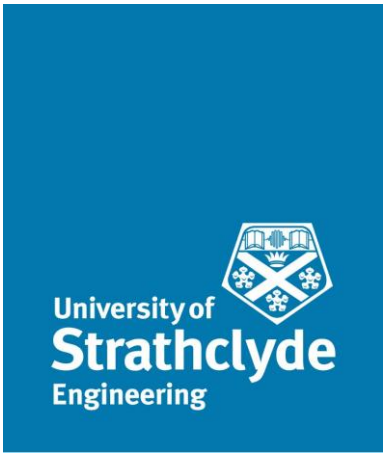
Development of a Superabsorber Based Haemofiltration Technology for use in Paediatric Cardiac Surgery



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Declaration

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Signed:

Date:

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Abstract

Cardiopulmonary bypass is known to cause great changes in a patient's physiological fluid homeostasis which frequently results in fluid accumulation in the interstitial space (edema). The associated inflammatory response can, in severe cases, result in systemic immune response syndrome, acute respiratory distress syndrome, and organ failure.

Modified Ultrafiltration (MUF) involves pumping blood through a haemofilter post-bypass to remove excess plasma, and has been shown to successfully moderate haemodilution and the inflammatory response and improve clinical outcomes in paediatric patients for whom edema is a serious morbidity.

In this research project, the current MUF technique is combined with a new superabsorber based haemofiltration technology to develop a novel haemofiltration device.

Design concepts were researched and the optimal device configuration was determined. Laboratory testing with bovine blood, computational modelling of blood flow and scanning electron microscope imaging of the control membrane highlighted the importance of both utilising flow disruption techniques to limit fouling, and controlling the swelling behaviour of the superabsorber material, in achieving rapid haemofiltration.

The device is able to achieve passive, chemically driven haemofiltration at a fixed blood flow rate and is designed such that it requires minimal input from clinical staff to be suitable for deployment in a clinical setting. The haemoconcentration time is close to current MUF procedures but with further refinement of the system, equalling the MUF time for current procedures is certainly achievable.

The blood haemoconcentrated by the device was of good quality with no significant change in cell counts, cell morphology measures, or haemoglobin level measures after 60mins of MUF indicating little to no impact upon the blood constituents or haemolysis induced by the device. The device did not demonstrate selective removal of pro-inflammatory mediators but similarly there was no increase in pro-inflammatory mediator concentrations observed. Further investigation of the inflammatory mediator impact is recommended.

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List of Abbreviations

ACT	–	Active Clotting Time
ADP	–	Adenosine Diphosphate
ATP	–	Adenosine Triphosphate
AV	–	Arterio-Venous
BP	–	Blood Pressure
BSA	–	Bovine Serum Albumin
BV	–	Blood Volume
CAD	–	Computer Aided Design
CAM	–	Computer Aided Manufacture
CBC	–	Complete Blood Count
CFD	–	Computational Flow Dynamics
CHb	–	Cellular Haemoglobin
CLS	–	Capillary Leak Syndrome
CNS	–	Central Nervous System
COP	–	Colloid Osmotic Pressure
CPB	–	Cardiopulmonary Bypass
CRP	–	C-Reactive Protein
CUF	–	Conventional Ultrafiltration
DC	–	Direct Current
DHCA	–	Deep Hypothermic Cardiac Arrest
DUF	–	Dilutional Ultrafiltration
ECC	–	Extracorporeal Circulation
EDTA	–	Ethylenediaminetetraacetic acid
ELISA	–	Enzyme-Linked Immunosorbent Assay
ET	–	Endothelin
FDA	–	US Food and Drug Administration
FFP	–	Fresh Frozen Plasma
GME	–	Gaseous Microemboli
Hb	–	Haemoglobin
Hct	–	Haematocrit
HMWK	–	High Molecular Weight Kininogen
ICU	–	Intensive Care Unit
IFV	–	Interstitial Fluid Volume
IH	–	Index of Haemolysis
IL	–	Interleukin
LUC	–	Large Unstained Cells
LV	–	Left Ventricular
LVAD	–	Left Ventricular Assistive Device
MCH	–	Mean Corpuscular Haemoglobin
MCHC	–	Mean Corpuscular Haemoglobin Concentration
MCV	–	Mean Corpuscular Volume
MIH	–	Modified Index of Haemolysis
MPV	–	Mean Platelet Volume
MUF	–	Modified Ultrafiltration
MVT	–	Mechanical Ventilation Time
MWCO	–	Molecular Weight Cut-Off
NIH	–	Normalised Index of Haemolysis
NS	–	Not Significant
OR	–	Operating Room
PA	–	Polyamide
PAA	–	Polyacrylic Acid
PAN	–	Polyacrylonitrile
PBS	–	Phosphate-buffered Saline
PCT	–	Plateletcrit

PCV	–	Packed Cell Volume
PCWP	–	Pulmonary Capillary Wedge Pressure
PDW	–	Platelet Distribution Width
PEG	–	Polyethylene Glycol
PES	–	Polyethersulfone
PICU	–	Paediatric Intensive Care Unit
PLT	–	Platelet Count
PMEA	–	Poly-2-methoxyethylacrylate
PS	–	Polysulfone
PTFE	–	Polytetrafluoroethylene
PVC	–	Polyvinyl Chloride
PVDF	–	Polyvinyldine Fluoride
RAP	–	Retrograde Autologous Priming
RBC	–	Red Blood Cell
RDW	–	Red Distribution Width
SAP	–	Superabsorbent Polymer
SEM	–	Scanning Electron Microscope
SIRS	–	Systemic Immune Response Syndrome
TBW	–	Total Body Water
TMP	–	Transmembrane Pressure
TNF	–	Tumor Necrosis Factor
TXB2	–	Thromboxane B2
UF	–	Ultrafiltration
VV	–	Veno-Venous
WBC	–	White Blood Cell
WHO	–	World Health Organisation

Chapter 1

Background

1. Background

1.1. CPB and Edema

1.1.1. Aetiology and Pathophysiology

Cardiopulmonary bypass (CPB) is a technique which combines a pump system and a gas exchange device (the oxygenator) to substitute the function of the heart and lungs respectively so that the cardiac and respiratory activity of a patient can be temporarily suspended to allow for intricate surgery to take place in a safe and controlled manner (Ghosh et al. 2009). This first successful deployment of CPB was by Gibbon et al in 1953 and since that early pioneering phase there has been a significant period of development and refinement of the CPB technique during which the major complications associated with the technique have been identified and suitable strategies to address these have been developed (Gibbon et al. 1953; Stoney 2009).

