transfusion Transmitted Disease
TTI	Transfusion-Transmitted Infections
WB	Whole Blood
WBC	White Blood Cells
WCBS	Western Cape Blood Service
WHO	World Health Organisation
YRBCs	Young Red Blood Cells

Keywords: Apheresis; conventional; haemochromatosis; haemoglobinopathies; haemolysis; *in vitro*; neocyte; senescence

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Glossary

Term	
Alloimmunisation	Blood group alloimmunisation occurs when an individual without a specific antigen is exposed to that antigen during transfusion or pregnancy
Apheresis	A medical procedure whereby whole blood is removed from a donor and separated into individual components, retaining the desired component, and returning the remaining components to the patient's bloodstream
Conventional	The standard practice or method used in a process and is widely accepted and practised within a field.
Ferroportin	A membrane protein that transports iron from inside of a cell to the outside.
Haematological	A specific characteristic or value related to blood or blood components
Haemochromatosis	Excess iron absorption or storage leading to organ damage
Haemoglobinopathies	A group of inherited genetic disorders which affects the haemoglobin section of red blood cells
Haemolysis	The rupture (breakdown) of red blood cells
Hypochromic	Red blood cells with a reduced concentration of haemoglobin in relation to their volume
Hyponatremia	Abnormally low sodium levels in the blood
Immunogenicity	The ability of a substance, such as an antigen, to provoke an immune response
In vitro	A process performed outside of a living organism
In vivo	A process performed inside a living organism
Neocyte	Young red blood cells which are 30 days and younger
Phagocytosis	A cellular process by which certain cells, such as macrophages, engulf and digest large particles, to remove them from an organism
Phenotype	The process of measuring an organism's traits, which results from the interaction of its genetic makeup. Blood phenotyping is the process of identifying specific antigens present on the surface of a red blood cell
Senescence	The aging process of a cell such as red blood cell

CHAPTER 1: INTRODUCTION

1 Introduction

Annually, nearly eight million people require a blood transfusion in Sub-Saharan Africa (SSA), resulting in the majority of countries experiencing a blood supply shortage (Bloch et al., 2012; Weimer et al., 2019; Diop & Pirenne, 2021). Despite the improvement in technology, several challenges regarding collection and blood safety persist. This requires a multi-pronged approach with several solutions being considered in the management of blood stock (Busch et al., 2019; Weimer et al., 2019).

Red Blood Cell (RBC) haemolysis, which occurs during component processing and storage, has serious clinical implications for patients receiving transfusions. Transfusing haemolysed blood can cause haemoglobinuria which may result in organ failure and even death (Sloan et al., 2009). The detection of elevated haemolysis is important to minimise the chances of a transfusion reaction and to monitor cell integrity and quality. During the process of red cell haemolysis, potassium and free haemoglobin are released into the circulation. This consequently may result in significant complications as potassium plays an important role in the normal sinus rhythm of the heart, while elevated free haemoglobin indicates increased haemolysis (Rastergar & Soleimani, 2001). For blood transfusion services to provide the best clinical outcome and reduce the adverse effects of transfusion, it is important for the quality and stored life span of red cell products to constantly be enhanced. Before the transfusion of a unit of blood, the patient's identification, any visual haemolysis of the blood product as well as the expiry date must be assessed. These checks are required to minimise any risk to the patient (WCBS & SANBS, 2023).

Patients diagnosed with chronic anaemias, such as Thalassaemia, Sickle Cell Disease (SCD) or Diamond Blackfan Anaemia (DBA) often require regular blood transfusions (Prati, 2000; Mcfarren el al., 2007; Weatherall, 2010). Due to the high amount of iron contained in a transfusion of red cells, these patients can develop iron overload (haemochromatosis). If not medically managed, the iron accumulates in tissues and can lead to organ failure (haemosiderosis) (Porter, 2013). Furthermore, those who receive multiple transfusions are at risk of becoming alloimmunised and develop antibodies against antigens, such as, Kell and Duffy which may lead to immediate or delayed life-threatening haemolytic transfusion reactions. These reactions can lead to cardiac failure, kidney failure and increased risk of mortality (Fasano, et al., 2019). Additionally, due to high exposure to multiple donors, these patients have an increased risk of contracting infections, such as,

Human Immunodeficiency Virus (HIV), Hepatitis B, Hepatitis C and Syphilis (Lee, Teh & Chan, 2005).

The transfusion of young RBC or neocytes could potentially reduce the number of transfusions in this group of patients as the intervals between transfusions would be extended. Initial studies utilising neocyte-enriched blood demonstrated encouraging results (Propper, Button & Nathan, 1980; Corash et al., 1981; Graziano, Piomelli et al., 1982; Cohen, Schmidt et al., 1984; Spanos et al, 1996), while a recent study of thalassaemic patients, reported that the mean interval between transfusions receiving conventional blood products was 26.1 days, and in those who received pooled neocytes, it increased to 45.8 days (Sharma et al., 2008; Kumbhakar, 2016a). In both research studies twelve patients participated and the conventional method of pooling neocytes was used. The results of these studies revealed that the yield for neocytes is considerably higher, and that after using this method the transfusion interval increased significantly.

Despite the prevalence of haemoglobinopathies and the need for chronic blood transfusion in Africa, very little work has been done to improve the viability of stored red cells and therefore, in an attempt to improve the longevity of stored RBC, this study aimed to isolate young red cells or neocytes and to examine the rate of haemolysis, haemoglobin, red cell count, red cell parameters as well as biochemical changes and viability as measures of survival. The results would be compared to the conventional filtered blood (leucocyte-poor) which are pre-stored over the standard 35–42-day period.

1.2 Hypothesis

Alternative hypothesis: Neocyte-enriched RBC can prolong the survival of stored red cell concentrates when compared to filtered blood.

Null hypothesis: No statistical difference has been observed with regards to RBC survival time when comparing neocyte-enriched blood to filtered blood.

1.3. Research Questions

1. How does *in vitro* RBC haemolysis in filtered blood at the Western Cape Blood Service (WCBS), South Africa compare to neocyte-enriched blood?
2. Can neocyte-enriched blood prolong the survival of RBC in storage?

1.4. Study Aim

The aim of this study is to determine whether the survival of neocyte rich blood in storage can be extended compared to filtered blood.

1.5. Study Objectives

The objectives of this study are to:

1) **compare the plasma haemoglobin levels of neocyte rich blood at day 1 and thereafter every 14 days during storage, and to compare this to routinely prepared leucocyte reduced RBC;**
2) **compare sodium levels of stored neocyte rich blood and routinely stored leucocyte RBC; and**
3) **compare red cell parameters including the reticulocyte count between the two unit types.**

This study emphasises the differences in red cell aging between young red blood cells (neocytes) and older RBC. Chapter 2 provides detailed background information, while chapter 3 focuses on the research design and methodology. Young red blood cells were collected at WCBS, SA, using the conventional top and bottom blood collection system approach. In chapter 4 the findings are presented. Significant differences were highlighted in some parameters. Chapter 5 and chapter 6 present the discussion and conclusion, respectively.

CHAPTER 2: BACKGROUND AND LITERATURE REVIEW

2.1 Introduction

Blood transfusion has become a lifesaving requirement for many patients who suffer from blood loss and anaemia. It is estimated that the annual global transfusion requirements are 85 million units (Lotterman & Sharma, 2023). Currently, 65% of children in low socio-economic income countries require a blood transfusion (Barro et al., 2018) and a recent study demonstrated that approximately 1,8% of females require at least one unit of blood while giving birth (Thurn et al., 2019.). The survival rate increases significantly for patients who receive a blood transfusion, despite the multiple risks (Smeets et al., 2018) which means that this form of therapy still plays a crucial role in saving lives.

There are, however, multiple risks when receiving a blood transfusion. These include: transfusion related circulatory overload; allergic reactions; transfusion related acute lung injury (TRALI); transfusion-transmitted Infections (TTIs); as well as febrile haemolytic reactions. Results have shown that 0.2% of transfused patients developed a transfusion reaction, with the majority being caused by allergies (33.6%), followed by febrile reactions (25.7%) (Negi et al., 2015). Multiple blood transfusions also significantly increase the risk of developing iron overload. Transfusion dependent patients can present with a high transferrin and ferritin saturation which can lead to non-transferrin bound iron. The unbound, circulating iron consequently results in organ and tissue damage and, if deposited in the organs and tissues, could result in death (Zhang et al., 2019). The transfusion associated risks are the motivation why the use of multiple blood units and increased frequency of transfusions, for a single patient, should be minimised as much as possible. The clinical guidelines of the WCBS indicates that red cell components should be infused in cases when a patient's haemoglobin (Hb) levels drop below 6 g/dl (WCBS & SANBS, 2023).

2.2 Chronic Blood Transfusions

Chronic blood transfusions are the most common treatment for several conditions. Some of these include myelodysplastic syndromes, patients undergoing chemotherapy, chronic kidney disease, β-thalassemia, DBA and SCD (Wood & Mcquilten, 2020; Prati, 2000; Gripp, et al., 2001; Weatherall, 2010; & Gill et al., 2013). Patients with chronic anaemia require regular transfusions of packed RBC or Red Blood Cell Concentrate (RBCC), to maintain a blood haemoglobin (Hb) concentration above 10 g/dL (100 g/L) and a haematocrit level above 30 percent. This is traditionally based on the criteria of the "10/30 rule" (Wang & Klein, 2010). The South African blood transfusion criteria indicate that blood products are

required once the Hb concentration drops below 6 g/dL (WCBS & SANBS, 2023). Frequent blood transfusions increase the risk of iron overload (Porter, 2013) and therefore, to reduce the risk of transfusion-induced haemochromatosis, transfusion with young RBC, also known as neocytes, has been proposed (Kumbhakar, 2016).

2.3 Hereditary Anaemias Requiring Regular Transfusions

Thalassemia is a common hereditary anaemia which is caused by a globin gene deficiency and is classified into α or β Thalassaemia. The β-Thalassemias are generally caused by point mutations or deletions which lead to a decrease in the production of the ß-globin chain or, in severe cases, no ß-globin chain production (Wang et al., 2022). The life risks for β-thalassemia patients range from low to severe, depending on whether the affected gene is homozygous or heterozygous. Generally, when the affected gene is heterozygous, the symptoms are milder, and the disease is referred to as β-Thalassemia minor. Homozygous individuals suffer severe symptoms which can lead to a complicated anaemia. This condition is either β-Thalassemia intermedia or major, depending on the severity (Buttari et al., 2020). β-Thalassemia is one of the most recurrent autosomal recessive diseases seen globally and has a high incidence in Africa (Cao & Galanello, 2010). Treatment for β-Thalassemia includes blood transfusions, gene therapy, splenectomy, and iron chelation therapy (Ali, Mumtaz et al., 2021) with the latter method of treatment considered to be expensive (Li et al., 2020). The use of multiple blood transfusions for patients leads to several risks which include, transfusion reactions, transfusion-transmitted infections and iron overload. This is due to the human body not being able to remove unnecessary high iron levels. Iron overload can cause serious complications such as heart failure, retardation, as well as liver disease. As such, iron chelation therapy is often required to regulate the iron levels in this group of patients (Cappelini et al., 2017).