CPB is known to cause great changes in a patient's physiological fluid homeostasis which frequently results in accumulation of fluid in the interstitial space, more commonly known as edema (Hirleman et al. 2008). In the 1960s, early in the development of CPB as a technique, edema was identified as a key concern with the intervention (Moore et al. 2000). In the early days of CPB, the pulmonary edema observed in most patients was known as pump-lung and was more or less accepted as a consequence of the procedure with an ill-defined aetiology (Baue 1975). The major consequences of edema are the impairment of both tissue perfusion and oxygen transfer (Ziegler et al. 1971). Edema in paediatric patients undergoing cardiovascular surgery is of particular concern due to the propensity to develop severe edema and thus experience its more harmful consequences (Brans et al. 1981; Maehara et al. 1991; Jones et al. 2006). During the development of the paediatric CPB technique De Leval and Stark published several papers describing early experiences with CPB in the first year of life (De Leval et al. 1974; Stark et al. 1974; Singh et al. 1976; Stark et al. 1978; Stark et al. 1980).

Brans et al in 1981 produced a report on a study into the identification of body composition changes in a group of 16 paediatric patients undergoing CPB for congenital heart defects. They report that their data suggests infants and young children experience similar changes in body composition from CPB to those observed in adults and speculate that these changes have an increased likelihood of becoming fatal when the lack of strong homeostatic capabilities of paediatric patients is considered. This is especially true for those who exhibit severe congenital defects (Brans et al. 1981). Brans et al also report that there is a greater interstitial volume expansion observed in paediatric patients compared to adults, and suggest that this may be due

to the difficulty in producing effective priming volumes which are also sufficiently small so as not to cause overhydration. In support of this evidence, in 1991 Maehara et al reported that younger age and smaller patient size are risk factors for edema (Maehara et al. 1991).

As many as 54% (Seghaye et al. 1996) of neonatal patients undergoing cardiovascular surgery will exhibit generalised edema which has been shown to cause organ dysfunction and lengthen procedure times by increasing the need for circulatory and respiratory support and delaying chest closure (Neuhof et al. 2003; McGuinness et al. 2008).

Paediatric patients who exhibit low body weight, low haematocrit and low temperature and those who undergo lengthy surgeries have been show to be at increased risk of edema formation (Maehara et al. 1991). One report that demonstrates this is from Maehara et al in 1991 where 20 paediatric patients undergoing open-heart surgery were part of a prospective study into the validity of using bioelectric impedance as a non-invasive measure of total body water (TBW) (Maehara et al. 1991). Maehara et al found a number of associations between TBW rise and procedural and patient factors at different points of the investigation. Significant TBW rises at the end of CPB compared to preoperative levels, were related to total fluid balance rise and lower weight, whilst significant increases in TBW recorded immediately prior to leaving the operating theatre compared to preoperative levels were found to be impacted on by longer CPB procedures, patients who were shorter in height and those of younger age. Additionally, significant TBW rises observed in the ICU compared to preoperative levels were also related to younger age, longer CPB time and lower temperatures during CPB. The report concludes that smaller and younger paediatric patients and those who undergo CPB at lower temperatures and for a longer duration have a greater inclination to TBW accumulation or edema. Despite the fact that Maehara et al concede that the study involves a small heterogeneous population they claim that their clinical experience supports these findings (Maehara et al. 1991).

In addition to this evidence, the fact that paediatric patients naturally have smaller circulatory volume, more permeable microvasculature and less developed organ systems means they are predisposed to a higher degree of edema formation and the resulting damage to the tissue (Jones et al. 2006).

The specific circumstances explaining why CPB in paediatric patients so often leads to edema are also not completely understood, but it is thought to be the result of a combination of the inflammatory response and capillary leakage brought about by exposure of the blood to the artificial surfaces of the CPB circuit (Miyaji et al. 2008) (involving a number of cascades including the complement cascade (Westaby 1983; Butler et al. 1993), the extended subjection to hypothermic conditions and increased haemodilution (Ungerleider 2005). Prevention or

minimisation of CPB induced edema formation may therefore be achieved by addressing one or a combination of these three issues.

1.1.2. Devising Edema Therapies

In order to begin devising worthwhile and beneficial approaches to the minimisation of edema formation by any of these approaches, it is important to have a good understanding of the underlying physiological fluid dynamics involved. The Starling equilibrium of transcapillary exchange is a model of transcapillary fluid first described by EH Starling in 1896 (Starling 1896). It describes how hydrostatic pressure difference between the tissue and capillary driving fluid out of the capillary is resisted by the protein osmotic pressure difference between the tissue and plasma, and that it is the balance between these two forces, in tandem with the surface area and permeability of the capillary itself, which determines the net flux of fluid from the capillaries into the interstitial space (Hirleman et al. 2008). The Starling equilibrium of transcapillary exchange is written as (Hirleman et al. 2008):

$$\Delta IFV = K_f[(P_c - P_t) - \sigma(pCOP - tCOP)] - Q_L$$

Where ΔIFV is the change in volume of the interstitial fluid, K_f is a coefficient describing capillary filtration based in its permeability and surface area, P_c and P_t are the hydrostatic pressures of the capillary and tissue respectively, σ is a coefficient describing a given macromolecule's solute reflection, pCOP and tCOP are the colloid osmotic pressure of the plasma and tissue respectively and Q_L is lymph flow. CPB will likely have an impact on one or more of these variables and could, provided the changes are sufficient to promote fluid transfer from the capillary to the tissue, lead to edema.

The Starling equation corroborates two of the three aforementioned suspected routes of edema formation (inflammatory response and capillary leak brought about by exposure of the blood to the artificial surfaces of the CPB circuit, the extended subjection to hypothermic conditions, and increased haemodilution) since decreased plasma osmotic pressure (pCOP) or increased tissue osmotic pressure (tCOP), e.g. by haemodilution, and increased capillary permeability or surface area for filtration (Kf), e.g. by capillary leak, would both lead to an increased interstitial fluid volume (ΔIFV) (Hirleman et al. 2008).

In 2002 Heltne et al produced a report which aimed to *"improve our understanding of fluid pathophysiology and to explore the implications of the changes in determinants of transcapillary fluid exchange with and without hypothermia"* (Heltne et al. 2002). Using a large body of published data on the aforementioned variables of the Starling equilibrium in conjunction with

related changes in tissue fluid contents, lymph flow and fluid extravasation, and by using reasoned estimations for the remaining unmeasured variables, the study analysed the Starling equilibrium to determine the prominent factors in edema formation. The analysis concluded that in normothermic CPB, changes in the filtration coefficient (K_f) should be addressed to limit fluid extravasation. Whereas in hypothermic CPB, both filtration coefficient (K_f) and the capillary hydrostatic pressure (P_c) should be the focus.

It is clear from Heltne et al's report therefore that filtration coefficient K_f and thus the surface area and permeability of the capillary are the predominant factors for concern when looking to limit edema formation in CPB. It should be noted that, K_f varies greatly among tissues which may be one reason for the tissue specific edema formation seen in patients (Hirleman et al. 2008).

It is important to note here the impact of pulsatile and non-pulsatile flow on edema formation. Work by Prior, Gourlay and Taylor on the pulse reverse osmosis theory of fluid exchange found that *"fluid balance is achieved when the mean capillary pulse pressure is equal and opposite to the osmotic gradient, and that fluid exchange is the result of the pulsing of the capillary pressure"* (Prior et al. 1995). An imbalance between mean capillary blood pressure and osmotic gradient could therefore lead to hypertension, hypotension, edema and shock (Prior et al. 1996). Pulse reverse osmosis suggests that when mean capillary pressure is raised and osmotic gradient is normal, during systole there will be a greater volume of fluid forced out of the capillary than is drawn back during diastole, leading to an overall movement of fluid into the interstitial space (Prior et al. 1995; Prior et al. 1999). This is shown in Figure 1.

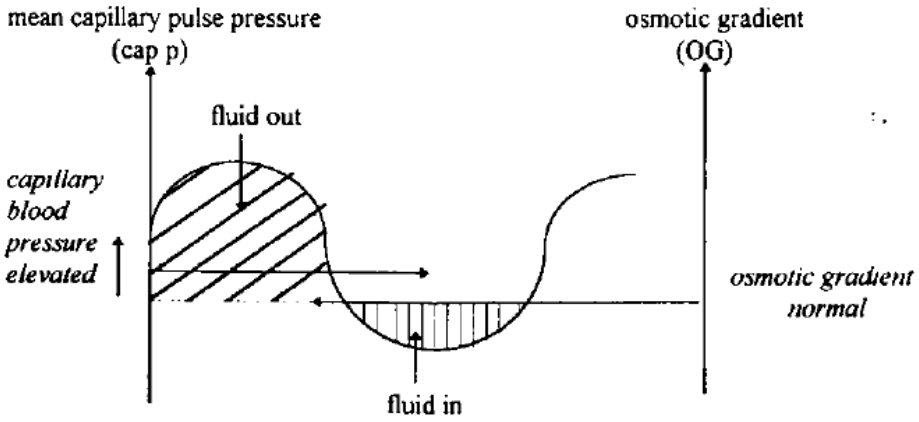


Figure 1: Edema formation according to pulse reverse osmosis (Prior et al. 1995)

The benefits of pulsatile flow over non-pulsatile flow in terms of fluid exchange, clearance and the controlling of edema formation were seen as early as 1938 when McMaster and Parsons,

utilising a rabbit model, demonstrated that lymph flow was greatly reduced with non-pulsatile flow and moreover that edema formation only occurred with the use of non-pulsatile flow (McMaster et al. 1938; Mongero et al. 2008).

1.1.2.1. Controlling Filtration Coefficient (K_f) and capillary leak

The importance of the role of K_f in edema formation is further highlighted when looking at capillary leak syndrome.

Capillary leak syndrome (CLS), is a syndrome characterised by generalised edema as a result of the microvasculature becoming more permeable to plasma proteins (Farstad et al. 2004) and is thought by many be to a significant factor in causing edema from CPB procedures, particularly in children (Hirleman et al. 2008).

Seghaye et al in 1996 performed a prospective study into the CPB related inflammatory reaction with respect to capillary leak syndrome (CLS) in a population of 24 neonatal patients, defining CLS as *"development of noncardiogenic generalized edema including pleural effusions or ascites (or both) and blood pressure instability necessitating volume substitution"*. Clinical outcomes showed that 13 of the 24 patients developed CLS representing an incidence of 54% (referred to earlier in *1.1.1. Aetiology and Pathophysiology*). Additionally, patients with CLS were found to have significantly lower serum concentrations of albumin during CPB, with serum levels of the proteins albumin, immunoglobulin G and α_2-macroglobulin dropping significantly within ten minutes of CPB being established. Reduced serum concentrations of these proteins are an indication of protein leak and thus increased permeability of the microvasculature. Additionally, patients with CLS had significantly higher liberation of the two microvasculature permeability mediators TNF-α and histamine indicating some form of perioperative capillary damage associated with the inflammatory reaction to CPB (Seghaye et al. 1996).