Sickle Cell disease (SCD) is a hereditary disorder which is most frequently caused when the β- globin gene is homozygously mutated. This mutation results in the substitution of the amino acid valine with glutamic acid in the β-globin chain with the formation of haemoglobin S. If the mutation is homozygous (HbSS), it is referred to as sickle cell anaemia, while a heterozygous gene mutation (HbAS) is referred to as sickle cell trait. In environments of deoxygenation, the abnormal Haemoglobin S will polymerise into fibres. The repeated oxygenation and deoxygenation of the RBC leads to RBC sickling and the cells lose their structure and become rigid resulting in their removal by the liver and spleen (Alli et al., 2014; Hoda & Cheng, 2017). SCD affects multiple organs, especially the lungs. It can be life threatening with a mortality rate of 50-90% in children, which is most commonly seen in

SSA (Grosse et al., 2011; Arigliani & Gupta, 2020). In Cape Town, South Africa an increase of 300%-400% of Sickle cell cases in children has been observed during the past decade. This is most likely due to migration from African countries in which SCD is endemic (Pule et al., 2017). The most common treatment for SCD includes red cell transfusions, hydroxycarbamide as well as stem cell transplant. A recent study has shown that the use of a Gardos Channel Inhibitor, prevents the dehydration of the RBC and consequently the sickling process (Tubman et al., 2016). The use of hydroxycarbamide (hydroxyurea) has resulted in significantly improved results as it increases the production of foetal haemoglobin, which then decreases sickle haemoglobin. Unfortunately, the treatment has not been used widely due to the lack of awareness by doctors and patients, and therefore blood transfusion remains the treatment of choice (Sheth, Licursi & Bhatia, 2013; Piccin et al., 2019).

Diamond Blackfan Anaemia (DBA) is a genetic disorder of the ribosomal protein gene S19. Some characteristics of DBA include reticulocytopaenia, with a decrease in erythropoiesis which leads to a reduction of erythroid precursor cells, while the leucocyte and platelet counts are normal (Engidaye, Melku & Enawgaw, 2019.). The decrease in erythropoietin causes apoptosis of the cells and eventually macrocytic anaemia. These patients usually suffer from anaemia from a very young age and require multiple blood transfusions, if they do not undergo a bone marrow transplant (Vlachos et al., 2008). The majority of DBA cases are diagnosed by the exclusion of other potential causes, but molecular testing is also available to confirm the diagnosis (Bartels & Bierings, 2019). There are multiple different options for treating DBA. These include blood transfusions, glucocorticoids as well as stem cell transplants. Long term use of glucocorticoids can lead to cataract formation as well as osteoporosis (Bartels & Bierings, 2019). Treatment by use of stem cell transplant can also lead to severe life-threatening conditions such as Graft-versus-Host disease. Furthermore, toxicities caused by stem cell transplant treatment can lead to an increased risk of infection (Bartels & Bierings, 2019). Although alternative treatment options exist for all hereditary and chronic anaemia, blood transfusion, despite its associated risks, remains the most common choice.

Aplastic anaemia is a condition where the bone marrow is unable to produce all haemopoietic cell lines, resulting in pancytopaenia. It has been hypothesised that this condition develops due to a dysregulated immune response, bone marrow degeneration or damage. Treatment options vary and include stem cell transplantation, immunosuppressive therapy and blood transfusions (Furlong & Carter, 2020; Scheinberg, 2021). Other

conditions leading to chronic anaemia include leukaemia, myelodysplasia and patients' undergoing chemotherapy. These conditions often require multiple transfusions. This means that RBC transfusion is seen as an effective way in treating multiple conditions despite the potential risks.

2.4 The Dangers of Chronic Blood Transfusions and Iron Overload

Since the first blood transfusion in 1665, this form of therapy has played an important role in saving lives. More than 60% of countries have a blood shortage each year (Roberts et al., 2019) and according to the World Health Organisation (WHO), the global number of donations is 118,5 million units annually (WHO, 2023). Every blood unit goes through multiple tests to minimise risk to the recipient. Despite these safety precautions, several risks remain which include TRALI, iron overload, alloimmunization and TTIs.

TRALI is known to be one of the major reasons for transfusion related deaths (Vlaar et al., 2019; Mermit Cilingir & Askar, 2021). TRALI is a broad name for an inflammatory response of unknown origin after a blood transfusion. It is believed leukocytes play a role in provoking an immune response (Tung et al., 2022). The initial inflammatory response causes elevated neutrophil activation, often leading to Acute Respiratory Distress Syndrome (ARDS) and pulmonary oedema. The initial response usually occurs four to six hours after a blood transfusion, and symptoms include fever, shortness of breath, a drop or increase in blood pressure, an irregular heart rate and even hypoxia (Vlaar *et al.*, 2019). The risk of developing TRALI has decreased with the use of filtered blood, as well as administering donor-specific blood where the exposure is limited to only a few donors per patient. Most cases recover and the prevalence of TRALI is 1:5000 transfusions (Toy & Lowell, 2007) .

Another risk of multiple transfusions is iron overload. Iron, is a crucial component of haemoglobin and therefore plays a role in the transport of oxygen throughout the human body (Kontoghiorghes, 2020). This means that once a person is anaemic and there is inadequate oxygen transport to the organs, a blood transfusion might be required to increase the haemoglobin levels of the patient. If a patient is treated with multiple blood transfusions over a period of time, this can lead to iron overload. This is due to the human body being unable to remove excess iron (Entezari et al., 2022).

The human body has several mechanisms which regulate iron metabolism. Iron is absorbed in the duodenum of the intestine where epithelial cells, called enterocytes, are responsible for the absorption of iron. Once iron is absorbed, it is released into the

bloodstream through a membrane molecule called ferroportin 1. The majority of the absorbed iron, is used by the bone marrow for the process of erythropoiesis. Hepcidin is a hormone which regulates and controls iron absorption and thus plays an important role in iron regulation (Vogt et al., 2021). It is released by the hepatocytes when iron stores are elevated or adequate, while during states of anaemia and iron deficiency production decreases. This provides a signal for the body to absorb iron. Once iron stores have been restored, hepcidin is again released by the hepatocytes, and the ferroportin 1 membrane protein is inhibited ensuring that iron is not released into the bloodstream (Fuqua, Vulpe & Anderson, 2012).

Old and damaged RBC are removed by macrophages which reside in the spleen. Macrophages play a significant role in regulating iron metabolism and are responsible for the removal of iron from phagocytosed erythrocytes. Once the old RBC are phagocytosed iron will be released back into the circulation to be recycled by the bone marrow. Hepcidin also plays a role in this process by inhibiting the release of iron and ensuring that less is released back into the circulation, especially in situations when it is not needed (Yiannikourides & Latunde-Dada, 2019; Camaschella, Nai & Silvestri, 2020).

Multiple conditions can lead to the breakdown of these mechanisms. During iron overload, transferrin becomes saturated, leading to an increase in non-transferrin bound iron which is absorbed and integrated into organ cells. This excess iron can cause significant damage, resulting in organ failure should treatment not be initiated. This can also contribute to the aggregation of Reactive Oxygen Species (ROS) within the bone marrow which results in damaged stem cells (Isidori et al., 2018; Knutson, 2019). When there is excess iron, patients present with high transferrin and ferritin saturation which translates into non-transferrin bound iron circulating throughout the body resulting in iron toxicity (Zhang et al., 2019). Iron toxicity can initiate cardiac function failure, liver cirrhosis as well as hepatocellular carcinoma (Shah et al., 2019; Shafique, Ali et al., 2023). Treatment is possible with chelation therapy or phlebotomy (Kontoghiorghes, 2020) which removes excess iron and thus prevents iron overload (Mobarra et al., 2016). There are three main types of iron chelation therapy, namely Deferoxamine, Deferiprone and Deferasirox (Reddy, Locke & Badawy, 2022). Each therapy uses its own specific mechanism for the removal of iron. The prolonged use of iron chelation therapy is expensive (Shah *et al.*, 2019) and has side-effects such as gastrointestinal and infusion reactions, resulting in less compliance from patients (Reddy *et al.*, 2022).

Alloimmunization is another major issue for chronically transfused patients. Studies have shown that between 5% and 30% of chronically transfused patients develop alloimmunization due to the development of antibodies (Romphruk et al., 2019). This occurs when a patient receives a transfusion and develops antibodies to the corresponding donor red cell antigen which is foreign to the host. Certain antibodies are more likely to develop due to their immunogenicity (Hendrickson & Tormey, 2016) and if clinically significant, they increase the risk of a haemolytic transfusion reaction occurring during or after a transfusion (Webb & Delaney, 2018). A haemolytic transfusion reaction poses a serious risk to the patient and is grouped into acute and delayed. One method in minimising the exposure to foreign antigens, is serological antigen typing which involves phenotyping the RBC of the donor and the recipient, and then matching the two (van Sambeeck et al., 2022). The challenges with this procedure are that it is time consuming, labour intensive and a matching unit is not always available for each patient. Antigenic typing is mostly done on the C, D, E and Kell antigens due to the strong immune response caused by these antigens and corresponding antibodies. Extended phenotyping, which would include testing for a broader group of antigens for each donor, would be costly and not feasible (Nickel et al., 2016). Extended phenotyping is currently thus only done on specific donors.

In the SSA region, 7% of blood donation units are discarded of which 8,7% are due to TTIs (Candotti, et al., 2021). These include Syphilis, Hepatitis B, Hepatitis C as well as HIV (Ainley & Hewitt, 2018; Busch *et al.*, 2019). One of the most important factors for blood services worldwide is the safety of the blood products. This means that the best available technology is generally used to minimise infection risks. Currently, the international standard recommends the use of Nucleic Acid Technology (NAT) (Candotti, Tagny-Tayou & Laperche, 2021) while the WHO requires blood transfusion services to test for HIV 1 and 2, Hepatitis B, Hepatitis C and Syphilis (Riaz et al., 2022). NAT technology reduces the window period for viral infections and thus increases the likelihood of detecting the virus in the donor. Unfortunately, NAT technology is not implemented globally and even if used, there is still a small underlying risk of contracting a TTI with each transfusion. This risk is increased when receiving chronic transfusions particularly in lower- and middle-income countries (Shah *et al.*, 2019).

2.5 The Effects of Storage on Red Cells

As RBCs age, they develop changes in their lipid membrane and become rigid which leads to an increase in haemolysis and cell death (Werre et al., 2004). A newly infused unit of blood is comprised of a range of red cells at different stages of their 120-day life span and

those which are older are quickly removed by the spleen. Thus, it is hypothesised that if the unit were populated by younger RBC, the lifespan of the unit would be lengthened, and thus the interval between blood transfusions would be decreased which would be of great benefit to chronically transfused patients.

RBC are biconcave which allows them to move effortlessly through the blood vessels. As RBC are stored *in vitro*, the cells begin to lose their structural integrity, surface area, as well as haemoglobin and there is an increase of echinocyte formation (Van De Watering, 2011). Echinocytes, also known as burr cells, are RBC with a spike shaped membrane and an excess is often observed in patients with kidney or liver disease (Ahmed & Patel, 2015). A change in morphology and structural integrity increases RBC breakdown during transfusion (Zubair, 2010). During storage, there is a decrease in Adenosine Triphosphate (ATP), pH as well as 2,3-Diphosphoglycerate (2,3-DPG). The pH decreases due to the increase in lactic acid in the unit (Arggawall, 2022). This causes a rise in phosphatase-3 enzyme which results in the breakdown of 2,3-DPG. The function of 2,3-DPG is to stabilise deoxyhaemoglobin and due to the loss of this molecule less oxygen will be transferred to the tissue or organ (Zubair, 2010). It is estimated that a unit of blood loses more than 90% of the 2,3-DPG within 42 days of storage (Beutler & Wood, 1969).