Causal factors of CLS and the associated systemic inflammatory response are thought to include surgical trauma, blood contact with the artificial surfaces of the extracorporeal circuit and reperfusion injury in previously ischemic areas, particularly the lungs, after bypass (Butler et al. 1993; Farstad et al. 2004). However, some groups have suggested that increased capillary leakage associated with hypothermic cardiopulmonary bypass may have a non-inflammatory mechanism as its cause (Farstad et al. 2004)

Farstad et al produced a report in 2004 which investigated three approaches to reducing cold-induced fluid extravasation in a group of 28 piglets (7 per group including a control). Each piglet in every group underwent an hour of normothermic CPB followed by 90 minutes of

hypothermic CPB. Farstad et al found that although the change in temperature did not increase haemodilution in the individuals, the fluid needs of individuals during the first 30 minutes of hypothermic CPB were nine times higher than at the end of normothermic CPB (Farstad et al. 2004). Farstad et al also found that this increased need was largely due to significantly increased fluid extravasation rates from the plasma to the interstitial space at a mean, which was indicated by rises in total body water seen at several tissue sites in every group. This evidence, in conjunction with the fact that there were no observed significant changes in albumin and protein masses, led Farstad et al to conclude that a non-inflammatory mechanism may be behind cold-induced fluid extravasation which leads to edema (Farstad et al. 2004).

The immune response associated with CLS involves activation of endothelial cells and neutrophils, increased complement and cytokine levels and initiation of the coagulation cascade (McGuinness et al. 2008). Reports have shown that following cardiovascular surgeries, edema can form in response to only modest increases in these mediators (Hirleman et al. 2008).

In 2002, Holmes et al investigated whether or not there was a correlation between the magnitude of inflammatory response seen in 29 adult CPB patients (mean age 63±11 years) and the adverse clinical outcomes witnessed thereafter such as bleeding, renal injury and pulmonary and cardiac dysfunction (Holmes et al. 2002). The investigation found that IL-6, IL-8 and C3a were independently associated with adverse outcomes, despite representing only a mild increase over the baseline levels. Furthermore, those patients deemed 'normal responders' (mediator levels below the group mean) were seen to experience adverse after effects, although to a lesser degree than the 'hyper responders' (mediator levels above the group mean). The report also concluded that patients who exhibited more severe inflammatory responses also suffered worse outcomes of CPB (Holmes et al. 2002).

If the inflammatory response is allowed to reach a severe level it will result in systemic immune response syndrome (SIRS), which can entail substantial edema along with serious morbidities such as acute respiratory distress syndrome and organ failure (Hirleman et al. 2008). The systemic inflammatory response is especially evident in paediatric patients in whom significant CLS and edema impair organ function and thus prolong recovery times (McGuinness et al. 2008). However, it is not yet known by which mechanism, or mechanisms, the immune response leads to increased capillary permeability or even if there is sufficient evidence to suggest a direct causal relationship (Hirleman et al. 2008). As a consequence, it is difficult to devise therapies focussed on controlling capillary permeability.

As mentioned earlier (*1.1.1. Aetiology and Pathophysiology*), exposure to the artificial surfaces of the CPB ultrafiltration circuits may be a root cause of the inflammatory response seen (Butler et

al. 1993; Farstad et al. 2004). Several studies have been carried out attempting to reduce the inflammatory response to artificial surfaces by coating them with biocompatible substances. In 2004, Jensen et al produced a randomised prospective trial of 40 paediatric patients undergoing CPB in order to determine whether use of biocompatible surfaces in a CPB circuit would result in less haemostatic activation (Jensen et al. 2004). The 40 patients were divided into two groups, one using a fully coated heparin system with a centrifugal pump in a closed circuit and the other using a system with non-coating, a roller pump and a rigid venous reservoir (Jensen et al. 2004). Four chemicals were measured: *"plasma thrombin- antithrombin (TAT), D-dimer, tissue plasminogen activator antigen (t-PA ag), and the complex consisting of tissue plasminogen activator and its inhibitor plasminogen activator inhibitor-1 (t-PA-PAI-1)"* (Jensen et al. 2004). The group found that there was significantly less fibrinolytic activation in the heparin-coated group. Furthermore there was a trend, but not statistically significant, between the biocompatible surface group and reduced d-dimer and TAT levels (Jensen et al. 2004). No trend was found with regards to t-PA-PAI-1. Jensen et al concluded that their heparin-coated system was associated with diminished fibrinolytic activation levels.

In the same year, Ueyama et al showed that PMEA surface coatings resulted in reduced levels of complement and c-reactive protein (CRP), a blood protein of which levels rise as an inflammatory response (Ueyama et al. 2004). Ueyama et al's prospective randomised study involved comparing the effectiveness of PMEA-coated, heparin-coated and uncoated perfusion circuits in 30 patients (10 patients per group) aged 42-74 years undergoing elective cardiac surgery (Ueyama et al. 2004). They found that post-CPB IL-6 levels were significantly higher in the non-coated group and that maximum CRP levels were significantly lower in the PMEA coated group when compared with the non-coated group (Ueyama et al. 2004). Ueyama et al also investigated the impact of biocompatible coating on the patients' respiratory function by measuring the alveolar-arterial exchange gradient and found that it was significantly lower (indicating improved oxygen transfer) in both the heparin and PMEA coated groups three hours post-CPB when compared with the non-coated control. As Ueyama et al conclude, this may indicate that PMEA coatings not only improve inflammatory response, but that they also improve respiratory function (Ueyama et al. 2004).

Ninomiya et al had previously demonstrated the beneficial impact of PMEA coating in their 2003 prospective randomised trial comparing PMEA-coated and non-coated circuits in a population of 22 adult patients (Ninomiya et al. 2003). They produced a prospective randomised clinical study investigating perioperative systemic inflammatory response, respiratory function and platelet levels (Ninomiya et al. 2003). In order to achieve this, the group recorded concentrations of C3a, polymorphonuclear- elastase (giving an insight into leukocyte activation) and IL-6 (related to acute phase inflammatory response) as well as platelet preservation

(Ninomiya et al. 2003). Their results showed that the PMEA-coated group exhibited significantly lower maximum levels of both C3a and polymorphonuclear- elastase but no significant difference between the groups in terms of IL-6 (Ninomiya et al. 2003). Platelets were significantly better preserved up until one hour post-CPB initiation but not beyond (Ninomiya et al. 2003). Due to these demonstrated benefits, Ninomiya et al concluded that PMEA-coated circuits were superior to un-coated alternatives (Ninomiya et al. 2003).

However, a more recent prospective randomised study by Kirshbom et al comparing the effectiveness of SMART-coated, PMEA-coated and uncoated circuits in a larger cohort of 69 paediatric patients undergoing first-time cardiac surgery found no significant difference between the groups in terms of postoperative bleeding, platelet counts, β-thromboglobulin levels or any thromboelastographic measures of platelet function (Kirshbom et al. 2006). The 69 patients, median age 6 months, were randomly assigned to one of three groups: a non-coated control group (n=22), a PMEA-coated group (n=24) or a SMART-coated group (n=23). The SMART coating is in actuality a *"combination of phosphorylcholine coating with polycaprolactone-polydimethylsiloxilane additives to the base polymer resin"* (Kirshbom et al. 2006). The group measured platelet count, thromboglobulin levels, and thromboelastography (with and without the platelet aggregation inhibitor abciximab) from blood samples collected at five time points (Kirshbom et al. 2006). The time points were: immediately after anaesthesia ('baseline'), five minutes after commencing CPB ('on-CPB'), between rewarming and weaning ('end-CPB'), five minutes after protamine was administered ('post-CPB') and on the morning following the procedure, between 18 and 24 hours later ('post-op day 1') (Kirshbom et al. 2006). In addition to these measurements, blood product use and post-op chest tube outputs were studied (Kirshbom et al. 2006). Results of the study showed that there was no significant difference between any of the groups in terms of: blood product use, time to extubation, CPB time, minimum CPB temperature, aortic cross-clamp time, post-op chest tube drainage or hospital stay. Furthermore with regards to blood sample measures, there was no significant difference between the groups in terms of platelet counts, β-thromboglobulin levels or platelet function measured by thromboelastograph (Kirshbom et al. 2006). The only significant difference between the groups found by Kirshbom et al was that at 'off-CPB' without abciximab the R-value, which represents the platelets' ability to aggregate in response to contact factors (Kakishita et al. 1989), was significantly lower in the non-coated control group than in the PMEA-coated or SMART coated groups (Kirshbom et al. 2006). Kirshbom et al explain that this suggests that clots could form more rapidly in the non-coated control patients after CPB weaning than in the coated groups (Kirshbom et al. 2006). Due to the distinct lack of significant differences found between the three groups, Kirshbom et al conclude that surface modified CPB circuits present no clinical benefit to paediatric patients. They suggest that advantages seen in adult populations *"may not translate to the paediatric population"* (Kirshbom et al. 2006).

Protection from exposure to artificial surface by means of biocompatible surface coatings is clearly not of uncontested benefit, particularly in the paediatric setting. However, since the PMEA-coated circuits are a third cheaper than heparin coated circuits and no more expensive than the uncoated circuits (Miyaji et al. 2008), it is difficult to see why clinicians would not utilise PMEA coatings unless they remain unconvinced of their efficacy.

It is clear therefore that due to incomplete understanding of the mechanisms involved, attempts to prevent or mitigate the consequences of the systemic inflammatory response are difficult to achieve. Addressing the problem of haemodilution may the next logical approach to mitigating adverse outcomes.

1.1.2.2. Controlling Colloid Osmotic Pressure (pCOP and tCOP) and Haemodilution

Haemodilution is an unavoidable consequence of even best current paediatric CPB procedures due to the disparity between the size of the patient's natural cardiopulmonary system and the CPB circuit. A crystalloid prime is used to dilute the patient's blood (generally at a prime to blood ratio of 2:1 or even 3:1 (Elliott 1993)) in order to maintain an adequate circulating volume, mitigate increases in blood viscosity from induced hypothermia, reduce red blood cell aggregation and increase cerebral blood flow velocity (Gruber EM et al. 1999; Jones et al. 2006). However, haemodilution leads to a perfusate which has a lower haematocrit, colloid osmotic pressure, clotting factor, serum albumin and serum protein content (Hirleman et al. 2008). Haemodilution has been shown to be a precipitate to edema formation by decreasing plasma colloid osmotic pressure (COP) by at least 25-50% (Hindman et al. 1990) thus encouraging movement of fluid into the inter- and intra-cellular space (Jones et al. 2006). Continued fluid accumulation in these areas can lead to impaired organ function (Jones et al. 2006).