Haemolysis is an important parameter used to assess the quality and survival of stored RBCs (Makroo et al., 2011). As RBC break down, chemical as well as haematological changes, can be observed. Haemolysis begins with the breakdown of the RBC membrane which loses some of its properties as red cell senescence occurs (Tarasev, Chakraborty & Alfano, 2015). This results in a rise in osmotic fragility and lactate dehydrogenase, potassium, as well as haemoglobin being released from inside the cell into the surrounding plasma (Hess, 2014; Arggawall, 2020). The RBC lose their biconcave shape and there is an increase in echinocytes and abnormally shaped RBC (Hess, 2014). This means that the RBC are unable to optimally transfer oxygen throughout the body.

The degree of haemolysis can be detected by measuring the presence of free haemoglobin in the red cell suspending media, such as Saline, Adenine, Glucose and Mannitol (SAGM) (Adams et al., 2018). As red cells age, chemical changes such as an increase in serum potassium occurs (Hess, 2014), and depending on the length of storage, sodium levels will decrease (Adams *et al.*, 2018). This is due to RBCs losing some of their structural properties and the development of lesions in the membrane of the RBC. Once the cells are stored at a cool temperature of 2-6ºC, the sodium/potassium pump halts (Van De Watering,

2011). Consequently, the haemoglobin within the RBC will seep into the supernatant or suspending media and can be measured to indicate the amount of haemolysis (Adams *et al.*, 2018). If a haemolysed unit is transfused into a patient, the patient's immune system can be activated resulting in a transfusion reaction which can lead to kidney failure, cardiac failure or even death (Strobel, 2008).

2.6 Neocytes

Neocytes, also known as young RBC of 30 days or younger, can be separated from whole blood (WB) through methods such as apheresis or the conventional method (Kumbhakar, 2016b). The apheresis method is used for the isolation of a specific required product, while infusing the rest of the blood back into the donor. Problems associated with the apheresis method are that it is expensive and can cause injury to the donor, such as vascular injury or citrate toxicity (Crocco et al., 2009). The conventional method is also known as the top and bottom method, where WB is processed from a quadruple bag system to form three products namely, RBC, buffy coat and platelets (Hardwick, 2008). Neocytes are the youngest RBC which are released by the bone marrow, and they have a lower density in comparison to mature red cells (Propper *et al.*, 1980). The difference between a reticulocyte and neocyte is that a reticulocyte is seen as immature, while a neocyte is a mature RBC. Reticulocytes are expected to have a higher mean corpuscular volume (MCV) compared to neocytes. Patients in need of chronic transfusions will benefit from neocyte transfusions as the lifespan of these younger red cells is longer (Spanos et al., 1996; Kumbhakar, 2016a). Thus, chronically anaemic patients will have a longer interval between blood transfusions, reducing the risk of developing iron overload.

Current techniques to prepare neocytes such as apheresis, also known as neocytapheresis, are technologically difficult, time consuming and expensive. They require costly equipment, such as cell washers or continuous-flow cell separators which have high maintenance costs and can only be used to collect a specific product (Montoya, 1993). Consequently, fewer products are produced from a single donor. As apheresis is a time-consuming process, this may result in donors being less willing to donate a unit. Other complications associated with apheresis are citrate toxicity (Crocco *et al.*, 2009) which occurs when citrate is infused during the blood collection process.

In contrast, the conventional method is more cost effective, the equipment requires less maintenance, and the donation time is approximately only 30 minutes. Additionally, WB is collected and therefore more products can be produced from a single donation and the

filtering of white blood cells (WBC) is more effective (Kumbhakar, 2016b). This is important as the presence of WBC in a transfused unit can cause a febrile non-haemolytic transfusion reaction (Da Ponte et al., 2005). Previous literature has also revealed that the yield of neocytes is higher and more consistent using the conventional method when compared to those where cell separators were used (Piomelli, Lurinsky & Wasserman, 1967; Montoya, 1993; Kumbhakar, 2016b). The risk of injuring donors is also less as compared to cell separators, which increases the risk of vascular damage as the pressure in which the blood is returned into the donor is high (Philip, Sarkar & Pathak, 2013).

2.7 Collection of Neocytes

At the WCBS in South Africa, blood is donated from a healthy donor who has given consent. The blood is processed to form three products namely platelets, plasma, and RBC. These products are distributed to the required hospital or doctor as requested. RBC products are used when a patient is anaemic, while platelets are used when a patient suffers from thrombocytopaenia, and plasma can be used when a patient is suffering from hypoproteinaemia. Generally, RBC transfusions are recommended when the patient's Hb levels are less than 6 g/dL. There are multiple types of red cell products, such as gamma irradiated RBC, which are mostly used for leukaemia patients, as well as washed cells which are provided to patients who are known to have recurrent allergic reactions post-transfusion (WCBS & SANBS, 2023). There are three available plasma products in South Africa, namely Fresh Frozen Plasma, Cryoprecipitate, and also Cryo-poor Fresh frozen plasma. The indication for use and the dosage for each product varies. There are two types of platelet products, namely pooled buffy coat platelet concentrates as well as single donor apheresis platelet concentrate (WCBS & SANBS, 2023).

Packed RBC have an expiry period of 42 days from the date of donation and are stored at 2-6 ºC. Plasma has a one-year expiry date from the date of donation and is stored at -18 ºC. Platelets have an expiry period of five days and must be constantly agitated as well as stored at room temperature (20 ºC to 25 ºC) (WCBS & SANBS, 2023). RBC units are prepared predominantly as a Red Blood Cell Concentrate (RBCC) which is re-suspended in additive solutions consisting of Saline, Adenine, Glucose and Mannitol (SAGM). This allows the red cell units to be stored for up to 42 days, thereafter, as a result of increased cell death and haemolysis, the unit is no longer viable and is discarded.

Piomelli et al. (1967) conducted a study that examined the relationship between cell age and density, following separation and centrifugation. It was observed that neocytes were

less dense than older cells and will, therefore, gather in the upper half of the concentrated RBC bag (Franco et al., 2013). Due to this difference in density, neocytes can be separated by means of centrifugation and can be collected by extracting them from the upper half of the bag. After centrifugation, the SAGM is removed from the original, filtered bag and transferred to a transfer bag using a manual extractor. The top 30 percent of RBC are then collected in a transfer bag by the use of a scale and a manual extractor. Once sufficient RBC are collected, the tubing is sealed, and the transfer bag is then removed from the original bag. SAGM is then added to the neocyte-enriched bag in a specific weight ratio. This process produces a neocyte-enriched unit of blood. Blood transfusion settings currently do not commonly use neocyte collection techniques. Most studies have focused on the collection of neocytes using the apheresis method (Graziano *et al.*, 1982; Montoya, 1993; Priddee, Pendry & Ryan, 2011). The major differences between the two methods of collection are depicted in table 2.1.

Table 2.1: The differences between blood collections from donors using the apheresis and conventional method for neocyte collection

	Apheresis Method	**Conventional Method**
Time	One donor can take up to 90 minutes	One donor can take up to 20 minutes
Price	Very Expensive	Less expensive
Safety	Increased risk of Citrate toxicity	Decreased risk of citrate toxicity
Equipment requirements	Specialised equipment required	Routine donation equipment required.
Neocyte Yield	Low	Higher

2.8 Current Settings using Neocytes

Neocytes, also known as young RBC, were first described in the 1980s (Propper *et al.*, 1980). Since then, multiple studies have been done to refine the collection method and to identify their application (Sharma et al., 2008; Kumbhakar, 2016b; Spanos et al, 1996). Previous studies have shown that the use of young RBC has multiple benefits for patients requiring chronic transfusions (Sharma *et al.*, 2008). Some of the conditions requiring multiple blood transfusions include Sickle Cell Anaemia and β-Thalassemia. Neocyte transfusion studies have been done to determine the benefit of infusing patients with young RBC. In India, twelve β-thalassemia patients were studied. Six patients were infused with conventional RBCC and six with pooled Neocytes. The results indicated an increase in the days between required transfusions for those transfused with pooled neocytes. The RBCC

cohort required an average transfusion every 24,5 days, while those receiving pooled neocytes required a transfusion every 45 days (Kumbhakar, 2016b).

A similar study, also performed in India, analysed twelve β-thalassemia participants. The Cohort with the RBCC infusion required a transfusion after 26,15 days while the pooled neocytes required a transfusion after 45,86 days (Sharma *et al.*, 2008). Further investigations performed on sixteen β-thalassemia patients in Canada, also reported a decrease in the required transfusions for patients after neocytes transfusions (Collins et al., 1994). A significant number of patients diagnosed with Sickle Cell Anaemia are found in SSA, and although the use of young RBC or neocytes have been utilised in the treatment, this has not been implemented or investigated in Africa. The use of neocyte-enriched blood in SSA could potentially be beneficial in the treatment for patients requiring multiple transfusions and therefore a study investigating the feasibility of this method is warranted.

2.9 Conclusion

Studies examining the role of neocytes in transfusion have been performed in other countries (Sharma *et al.*, 2008). There are, however, no studies examining the potential use of neocytes in SSA (Roberts *et al.*, 2019). This current study may result in neocyte-enriched blood being utilised for patients with chronic anaemia. Due to an increase in the incidence of Sickle cell anaemia and other inherited haemoglobinopathies in South Africa (Thompson, et al, 2019), the possibility for a blood bank to expand the lifespan and quality of RBC concentrate units may be important to improve clinical results and reduce the interval between transfusions.

Centrifugation for the preparation and collection of neocytes is an easy process with low cost and requires no special equipment. It is also convenient for the donor with less risk and potentially will increase the interval between transfusions and reduce the risk of transfusion related complications (Spanos *et al.*, 1996; Nishi, 2005; Sharma *et al.*, 2008).

The purpose of this study therefore is to examine RBC haemolysis and survival in traditionally stored filtered blood and to compare these results to neocyte-enriched blood from the same donor, in an effort to investigate the feasibility of using neocytes to prolong the lifespan and storage of red cell concentrate units. This could possibly also mean that the interval between future required transfusions could be increased.

CHAPTER 3: RESEARCH DESIGN AND METHODOLOGY

3.1 Study Design

This was a laboratory based prospective cohort study in which two different red cell products from 30 group AB positive donors were compared. The study was conducted at the headquarters of the Western Cape Blood Service (WCBS**)** in Ndabeni, Cape Town. The donor interval between consecutive blood donations was not less than 56 days. All donated blood that screened positive for HIV, Hepatitis B, Hepatitis C or syphilis were excluded, and thereafter only AB positive units were used due to the low demand for this blood type (Hulley et al., 2013.).

After separating the neocyte red cell population from the filtered blood unit, multiple parameters were tested at regular 14-day intervals to determine the level of haemolysis and lifespan of the RBC within a controlled environment. The results of Neocyte enriched cell population were compared to the original filtered blood cells from the same bag which were stored under the same conditions.

3.2 Study Population and Sample Size

The study population was routine donors from the Western Cape Blood Service, who donated blood between October 2021 and December 2022 and who met the minimum criteria to donate. From these donors, only fresh, blood group AB positive units were used for the study.

The units obtained from the donors followed standard operating procedures at the Western Cape Blood Service. Each donor had to fill in the required questionnaire prior to donation and was tested for any transfusion transmitted diseases (TTDs) after donation. The WB was then processed to produce multiple different blood products.

The formula used to determine the sample size was: $n = z^2(pq)/e^2$ where n= sample size, z= standard error associated with the chosen level of confidence(1.96), p= the estimated percentage within a population (AB blood prevalence is 4% in South Africa), q is 100-p and e is the acceptable sample error (5%). This means that $n= 1.96^2(4x96)/5^2$. Thus n =59.01. This result was then rounded up to a sample size of 60.

3.3.1 Inclusion criteria

It is essential that blood transfusion institutions protect their donors from being compromised. Therefore, certain criteria were followed to protect the donor. Blood donations were given on a voluntary basis, and donors were not remunerated for their donation. The following standards were documented.