Gourlay et al demonstrated a linear relationship between inflammatory response and both haemodilution and circuit surface area (Gourlay et al. 2001; Gourlay et al. 2003). In 2001 Gourlay et al demonstrated that in a cohort of rats exposed to different surface areas of PVC in extracorporeal circuits, those who had been exposed to the largest surface area had significantly ($p<0.001$) higher expression of CD11b (an adhesion molecule on neutrophils) than those exposed to the smallest surface area (Gourlay et al. 2001). Additionally, in 2003, Gourlay et al used a second cohort of rats this time at different mean induced haematocrit and found that rats which had the lowest Hct had a significantly ($p<0.05$) higher percentage change in CD11b expression after biomaterial contact compared to rats with higher Hct (Gourlay et al. 2003).

Attempts have been made to mitigate the adverse effects of haemodilution, mostly focused on reducing prime volumes emanating from recent successes in creating miniaturised bypass systems which have been shown to not only reduce haemodilution but also postoperative bleeding and the need for blood constituent therapy (Perthel et al. 2007a; Perthel et al. 2007b; Miyaji et al. 2008).

Perthel et al demonstrated that mini bypass systems used routinely in extracorporeal circulation were associated with a reduction in blood product usage (Perthel et al. 2007a). The prospective randomised study of 60 patients compared control (conventional bypass, n=30) and mini bypass (n=30) groups in terms of postoperative bleeding and blood product usage - fresh frozen plasma and homologous blood from operating room (OR) to intensive care unit (ICU). The group found that in the mini bypass group there was a statistically significant reduction in homologous blood product use compared with the control (Perthel et al. 2007a). They found that not only were there fewer occurrences of blood product requirement, but that in the mini bypass group smaller volumes were necessary (Perthel et al. 2007a). This was reflected with fresh frozen plasma (FFP) requirements with three patients in the control group requiring at least one unit of FFP whereas in the mini bypass group there was no requirement (Perthel et al. 2007a). Additionally, Perthel et al saw that there was a significant reduction in cumulative postoperative bleeding during ICU stay (Perthel et al. 2007a). Based on these positive results Perthel et al concluded that mini bypass systems, which by their nature aim to reduce priming volumes, are a "favorable alternative to conventional ECC in all revascularization cases" (Perthel et al. 2007a).

In another publication in the same year, Perthel et al present the results of the earlier paper but this time in conjunction with figures for gaseous microemboli (GME) activity (which alongside impairment of neurocognitive function forms an important part of post-CPB morbidity and mortality) (Perthel et al. 2007b). Perthel et al found that there was a significantly smaller GME volume in the mini bypass group compared with the conventional bypass control group but only in the post-arterial filter section of the circuit (Perthel et al. 2007b). Perthel et al thus concluded that mini bypass systems could reduce exposure to GME (Perthel et al. 2007b).

In 2008, Miyaji et al produced a report investigating whether or not their miniaturised bypass system was capable of reducing the perioperative systemic response in neonatal and infant patients (Miyaji et al. 2008). The study involved retrospectively viewing 80 consecutive patients' inflammatory response by measuring body weight gain as a percentile of body weight %BWG, postoperative mechanical ventilation in days (MVT) and blood constituents including postoperative C-reactive protein (P-CRP), white blood cell (WBC) and platelet counts (PLT). Their results showed that those patients who underwent CPB using a conventional system

exhibited significantly higher %BWG, MVT and P-CRP when compared with those using the mini bypass system in which the priming volume had been reduced from 500ml to 140ml (Miyaji et al. 2008). The group also found that MVT was most influenced by a combination of priming volume and age, %BWG was most influenced by priming volume and bypass time and P-CRP was most influenced by priming volume alone (Miyaji et al. 2008).

Another approach to CPB that aims to reduce the priming volume is retrograde autologous priming (RAP). RAP is a technique in which the crystalloid prime is removed from the pump system at the initiation of bypass in lieu of the patient's own blood column (Rosengart et al. 1998). Rosengart et al investigated the effectiveness of RAP in a 1998 prospective randomised study involving 60 first time CPB patients. They found that RAP was associated with significantly more 'lowest Hct values', significantly fewer intraoperative transfusion occurrences and significantly fewer homologous red blood cell transfusions during the entire hospital stay. The group concluded that RAP is a "safe and effective means of significantly decreasing haemodilution and the number of patients requiring red cell transfusion during cardiac operations" (Rosengart et al. 1998).

Reducing prime volumes has been clearly demonstrated as being advantageous to the clinical outcomes of paediatric patients undergoing CPB. However, there is only so far the prime volume can be safely reduced with currently available technology, thus, there is still a need for complimentary techniques which alleviate the problems brought about by haemodilution. A further system, which aims to alleviate the problem of haemodilution brought on by CPB but without attempting to reduce priming volumes as its driving method, is modified ultrafiltration.

1.2. Conventional Ultrafiltration

In order to understand modified ultrafiltration it is important to have a good comprehension of its predecessor, which became referred to as 'conventional' ultrafiltration (CUF).

CUF works on the basis of Ultrafiltration (UF) which is a convective mass transfer process in which a hydrostatic pressure gradient is applied across a semi-permeable membrane in order to selectively remove water and low-molecular-weight solutes from a fluid (Wang et al. 1996). A diagrammatical representation of pressure driven ultrafiltration is shown in Figure 2.

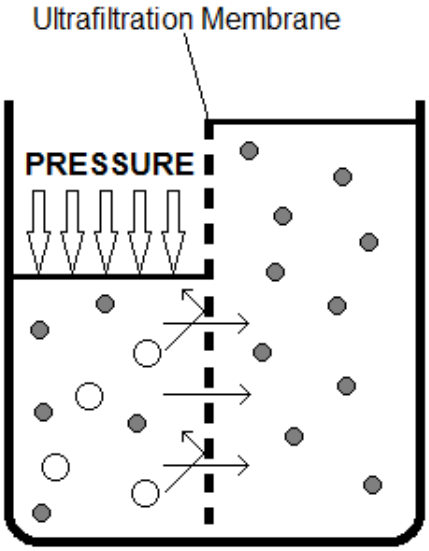


Figure 2: Pressure driven ultrafiltration separation process

In this way, plasma water (along with any low-molecular weight solutes) can be separated from the formed elements of the blood and thereby reversing haemodilution in that, as the procedure take place, the volume of these formed elements increases relative to the volume of the remaining plasma water.