All first-time donors were given verbal or written information about the procedure (whole blood donation) so that they could give written consent for the procedure prior to each donation. A donor completed and signed a questionnaire (Addendum B) and donors were only included if they met the following criteria:

- were healthy and between the ages of 16 and 75;
- weighed more than 50kg;
- the interval between consecutive blood donations was not less than 56 days;
- were in good normal health and did not suffer, nor were suffering from any serious illness;
- were not taking any medication which could affect the quality of the blood donation;
- the haemoglobin level was not less than 12, 5 g/dl (125 g/L);
- the pulse rate did not indicate any irregularity and fell within the parameters as set by the institution of 50 to 100 beats per minute;
- the systolic blood pressure was between 90 mmHg and 180 mmHg while the diastolic pressure was between 50 mmHg and 100 mmHg; and,
- were not pregnant and had not given birth within the preceding three months.

3.3.1.1 Exclusion criteria

Any donor who did not meet the minimum criteria to become a donor at the WCBS or did not meet any of the criteria listed above.

3.3.2 Donation process

Once a donor met the requirements to donate, the donor was registered on the system with a serial number which was linked to the unique donor code of the individual. If the donor was not eligible to donate, a serial number was not produced for the donor and thus a unit would not have been collected from the donor. A plasma low Hb analyser (Hemocue, Sweden), was used to determine the haemoglobin level prior to donation. A donor was only allowed to donate if the haemoglobin level was within the required specification of >12.5 g/dL. The personal information of the donor was then verbally confirmed, and the serial

number was checked to ensure that the number on the donation bag, sample tubes and donor questionnaire matched.

The needling site was sterilised with 70% isopropyl alcohol and a needle was inserted into the vein of the donor. The donation sample pouch was then filled with blood after which two 10 mL Ethylenediaminetetraacetic acid (EDTA) and one 6 mL EDTA was filled. The 10 mL samples were sent for virological testing on a molecular as well as serological level. The molecular sample was tested on the Panther Nucleic Acid Testing analyser (Grifols, Spain) and the serological sample was tested on the Cobas e801 analyser (Roche, Germany) . The 6 mL sample was sent to the RBC serology and haematology department where the blood group was tested, and syphilis testing was performed on the Beckman-Coulter PK analyser (Beckman-Coulter, USA). When all three samples were collected, the sample pouch was closed. The rest of the donation process was then continued until the required weight of WB was reached. The tubes were clamped, and the needle was removed from the donor's arm.

3.3.3 Processing of whole blood units

WB was collected by standard procedures from 30 normal healthy donors who had given their informed consent. The blood was collected into Terumo quadruple blood bags, consisting of three satellite bags which is a closed system. All units were weighed before processing and were processed within 24 hours of collection. WB units were centrifuged using the Thermo Scientific Sorvall RC 12BP9+ centrifuge at 45 RCF (Relative Centrifugal Force) for twelve minutes, maintaining a temperature of + 4° C on the Sorvall RC 12BP9+ centrifuge. The unit was then processed into plasma, platelets and RBCC, using the Terumo T-ACE Ⅱ extractor. RBC were removed into the first satellite bag which contains saline, adenine, glucose and mannitol (SAGM) and is known as RBCC. The plasma was extracted into the second bag and the third bag was used for any by-products, such as cryoprecipitate. The AB positive units were kept aside for this research study until all testing results confirmed that the unit was TTD negative.

3.3.4 Filtration of units

Once the AB positive RBCC unit was collected by the researcher, the required transfer bags, sample pouches and sample tubes were labelled as required. Each unit required 5 X 4mL EDTA samples (1 for Reticulocyte count and 4 for full blood cell count), 4 X 10mL EDTA (separation of plasma from red blood cells), 8 X 5 mL cryo tubes (4 for plasma sodium testing and 4 for plasma haemolysis testing). Each unit also required 8 sample

pouches (4 for neocyte-enriched and 4 for filtered blood), 2 X transfer bags (1 for neocyte-enriched blood and 1 for SAGM) and an Imugard Ⅲ-RC filter pack.

After collection, the unit was sealed with a Imugard Ⅲ-RC filter pack by using a Terumo sterile tubing welder TSCD-Ⅱ. Once the filter pack was sealed to the unit, the red clamp, blue clamp and white clamps were closed. The sealer (found at the bottom of all RBCC units) was broken and the unit was hung upside down. The red and white clamps were opened, and the transfer bag was dropped to the bottom. After approximately 10 minutes, the unit filtration was complete. This was confirmed by visually checking that there were no more RBC in the original RBCC unit. The white clamp was closed, and the blue clamp was opened. This allowed a passageway for excess air to be removed from the transfer bag. The transfer bag was squeezed to remove any excess air. Once all the air was removed, all the clamps were closed, and the tubes were sealed by using the Terumo T-Seal Ⅱ. The filtered product is called filtered blood.

3.3.5 Isolation of neocytes

In order to isolate and enrich the neocyte population, two transfer bags were attached to the filtered blood bag by using a Terumo sterile tubing welder TSCD-Ⅱ. These transfer bags were used to extract the neocytes as well as the SAGM from the filtered blood. The filtered blood unit with the attached bags were spun at 4500 relative centrifugal force (RCF) for 12 minutes, maintaining a temperature of + 4° C on the Sorvall RC 12BP9+ centrifuge. After centrifugation, the two transfer bags were placed on separate scales and the scales were tared. The tube leading to the neocyte transfer bag was sealed using a manual clamp. A manual extractor was used to remove SAGM from the filtered blood bag into the SAGM transfer bag. The SAGM transfer tube was manually clamped. The tube leading to the SAGM transfer bag was sealed using the Terumo T-Seal Ⅱ. The manual clamp leading to the neocyte transfer bag was removed. Approximately, 35 g of the top 30% of the remaining RBC was extracted into the neocyte transfer bag. The neocyte transfer tube was manually clamped. The neocyte transfer tube was also sealed using the Terumo T-Seal Ⅱ. The sealed tubes of both neocyte, as well as SAGM transfer bags, were welded together using the Terumo sterile tubing welder (TSCD-Ⅱ). A fixed ratio (1:2,6) of SAGM to neocytes was applied, meaning that approximately 13,46 g of SAGM was transferred from the SAGM transfer bag into the neocyte transfer bag. Once the SAGM was added, the transfer tube between the two bags was sealed using the Terumo T-Seal Ⅱ. The SAGM transfer bag

was discarded and the neocyte bag was the final product. The process of extracting the neocyte fraction is depicted in figure 3.1.

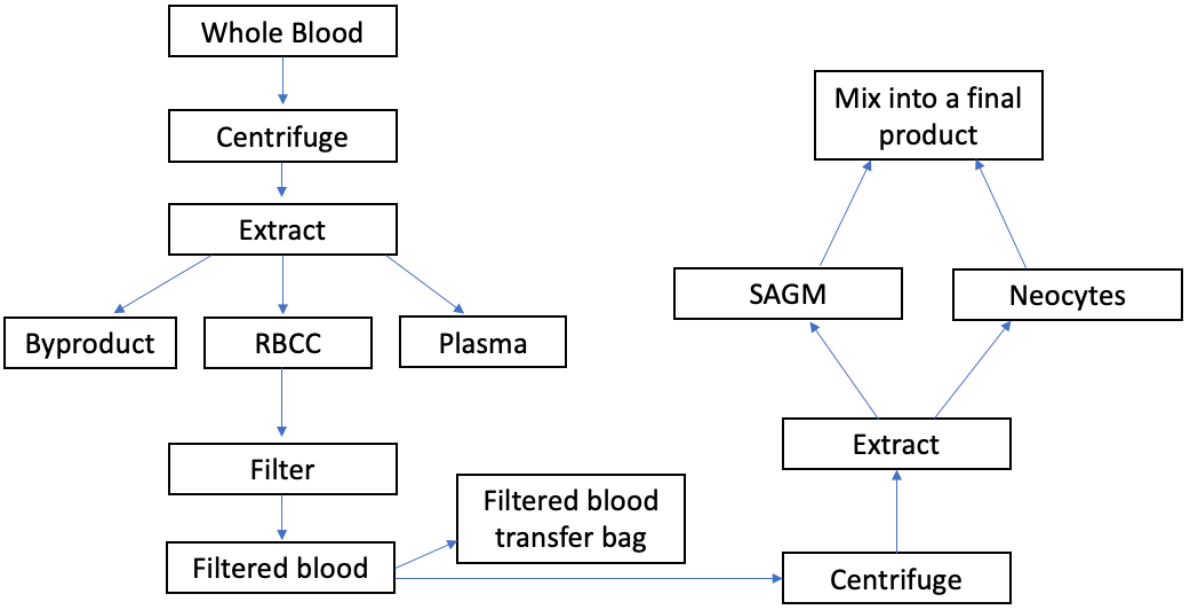


Figure 3.1: Flow diagram of methodology to extract neocytes. RBCC: Red Blood Cell Concentrate; SAGM: Saline Adenine Glucose Mannitol

3.3.6 Storage

Both the neocyte-enriched and filtered blood labelled bags were stored in a temperature-controlled fridge (2-6^{o}C) for 42 days. Analysis of haemolysis and biochemical tests were performed on days 1,14, 28 and 42 of storage.

3.3.7 Analysis of neocyte and filtered blood

Preparation of samples for testing was achieved by welding a new sample pouch to the labelled filtered blood neocyte bag every 14 days. This process maintained the closed system and ensured sterility. Seven (7) mL of neocytes was transferred into the sample pouch and the tube between the sample pouch and neocyte transfer bag was resealed. This sample pouch was used for preparing samples. On Day 1, a reticulocyte count was performed on the neocyte-enriched unit. Samples from both bags were analysed for sodium, haemoglobin, red cell count, red cell indices and free haemoglobin at each time point.

3.3 Laboratory Analysis

3.4.1 Sodium levels

A serum separator tube (SST) was filled with 2 ml of blood. This was sent to a private ISO 15189 accredited pathology laboratory where it was analysed using the Architect c8000 analyser (Abbot, USA). The instrument analysed the sodium using an Ion Selective electrode. This method functions by using a membrane which is placed between the sample and the analyte's ion. The potential difference is analysed, and a value is calculated based on the potential difference (Zoski, 2007).

3.4.2 Haematological parameters

Three (3) mL of blood was collected into an EDTA tube for the haematological tests. The full blood count was performed using the Sysmex, XN-3000 (Sysmex, Kobe Japan). The Sysmex XN-3000 tags the intracellular nucleic acids with a fluorescence tag. This tag is directly proportional to the content of nucleic acid. Reticulocyte cells have a much higher fluorescence tag compared to mature RBC and are much lower than WBC. There are three levels of maturation stages for reticulocytes. These include the low fluorescence ratio, medium fluorescence ratio and high fluorescence ratio.

The sample was also run on the Sysmex, XN-1000 (Sysmex, Kobe Japan). The Sysmex XN-1000 has an RBC/PLT channel which is used to read the RBC and platelets. This is read by using the sheath flow direct current method. The sample is transported into the sheath flow and pushed through the detector. The function of the sheath flow is to control the route of the cells at the detector unit. The cells will then cause an electrical disturbance which is directly proportional to the volume inside the cells. This is read in the form of a pulse. The result is then transformed into graphs. The RBC indices are formulated based on calculations. The haemoglobin is determined by using cyanide-free sodium lauryl

sulphate (SLS). The reagent lyses RBC and WBC in the sample. The chemical reaction changes the globin and oxidises the haem group. The SLS hydrophilic group can bind to the haem group and forms a complex called SLS-HGB, which is analysed by photometry. An LED light is emitted and absorbs the SLS-HGB complex. The absorbance is measured by a photo sensor which is directly proportional to the haemoglobin concentration of the sample.