This relates to perhaps the most important measure of the performance of CUF, haematocrit (Hct). Haematocrit (also known as Packed Cell Volume or PCV) is the measure of the percentage of a given volume of blood which is comprised of cells, the majority of which are red blood cells (Burton 2000). The normal levels of which are approximately 46% for men and 41% for women (Burton 2000). Hct is a useful measure of how diluted a patient's blood has become, particularly post-CPB, and thus can be used to measure how well a given ultrafilter or ultrafiltration technique re-concentrates the blood to more normal levels.

The CUF procedure involves UF during the rewarming phase of CPB with the ultrafilter inlet positioned distal to the oxygenator and the outlet positioned in the venous reservoir (Wang et al. 1996). Once flow through the ultrafilter has been established, a negative pressure is applied across the membrane by use of a vacuum pump or similar apparatus (Naik et al. 1991a). The blood/prime mixture in the bypass circuit is thus filtered whilst the patient is still on bypass in an attempt to return Hct back towards pre-operative levels (Elliott 1993). Figure 3 shows the CUF circuit and the position of the ultrafilter.

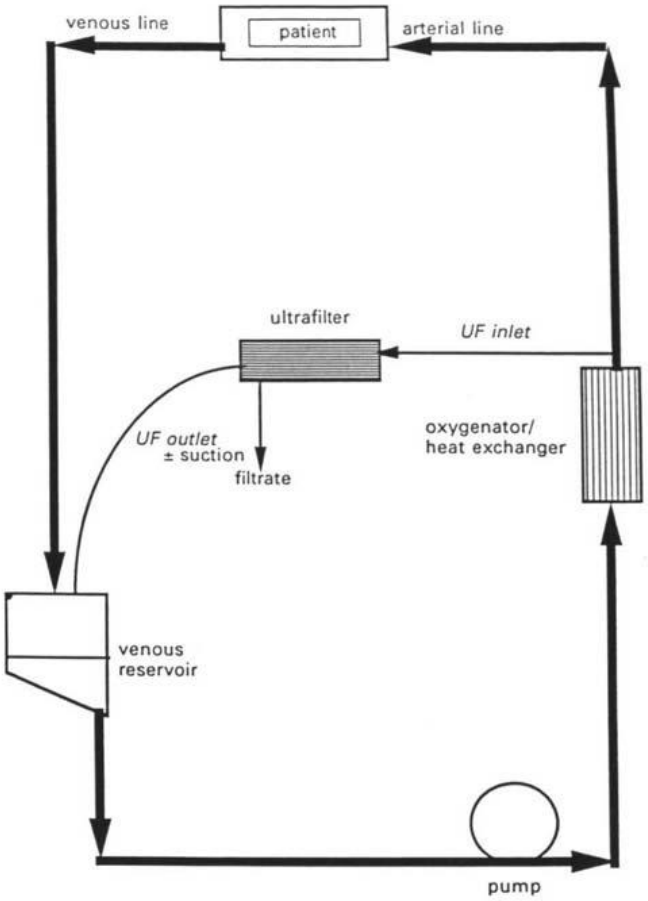


Figure 3: Position of ultrafilter in CUF (Naik et al. 1991a)

CUF continues (continuously or intermittently) until the volume of blood in the venous reservoir reaches its safe minimum level, at which point the patient is weaned from bypass with the arterial and venous lines being clamped, isolating the ultrafilter and preventing further use in the procedure (Naik et al. 1991a).

The term 'conventional' ultrafiltration was retrospectively coined following Naik, Knight and Elliott's modification of the technique which in turn became known as 'modified' ultrafiltration (MUF) (Naik et al. 1991a).

The earliest description of the UF as a treatment for edema reduction was by Lunderquist et al in 1952. The paper was part of a series describing 'The Artificial Kidney' and describes human patients with renal failure undergoing a treatment in which *"the application of negative pressure outside the tubing acts as an ultrafilter i.e. ultrafiltrate is sucked from the blood which flows through the tubing"* (Lunderquist 1952). This technique is recognisable as a loose description of the UF procedure.

After demonstrating its value in renal failure treatment, UF was first used for bypass management in adult patients in the 1970s (Elliott 1993).

Romagnoli et al in 1976 were the first (Moore et al. 2000) to report the use of UF in cardiac surgery. In their study UF was successfully utilised after CPB in a cohort of 24 paediatric patients aged 2-7 years with an average increase in haematocrit from 21% to 31% (Romagnoli et al. 1976).

Following early work from Magilligan (as described in a later publication) (Magilligan 1985) CUF became a subject of interest for a number of research groups to study (Elliott 1993).

Walpoth et al in 1979 produced a report on their investigation into the reduction of post-bypass haemodilution by UF. Five dogs underwent one hour of CPB during which their Hct reduced below 20%. Subsequently 1L of fluid from the bypass circuit was ultrafiltered for 16mins. Hct rose significantly from 19% - 29% on average with no deleterious alterations in haemodynamics, osmolality or electrolyte balance (Walpoth et al. 1979).

In 1982, Vertrees et al described their intra-operative method of haemoconcentration by UF. The group state that in five procedures an average of 6L of ultrafiltrate was removed with 500cc of the patients own blood, reconcentrated to 55% Hct, being returned to them (Vertrees et al. 1982).