3.4.3 Plasma haemoglobin

The last 2ml of the sample bag was used for the free haemoglobin test, on day 1, 14, 28 and 42. Each time the samples were collected they were centrifuged at 4000 rpm for 15 minutes. A small drop of the supernatant was pipetted onto a slide and the plasma/low Haemoglobin (Hb) cuvette was filled. The cuvette can only be used once and 20 µL of sample is drawn into the chamber by capillary action. The photometer measures two wavelengths in order to compensate for a certain degree of turbidity, and the haemoglobin level is calculated and presented. The system uses the international reference method for calculating haemoglobin. The percentage plasma haemolysis was calculated using a formula: $\frac{(100-HCT)\ X\ plasma\ Haemoglobin(g\ dl-1)}{Total\ Haemoglobin\ (g\ dl-1)}$. This is a formula used in literature to determine the percentage plasma haemolysis (Makroo *et al.*, 2011). According to the Council of European guidelines, the upper limit of percentage plasma haemolysis is 0,8 (Cardigan, 2007).

3.4.4 Quality control of analysers

Daily quality control was done to ensure that all results were within recognised standards. The Sysmex XN-3000 as well as Sysmex XN-1000 measured a low, normal, and high control which were run three times a day. Calibration was done after the annual service. Quality Control on the HemoCue® Plasma/Low Hb, was run on two levels, namely, high and low level. Monthly external quality control was performed on all instruments. All samples were run in a SANAS (South African National Accreditation System) accredited laboratory.

3.4 Statistical Analysis

All the results were transcribed onto Excel version 16.84. The statistical consultation centre of CPUT was approached for statistical assistance. The program SPSS, version 28 was used for the Analysis of Variance, while Microsoft visual studio code, version 1.60, was used for descriptive statistics and Spearman correlation coefficient. The median formula

used was: $\frac{\frac{n}{2}Obs.+\left(\frac{n}{2}+1\right)Obs}{2}$, while the IQR formula used was: IQR = Q3 $(\frac{3(n+1)}{4}) - Q1\left(\frac{n+1}{4}\right)$. A Shapiro-Wilk test was used to determine the normality of the results. The programming language used was Python and the library packages were Pandas, Seabourne and Matplotlib. The results for day 1, day 14, day 28 and day 42 were analysed and compared between the neocyte enriched blood and filtered blood using the t-test. The Spearman correlation coefficient was used to determine the correlation of the different variables. The overall statistical significance for all the parameters was calculated using the Analysis of Variance (ANOVA) test. The results were assessed using the *p*-values and the x-values. A significant difference was found if the *p*-value was less than 0.05. Moreover, a positive linear correlation was suggested if the x-value was more than 0, while a result below 0, would suggest a negative linear correlation.

3.5 Ethical Considerations

The Western Cape Blood Service is a non-profit organisation which provides a high standard of blood products to all private and public hospitals in the Western Cape province. Each year, roughly 155,000 units of blood are donated in the Western Cape from voluntary, non-remunerated donors. Prior to donation, each donor is required to fill in a donor acceptance questionnaire. In this questionnaire, the donor is required to respond to multiple lifestyle and health related questions. This is to determine whether the donor meets the minimum criteria to become enrolled as a blood donor for the Western Cape Blood Service. This questionnaire also requests permission from the donor to use their blood for reagent preparation or research purposes.

All information required from the donors for this study was kept confidential by removing all identifying information. All information acquired from this study was kept in a safe, password-protected environment which was accessible by the researcher only.

Ethical approval was obtained from the Cape Peninsula University of Technology Health Research Council (HREC) on the 24th of February 2021 with the reference no: CPUT/HW-REC 2021/H5 (Addendum A).

CHAPTER 4: RESULTS

4.1 Introduction

During this study, 30 neocyte-enriched and 30 filtered blood units were processed. The two unit types were analysed and compared using different parameters. As expected, significant changes in the MCV, plasma haemolysis and sodium levels were observed in both unit types over the 42-day storage period. A significant and steady increase in the MCV and percentage plasma haemolysis was observed. Sodium levels were highly variable but steadily decreased in both groups. All other parameters remained stable at all timepoints. The Shapiro-Wilk test confirmed that the collected data exhibited a normal distribution. Therefore, to assess central tendency and variability, the mean and standard deviation were employed for further analysis. Overall, using the one way ANOVA statistical test, there was no significant difference between the means of any of the parameters measured across all time points. Variations within the results have been noted for MCV, MCHC, sodium and percentage plasma haemolysis. These results are depicted in table 4.1 below.

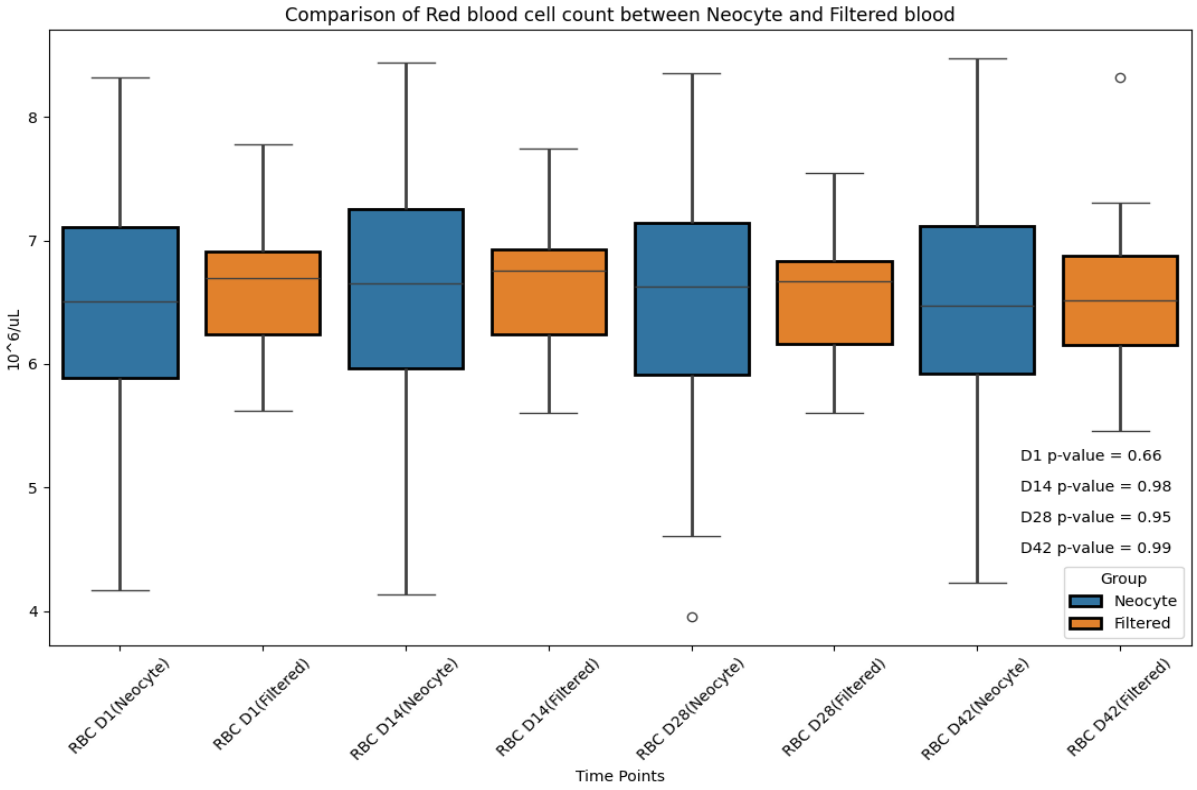
Table 4.1: Mean and standard deviation results of filtered blood and neocyte-enriched blood

	Filtered blood				**Neocyte-enriched**					
Analyte	**D1 Mean (± SD)**	**D14 Mean (± SD)**	**D28 Mean (± SD)**	**D42 Mean (± SD)**	**D1 Mean (± SD)**	**D14 Mean (± SD)**	**D28 Mean (± SD)**	**D42 Mean (± SD)**	***p*-value**	***F*-Ratio**
Red blood cell count (10^6/uL)	6,59 (0.53)	6,64 (0.57)	6,52 (0.53)	6,54 (0.60)	6,50 (1.00)	6,63 (1.03)	6,54 (1.09)	6,54 (1.03)	0,27	0.02
MCV (fL)	92,27 (6.39)	95,85 (6.47)	99,09 (6.83)	101,6 (6.48)	94,86 (6.46)	98,44 (6.49)	101,8 (6.70)	104,11 (6.47)	0,87	253.87
MCHC (g/dL)	30,29 (1.39)	29,11 (1.00)	28,3 (0.89)	27,53 (1.09)	29,32 (1.17)	28,33 (0.98)	27,43 (0.99)	26,9 (1.03)	0,44	3.89
Hb (g/dL)	18,29 (1.27)	18,46 (1.45)	18,23 (1.32)	18,28 (1.30)	18,03 (2.50)	18,41 (2.65)	18,15 (2.73)	18,20 (2.55)	0,75	0.05
Sodium (mmol/L)	141,1 (3.18)	124,37 (4.05)	114,07 (5.12)	107,13 (4.31)	140,13 (5.46)	121,57 (8.48)	110,87 (9.07)	105,90 (7.28)	0,14	0.65
Plasma haemolysis (%)	0,15 (0.07)	0,28 (0.09)	0,44 (0.12)	0,64 (0.13)	0,19 (0.08)	0,33 (0.07)	0,51 (0.14)	0,72 (0.18)	0,65	7.52
Average reticulocyte (10^9/L)					75,79					

MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Haemoglobin Concentration; Hb: Haemoglobin; SD: Standard Deviation.

4.2 Red Blood Cell Count and Reticulocyte Count

Both neocytes enriched blood and filtered blood demonstrated an increase in the mean RBC count between day 1 and day 14. However, despite a further decline in the number of RBC by day 42, no significant difference was observed (all $p > 0.05$). As expected, the reticulocyte count was 76% in the neocyte-enriched blood confirming the presence of young immature red cells. The red cell count at each time point is depicted in Figure 4.1.



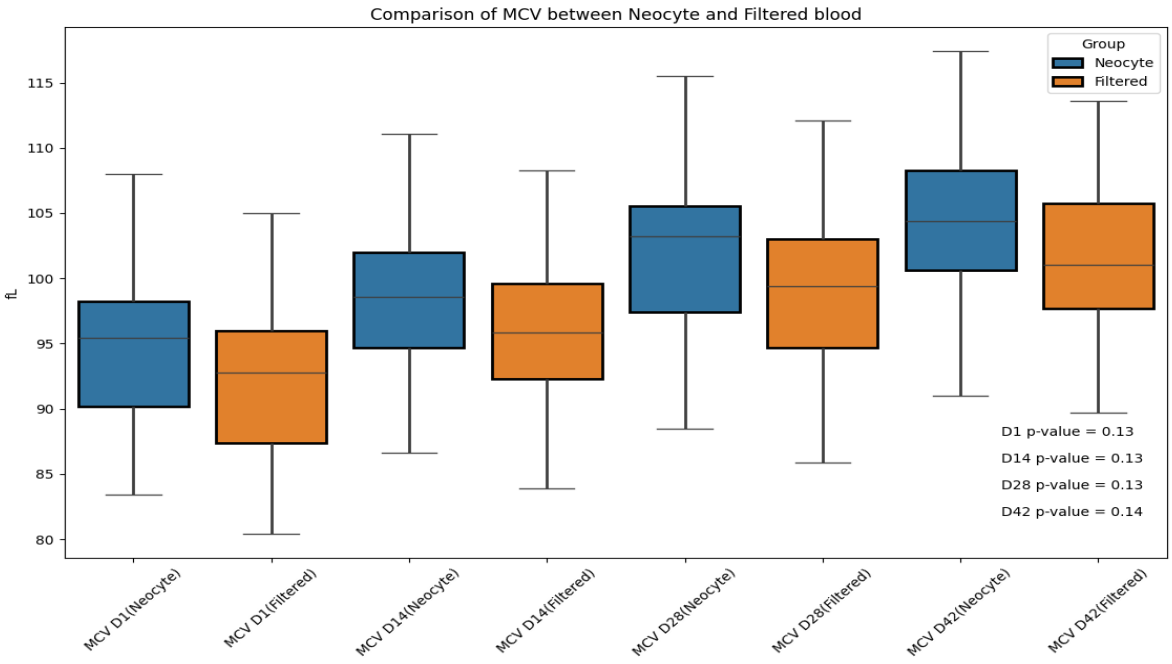
RBC: Red blood cells; D: Day

Figure 4.1: Red blood cell count of filtered blood and neocyte-enriched blood over 42 days

The box-and-whiskers plot compared red blood cell counts between neocyte-enriched and filtered blood at four different timepoints. The blue boxes represent the neocyte-enriched blood while the orange boxes represent the filtered blood. The p-values for each timepoint indicated no significant differences between the two groups at any timepoint, suggesting that RBC counts are similar for neocyte and filtered blood throughout the observed period.

4.3 Mean Corpuscular Volume

A steady increase in mean corpuscular volume (MCV) was observed in both the filtered and neocyte-enriched blood. Although the MCV of the neocyte-enriched blood was consistently higher, the values at each timepoint were not significantly different to the filtered blood ($p>0.05$). However, high variances in the results were observed (F ratio = 253.87)



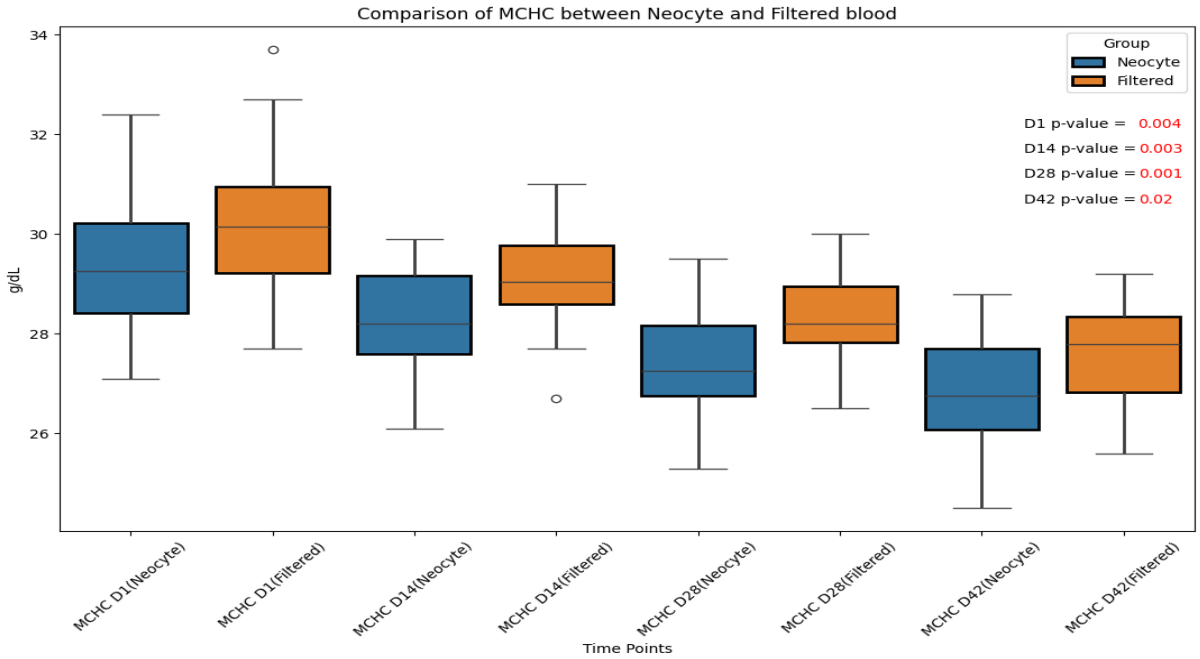
MCV: Mean Corpuscular Volume; D: Day

Figure 4.2: Mean corpuscular volume of filtered blood and neocyte-enriched blood over 42 days

The box and whiskers plot in figure 4.2 compared the MCV between neocyte-enriched blood and filtered blood. The blue boxes represent the neocyte-enriched blood while the orange boxes represent the filtered blood. The p-values for each timepoint indicate no significant differences in MCV between the two cohorts. Both cohorts experienced a slight increase in MCV over the observed period.

4.4 Mean Corpuscular Haemoglobin Concentration

The MCHC steadily decreased in both blood types over the 42-day study period reflecting the change in the red cell morphology. Although the MCHC in both the neocyte-enriched and filtered RBC was within the reference range, levels were significantly lower in the neocyte-enriched blood at day 1 ($p = 0.004$), day 14 ($p = 0.003$), day 28 ($p = 0.001$) and day 42 ($p = 0.02$). Significantly high variability was observed for the MCHC (F ratio = 3.89)



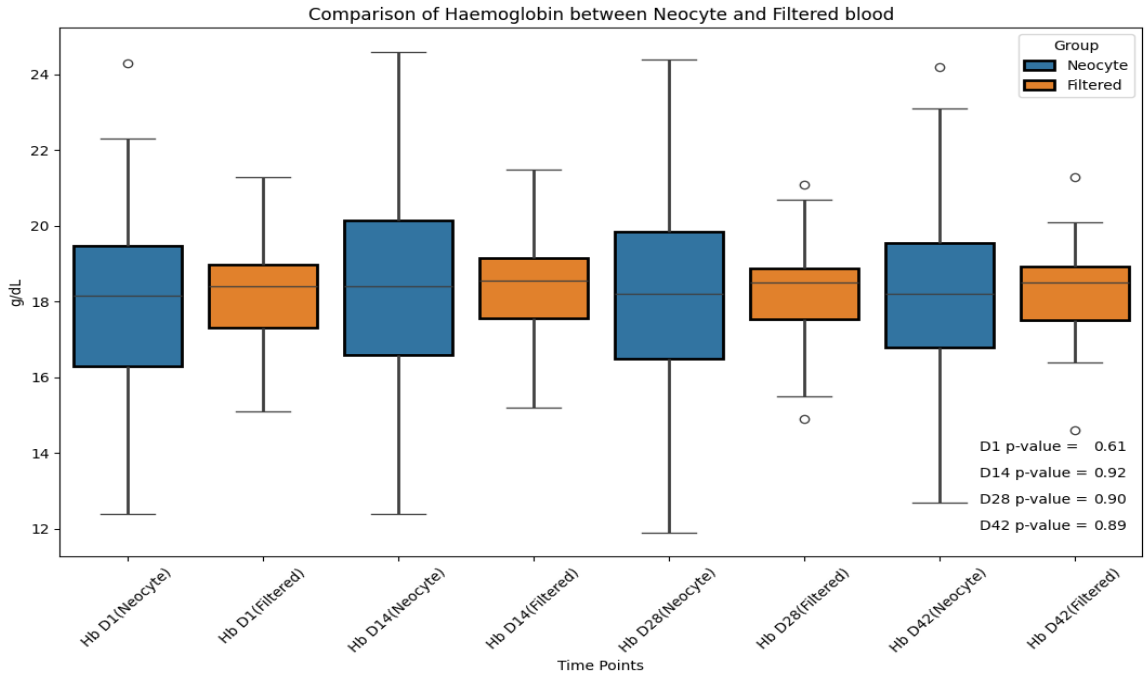
MCHC: Mean Corpuscular Haemoglobin Concentration; D: Day

Figure 4.3: Mean corpuscular haemoglobin concentration of filtered blood and neocyte-enriched blood over 42 days

The box and whiskers plot in figure 4.3 compared the MCHC between neocyte-enriched blood and filtered blood at four different timepoints, with blue representing neocyte-enriched blood and orange representing filtered blood. Across all timepoints, neocyte-enriched blood consistently showed lower median MCHC compared to filtered blood with statistical differences observed at each timepoint. The plot also highlights an outlier in the neocyte group at day 1 above the upper whisker and another at day 28 below the lower whisker, indicating slight variability in the neocyte samples. Despite these outliers, the overall trend shows that neocyte blood has a consistently lower MCHC than filtered blood over the observed period.

4.5 Haemoglobin

Haemoglobin levels in both the neocyte-enriched and filtered blood fluctuated significantly at each timepoint, however there was no significant difference in the haemoglobin levels between the two blood unit types.



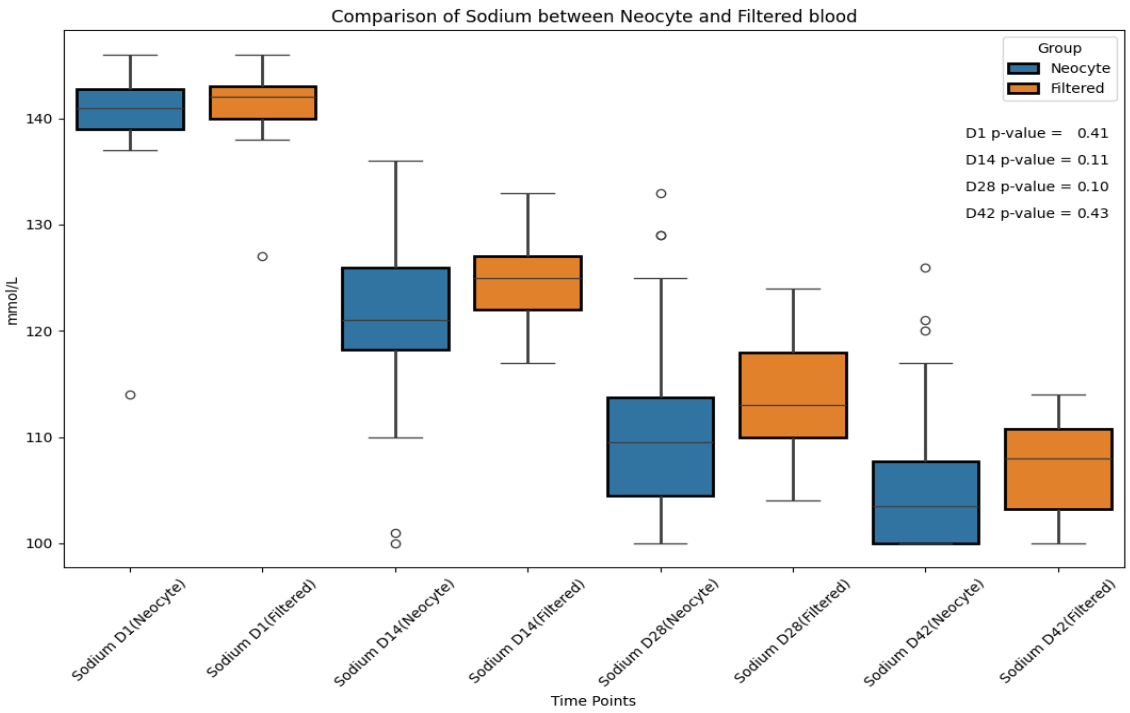
Hb: Haemoglobin; D: Day

Figure 4.4: Haemoglobin of filtered blood and neocyte-enriched blood over 42 days

The box and whiskers plot in figure 4.4 compared the haemoglobin levels between neocyte-enriched blood and filtered blood at four different timepoints, with blue representing neocyte-enriched blood and orange representing filtered blood. No significant difference was observed across the different timepoints. The median haemoglobin levels and interquartile ranges are similar for both groups at each timepoint. Outliers are present in the neocyte-enriched cohort at day 1, day 28 and day 42, but these do not affect the overall lack of significant difference between the groups.

4.6 Sodium

Sodium levels decreased steadily in both units, whereas the filtered blood had consistently higher levels at each timepoint. This difference was not significant. The percentage change decreased between day 1 and day 42 for filtered blood and neocyte-enriched blood. By day 42, filtered blood had an average sodium level of 107.93 mmol/L and neocyte-enriched blood had 105.90 mmol/L. No significant difference was found between neocyte-enriched and filtered blood for the overall sodium levels ($p = 0.14$) and no significant difference was identified at the individual timepoints between the neocyte-enriched and filtered blood at each timepoint. However, significant variability was observed (F ratio = 0.65).



D: Day

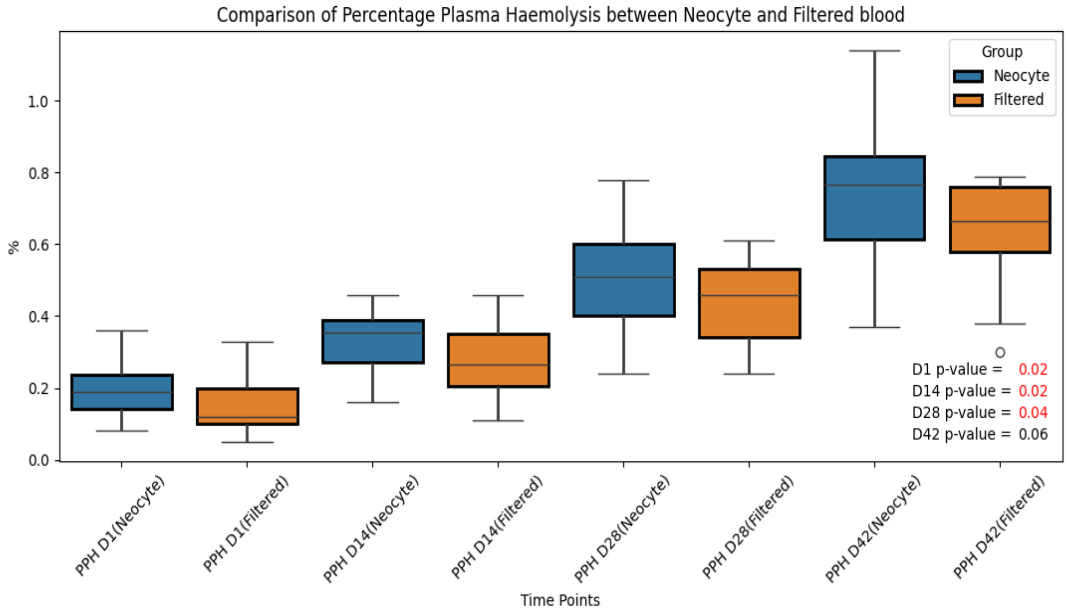
Figure 4.5: Sodium of filtered blood and neocyte-enriched blood over 42 days

The box and whiskers plot in figure 4.5 compared the sodium levels between neocyte-enriched blood and filtered blood at four different timepoints, with blue representing neocyte-enriched blood and orange representing filtered blood. Both groups exhibited a general decrease in sodium levels over time, neocyte-enriched blood had a higher median on day 1 than filtered blood, but this changed by day 14, where filtered blood had a higher median. The variability in sodium levels is consistently higher in the neocyte-enriched blood, compared to filtered blood. Despite these observations, the p-values indicate that the differences in sodium levels between the two cohorts are not statistically significant at any timepoints

4.7 Percentage Plasma Haemolysis

Plasma haemolysis increased steadily throughout the 42-day time period. The neocyte-enriched blood had a higher percentage of plasma haemolysis compared to filtered blood at each timepoint. By day 42, the filtered blood had 0.64%, whereas neocyte-enriched blood had 0.72%. This change in percentage between day 1 and day 42 was 0.49% for filtered blood and 0.53% for neocyte-enriched blood. However, no overall significant difference was found between neocyte-enriched and filtered blood ($p = 0.65$). Significant

differences were found between neocyte-enriched blood and filtered blood at the individual timepoints. These differences were at day 1 (0.02), day 14 (0.02) and day 28 (0.04). Significantly high variances were observed in the results (F = 7.52).



PPH: Percentage Plasma Haemolysis; D: Day

Figure 4.6: Percentage plasma haemolysis of filtered blood and neocyte-enriched blood over 42 days

The box and whiskers plot in figure 4.6 compared the percentage plasma haemolysis between neocyte-enriched blood and filtered blood at four different timepoints, with blue representing neocyte-enriched blood and orange representing filtered blood. The data show that neocyte-enriched blood had a significantly higher percentage of plasma haemolysis compared to filtered blood at day 1, day 14 and day 28. Neocyte-enriched blood also consistently showed a higher median haemolysis percentage across all the timepoints, with the difference being statistically significant at three timepoints. Overall, the plot indicates that neocyte-enriched blood tends to have a higher percentage of plasma haemolysis compared to filtered blood over the time period.

4.8 Correlation Studies

A Spearman correlation was also performed. The results are presented where a result (x) above 0 is seen as a positive correlation, and a result below 0 is seen as a negative correlation.

The results reveal significantly positive correlations between the Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), sodium levels and percentage plasma haemolysis exhibits a strong linear correlation (x = 1.00) within both neocyte-enriched and leucocyte-poor blood samples. This finding suggests highly similar relationships among these parameters, indicating that as one variable increases or decreases, the others follow in a predictable manner.

Moreover, when examining the correlation between haemoglobin (Hb) levels for neocyte-enriched and leucocyte-poor blood, a slightly weaker correlation is observed (x = 0.60). While this correlation is not perfect, its strength still underscores a meaningful association between Hb levels.

These correlations suggest a consistent interplay between the measured parameters. Furthermore, the consistent correlations observed across all variables indicate that changes in these parameters over the 42-day period are not isolated occurrences, but rather occur concurrently in both neocyte-enriched and leucocyte-poor blood samples. This indicates a common underlying mechanism, senescence, driving the fluctuations in these variables over time.

CHAPTER 5: DISCUSSION

5.1 Introduction

In this comparative laboratory study of 30 neocyte-enriched and 30 filtered units, some significant differences were found between the measured parameters over the 42-day storage period. Although both groups displayed significant increases in percentage plasma haemoglobin and a steady decrease in sodium levels, this was similar for both units.

The standard storage duration for RBC, is 42 days. Roughly 25% of RBC are removed by the spleen after transfusion (Bosman, 2013). This is due to the aging of RBC during storage, which undergo significant changes such as cellular shape. The RBC lose their biconcave shape and there is an increase in echinocytes and abnormally shaped RBC (Hess, 2014), therefore the RBC are unable to move and transport oxygen optimally throughout the body. Multiple biochemical changes, involving sodium and potassium ions, also occur. Sodium levels decrease while the potassium levels increase, which can lead to hyperkalaemia after transfusion (Namjoshi et al., 2021). These ionic changes influence the red cell volume and shape resulting in changes to multiple haematological parameters such as the MCV and MCHC.

5.2 Haematological Parameters

RBC rely on anaerobic glycolysis to generate energy. This process produces adenosine triphosphate (ATP), 2,3-diphoshoglocyrate (2,3-DPG) and reduced nicotinamide adenine dinucleotide (NADH) 2,3-DPG which significantly decreases during storage, causing the red cells to lose the ability to release oxygen (Scott et al., 2016; Ghezelbash et al., 2018a; McMahon et al., 2021; Belousov et al., 2023). Prolonged oxidative stress leads to irreversible damage such as lipid and protein breakdown which results in the removal of the RBC from the circulation (Zubair, 2010).

The results of this study have confirmed that the RBC count remained stable throughout the storage period, which is consistent with the findings of previous studies in Indonesia and Kenya (Sivertsen et al., 2020; Maulidan et al., 2022). These findings suggest that the RBC count is not significantly affected at different storage time intervals. An increase in MCV was, however, observed during the storage period. This increase is attributed to a decrease in ATP levels, which impairs the cell's ability to maintain ionic homeostasis, resulting in cellular swelling. This observation aligns with studies conducted in Iran and

South Africa, which also reported significant increases in MCV over similar periods (Adams et al., 2015; Tayer, 2017).

The MCHC has decreased during the 42-day storage period. This decrease results from the increase in cellular volume leading to hypochromic red cells. These findings are in line with studies from Portugal and Iran, which reported similar decreases in MCHC (Nogueira et al., 2015; Ghezelbash et al., 2018). Blood products with low MCHC contain red blood cells with decreased Hb concentration, which reduces their ability to carry oxygen effectively. This can lead to a lower increase in the patient's haemoglobin post-transfusion, compared to products with normal MCHC levels (Choy et al., 2023). The haemoglobin concentration initially increased, before declining over the 42-day storage period. These findings are expected as RBC break down and release haemoglobin from within the cell into the surrounding plasma (Arif et al., 2017). This pattern was slightly different from a study conducted in Italy, which observed a slight increase over the 42-day period (Blasi et al., 2012; Arif et al., 2017). These variations may be due to differences in study durations and methodologies. One notable difference is that the study conducted in Italy had a smaller sample size of eight units, whereas this study processed 30 units.

The study highlights the consistent changes that occur to the RBC count, MCV, MCHC and haemoglobin concentration over extended storage periods. These findings collectively highlight the critical importance of continuously improving storage periods to preserve the quality of stored blood. Ensuring high-quality stored blood is essential for transfusion therapy, particularly in emergency medicine, surgeries, and for patients with chronic conditions requiring regular transfusions. Thus, optimising these conditions can significantly enhance the patient outcomes and reduce the risk of transfusion-related complications.

5.3 Sodium

The storage of RBC significantly impacts electrolyte balance, particularly sodium levels. Electrolyte imbalances can have severe consequences, including muscle spasms, fatigue, hallucinations, irregular heart rate and even cardiac arrest (Namjoshi *et al.*, 2021). Once RBC are stored, the functioning of the sodium-potassium pump starts decreasing (Opoku-Okrah et al., 2015). The sodium-potassium pump becomes ineffective during blood storage, which leads to potassium moving in and sodium moving out of the semi-permeable RBC membrane (Adams et al., 2015; Marabi et al., 2021). A change in the intracellular sodium concentration affects the cell volume and shape, which can lead to an increase in the MCV,

as seen in this study. This suggests that the cells are deformed and thus lose their viability (Orlov & Karkouti, 2015). Reduced sodium levels during storage can lead to hyponatremia, which in turn can affect cardiac functions (Opoku-Okrah et al., 2015b; Antwi-Baffour et al., 2019). Studies have shown that extended storage of blood leads to major changes in sodium, which can lead to severe outcomes for patients.. This means that the management of sodium levels is crucial to the safety of the patient (Namjoshi *et al.*, 2021).

In this study, both neocyte-enriched and filtered blood exhibited a decrease in sodium levels over the 42-day period, with a high Spearman correlation coefficient (x = 1.00). A study conducted in Ghana, involving 28 blood units, similarly noted a decline in sodium levels during *in vitro* storage (Opoku-Okrah et al., 2015). These findings align with research in India, where analysis of 30 blood units, over 21 days, revealed a decrease in sodium levels (Verma & Dahiya, 2015). Additionally, a study involving 30 blood units over 28 days confirmed a reduction in sodium levels (Antwi-Baffour *et al.*, 2019). A high standard deviation (more than 14 mmol/L) for both neocyte-enriched and filtered blood sodium levels was found and suggests substantial variability of the results. These results show that there were many inconsistent sodium levels over the 42-day period for both cohorts. Moreover, the elevated interquartile range (more than 16 mmol/L) strengthens the suggestion of substantial variability in the sodium levels.

5.4 Percentage Plasma Haemolysis

Haemolysis refers to the release of intracellular components from RBC and indicates the damage that has occurred to the RBC membrane (Kirschbaumweg, 2002.). Haemolysis can be visually assessed as a discolouration of the plasma. A percentage plasma haemolysis can be calculated and used as a quality standard (Fernando et al., 2023). As RBC age the percentage plasma haemolysis increases. A high percentage of haemolysis may have devastating consequences for patients, especially those who are chronically transfused. After a transfusion, the spleen removes damaged RBC. This process can lead to elevated levels of non-transferrin-bound iron (NTBI), which can be toxic (Rapido, 2017). High levels of NTBI can lead to cardiac, hepatic and pancreatic damage, meaning that the percentage plasma haemolysis can be crucial in the patient (Patel & Ramavataram, 2012). The current European Council standard for the maximum acceptable percentage plasma haemolysis is 0.8% (Cardigan, 2007).

The findings of this study indicated that over the 42-day period, both filtered blood and neocyte-enriched blood showed an increase in percentage plasma haemolysis, with a

strong correlation between the two cohorts (x = 1.00). In contrast, a study from India, involving 25 units, reported a rise in percentage plasma haemolysis over 28 days, reaching 0.29% (Sawant et al., 2007) which was substantially lower. One notable difference in the Indian study is that samples were only tested up to day 28, while this study extended the testing to day 42. Additionally, the Indian study used packed RBC, whereas this study utilised filtered blood, a process that can potentially cause mechanical damage, leading to an increased percentage of plasma haemolysis.

Multiple studies indicate that extended RBC storage before transfusion is associated with an increased mortality rate, and an increased chance for major infections, as well as multiple organ failure (Leal-Noval et al., 2001; Koch et al., 2008). Furthermore, the use of aged RBC increases the risk of iron overload which affects the oxygen transportation, pH level and leads to increased breakdown of RBC (Aubron et al., 2013). Literature thus suggests that using younger RBC could potentially prevent or limit the possibility of developing iron overload (Aubron *et al.*, 2013). Multiple parameters were assessed in this study to determine whether neocyte-enriched blood, collected using the conventional top and bottom system, improves RBC survival, compared to filtered blood. The RBC count, mean corpuscular haemoglobin concentration, haemoglobin and sodium did not show any difference between the neocyte-enriched and filtered blood. However, differences were identified between neocyte-enriched blood and leucocyte poor blood for the mean corpuscular volume, as well as percentage plasma haemolysis at each individual timepoint and overall. Strong positive correlations were identified for all the parameters. These correlations suggest that the changes in parameters are not isolated incidents, but occur simultaneously for both neocyte-enriched and filtered blood. This implies a common underlying mechanism, such as red cell senescence, which drives the fluctuations of these parameters over time. Furthermore, an increase in the percentage plasma haemolysis could be the result of RBC experiencing additional mechanical damage due to the extra centrifugation cycle or the manual extraction of the neocytes.

Numerous research studies have utilised a variety of different methods in the collection of the young RBC and assessed the red cell senescence through different parameters (Graziano et al., 1982; Middelburg et al., 2013; Risso et al., 2014; Veale et al., 2014). Some of these methods include the conventional top and bottom method and the apheresis method. The conventional method involves centrifuging blood to separate the different RBC based on density. This method is cost-effective, simple and requires minimum specialised equipment. However, it offers limited precision in isolating the young RBC and can cause

potential mechanical damage due to multiple centrifugation steps. In contrast, apheresis is an automated process that selectively collects RBC based on their size and density. This process is much more precise in collecting the desired RBC and has a reduced likelihood of causing mechanical cellular damage. Nevertheless, the apheresis method is more expensive and can be less feasible. Therefore, while the conventional method is more accessible and cost-effective, apheresis provides greater accuracy and cell integrity, but at a higher cost and operational complexity.

Multiple research studies have been conducted to investigate the storage of young RBC. As expected, contrasting results have been found. For instance, a notable study conducted in Italy, assessed the viability of young, middle-aged and older RBC. Blood samples were collected in K-EDTA vacutainer tubes. The samples were centrifuged to separate the different blood populations. The results suggested that young RBC are more sensitive to survival signals, such as erythropoietin, compared to older RBC (Risso *et al.*, 2014). However, the results of a study conducted in Australia, have found no notable differences between young and old RBC aging over a 42-day period. Filtered blood was centrifuged to separate the young and old red blood (Veale *et al.*, 2014). The *in vivo* survival of neocytes, collected via apheresis, has demonstrated promising results, such as an increase in red cell survival (Sharma *et al.*, 2008; Kumbhakar, 2016b). These differences highlight the need for standardisation in young RBC collection methods as well as monitoring of red cell senescence.

5.5 Research Strengths and Limitations

The results of this study provide valuable insights into red cell senescence differences between neocyte-enriched and filtered blood. The use of the conventional top and bottom system for neocyte collection offers a more practical and cost-effective choice to alternative methods such as the apheresis method. This study maintained a controlled environment for testing, ensuring consistent conditions for the analysis of haemolyses and lifespan of the RBC.

However, this study also has limitations. The sample size was small, which could have affected the statistical analysis of the findings. Furthermore, the focus was solely on the storage duration of RBC, without assessing the *in vivo* survival, which could impact the patient's outcome. Moreover, this study only investigated the top and bottom system, instead of comparing the top and bottom system with the apheresis system. The use of flow cytometry has become an international standard for the identification of cell

populations (Xu, 2019). Some studies have suggested that the expression of CD47 decreases as red cells age. Young RBC also express CD35, CD44 and CD71 (Chang et al., 2009.; Ensinck et al., 2019). A reticulocyte count was used to determine the neocytes population, and in order to accurately study the young red cell population, the analysis of red cell surface antigens using flow cytometry is recommended. Limited resources and feasibility restricted the ability to precisely characterise the cell population. However, despite these limitations this study was able to clearly demonstrate that using the conventional method to collect neocytes provided no significant survival advantages over the 42-day storage period.

CHAPTER 6: CONCLUSION

6.1 Introduction

Chronic blood transfusions are essential for treating various conditions like myelodysplastic syndromes, chronic kidney disease, β-thalassemia, DBA and SCD. Patients with chronic anaemia need regular transfusions to maintain haemoglobin and haematocrit levels. Repeated transfusions increase risks for patients, including iron overload, transfusion reactions and infections. Iron overload can cause severe complications such as cardiac failure and liver disease. Neocytes could have a longer lifespan than mature red cells, meaning that the use of neocytes could be beneficial for patients in need of chronic transfusions. Current literature mostly focuses on utilising the apheresis method for collecting neocytes. However, this method has proven to be both expensive and time-consuming. The purpose of this study was to determine whether neocyte-enriched blood, collected by using the conventional top and bottom system, has a longer lifespan when compared to filtered blood.

The results suggest that young RBC collected using this system do not have an extended lifetime compared to filtered blood. Although there were significant differences at individual time points for MCHC, the ANOVA test indicated that the overall difference was not significant for all parameters. Significant differences were found between neocyte-enriched and filtered blood for sodium and percentage plasma haemolysis. This suggests that neocyte-enriched blood do not prologue the survival of red blood cells, compared to filtered blood. These results were further supported by the Spearman correlation coefficient, which showed a strong positive correlation between neocyte-enriched blood and filtered blood. The null hypothesis stated no significant difference between neocytes enriched and filtered, whereas the alternative hypothesis suggested that neocyte-enriched blood would prolong the storage life of red blood cell products. Although differences were observed in this study's results, these were not found to be statistically significant. Therefore, the results support the null hypothesis and reject the alternative hypothesis.

This study contributed to literature by demonstrating that neocyte-enriched blood, collected using the top and bottom system, does not extend the storage duration of RBC compared to filtered blood. Despite significant differences found between neocyte-enriched and filtered blood at individual time points, overall results suggest that neocyte-enriched blood would not be beneficial for the extended survival of red blood cells in storage. These findings underscore the need to reassess the potential benefits and practicality of using

neocyte-enriched blood for chronic transfusions and suggests that more efficient and cost-effective collection methods should be explored.

6.2 Future Research

Future research should involve conducting studies with larger sample sizes to improve the statistical analysis. Investigating the *in vivo* survival and clinical efficacy of neocyte-enriched blood compared to filtered blood will help understand the potential benefits to the patients' outcomes. Future studies should also explore alternative methods for neocyte collection to identify more efficient, cost-effective and practical approaches. Flow cytometry should be included to identify and further understand the antigens expressed on red cells and various stages of their lifespan. Finally, performing a cost-benefit analysis as well as risk analysis in clinical practice will be crucial for understanding both short-term and long-term healthcare costs and risks.

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Addendum A: HREC Approval

Cape Peninsula University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)
Registration Number NHREC: REC- 230408-014

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24 February 2021
REC Approval Reference No:
CPUT/HW-REC 2021/H5

Faculty of Health and Wellness Sciences

Dear Mr A Meyer Meyer

Re: APPLICATION TO THE HW-REC FOR ETHICS CLEARANCE

Approval was granted by the Health and Wellness Sciences-REC to **Mr A Meyer** for ethical clearance. This approval is for research activities related to research for **Mr A Meyer** at Cape Peninsula University of Technology.

TITLE: **An in vitro comparison of RBC haemolysis in neocyte-enriched and leukocyte-poor blood**

Supervisor: Assoc Prof Glenda Davison

Comment:

Approval will not extend beyond 25 February 2022. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the HWS-REC in December of that particular year, for the HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards

Carolynn Lackay
Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

Addendum B: Donor Questionnaire

Please read and sign the Declaration and Consent before donating blood.

Declaration

1. I confirm that I am 16 years of age or older.
2. I confirm that I have read 'Important Information for Blood Donors' and WCBS' Privacy Statement, and understand and accept the donation process and the related risks as explained to me.
3. To the best of my knowledge, all the information I have supplied is the truth. I understand that if I have not answered the questions truthfully, it may endanger patients and lead to legal proceedings against me.
4. I undertake to inform WCBS immediately if I think that my blood may not be safe for use.

Consent

1. I consent to the testing of my blood for blood group, syphilis, Hepatitis B, Hepatitis C and HIV as well as additional testing that may be necessary to ensure the safety of myself or patients.
2. I consent to being contacted using any contact details I have supplied in order to be informed of test results that are important to my health or affect my ability to donate blood.
3. I consent to my test results, personal information, and special personal information being kept in a strictly confidential manner for periods in accordance with WCBS' policies, Privacy Statement and legislative requirements.
4. I consent to samples of my blood and/or donation data being used anonymously for scientific research aimed at improving the safety of the blood supply and donor health, and that on occasion WCBS may permit researchers to request additional samples from me with my specific consent.
5. I consent to my blood products or samples being used for the preparation of diagnostic reagents utilised by blood banks and related medical facilities, and for the production of plasma-derived medicinal products manufactured by the National Bioproducts Institute.
6. I consent to receiving medical care (including infusion of fluids and medication) in the event of or to prevent an untoward donor reaction.

NAME AND SURNAME: _____ SIGNATURE: _____

RSA ID NUMBER / FOREIGN PASSPORT NUMBER: _____ CELL PHONE NUMBER: _____

FOR OFFICE USE: Interview done | YES | NO | Signature (Interviewer): _____

CLN12 (02 May 23)