Klineberg et al produced a report in 1984 describing their experience with haemoconcentration by UF in two case study patients. The first was a 42 year old woman with anuric renal failure, congestive cardiac failure and peritonitis, and the second a 48 year old woman with rheumatic aortic and mitral valve disease. In both cases UF was used during bypass with 850ml and 1000ml ultrafiltrate removed from the first and second patients respectively. Hct was improved by UF in both cases and Klineberg et al recommended the procedure for patients with cardiac and/or renal failure who are likely to undergo CPB for longer than one hour (Klineberg et al. 1984).

In 1984, Magilligan et al reported on two studies related to UF during CPB, the first being an animal study laboratory evaluation involving two groups of mongrel dogs, and the second being the clinical experience of the group with regards to ten patients undergoing UF during CPB. In the animal study, there were two groups; in one dogs underwent 2 hours of empty beating heart CPB, and in the other dogs underwent 90 minutes of cold cardioplegic arrest followed by 30 minutes of recovery time. Animals in both groups that received ultrafiltration exhibited less accumulation of extravascular lung water. The group describe clinical experience of ten patients

undergoing CPB with ultrafiltration with an average of 3420ml of fluid removed and a significant decrease in extravascular lung water from 1132 ± 183ml to 919 ± 267ml on average. Magilligan et al describe UF as *"a safe, effective means of removing body water and of preventing further accumulation of such water during haemodilution CPB"* (Magilligan et al. 1984).

Morgan et al described a case study in 1985 in which they utilised UF in a preoperative setting to control edema in a 60 year old patient who was too ill to undergo cardiac surgery. UF was administered for four hours on three consecutive days resulting in a net weight loss of 7kg. Morgan et al state that *"her clinical condition improved considerably and was accompanied by a pronounced reduction in cardiac size on chest radiograph"* and subsequently she was able to undergo a successful tricuspid valve replacement. Morgan et al report that they have used the same technique in over 30 patients with no complications and that they have also successfully used CUF during CPB (Morgan et al. 1985).

Coraim et al used UF as a postoperative treatment for severe acute pulmonary failure in 36 cardiac surgery patients. UF was administered continuously for 24 hours and patients showed greatly decreased levels of cardiopulmonary toxic substances which are partly responsible for shock, leading to improved haemodynamic and respiratory function (Coraim et al. 1986).

Nakamura et al compared CUF with 'Cell Saver' a centrifugal type of blood concentrator. Each haemoconcentrator was used in a group of six patients undergoing CPB. Nakamura et al found that despite the fact that both groups successfully raised Hct, patients treated with cell saver exhibited significantly lower total protein serum concentrations, including fibrinogen and immunoglobulin. Moreover, the group state that platelets levels were better preserved by CUF. This led Nakamura et al to describe CUF as *"preferable for hemoconcentration in open heart operations performed without transfusion of donor blood when the patient has neither severe hemolysis nor preoperative renal dysfunction"* (Nakamura et al. 1990).

During this period in the 1980s, CUF transitioned from use in isolated academic centres to more generalised clinical practice and, as a consequence, became more commonly used during paediatric bypass surgery (Elliott 1993; Moore et al. 2000).

However, Naik, Knight and Elliott et al were unconvinced of its efficacy and consistency, stating that *"there did not appear to be any subjective difference in the degree of edema present postoperatively between patients subjected or not subjected to ultrafiltration"* (Elliott 1993). This scepticism was partly due to the natural limitations of the technique, namely that the amount of fluid which can be removed is restricted by the need to maintain a safe minimum level in venous reservoir in order to ensure adequate arterial inflow (Naik et al. 1991a; Thompson et al. 2001;

Perez-Vela et al. 2008). This is of particular concern in paediatric patients for whom minimum CPB prime volumes are used, the safe minimum level for paediatric patients can be 25% of cardiac output (Naik et al. 1993). Theoretically, as filtrate is extracted from the bypass circuit, accumulated fluid from the "third space" (areas of physiologically non-functional fluid accumulation such as the interstitium (Seifter et al. 2005)) should replace it so that effectively the fluid is being removed from potentially problematic areas (Naik et al. 1993). However, the rate of transfer between the third space and the intravascular space often lags behind the rate of fluid extraction from the bypass circuit by the ultrafilter meaning that the venous reservoir level quickly drops below the 'safe minimum' level and either fluid must be added to 'top up' the venous reservoir (negating the purpose of CUF), or CUF must be halted (Naik et al. 1993). Naik, Knight and Elliott et al began seeking to modify the method to improve upon its performance and consistency.

1.3. Modified Ultrafiltration

1.3.1. The Emergence of MUF

Modified ultrafiltration (MUF) was first described by Naik, Knight and Elliott of The Hospital for Sick Children, Great Ormond Street, London in 1991. Their report concerned a pilot study intended to assess modifications to the 'conventional' ultrafiltration circuit in terms of reducing total body water rise and elevating haematocrit (Naik et al. 1991a). The modifications made involved changing the positioning and operation time of CUF. In CUF, the haemofilter is placed between the arterial line and the inlet of the venous reservoir (Wang et al. 1996) whereas Naik, Knight and Elliott repositioned it so that the inlet cannula was in the arterial line and the outlet was at the venous line. Additionally, ultrafiltration was performed immediately after the cessation of CPB rather than during it.

Naik, Knight and Elliott describe their dissatisfaction with CUF and in their study show that it *"consistently failed to lower the rise in TBW after CPB and to elevate the postoperative Hct"*. They concede that in this study CUF did significantly reduce the total body water (TBW) rise in some patients but attest that it was not efficient, reproducible or uniform.

In Naik, Knight and Elliott's pilot study, data was recorded on 21 patients in need of surgery with CPB. The patients were not randomized and were instead allocated to a control group (n=6), a CUF group (n=7) and a MUF group (n=8) in sequence. The patients' age, weight, prime volume, bypass time and bypass temperature were all recorded and the groups comparatively similar on these characteristics but no test of statistical significance was performed.

Plasma osmolarity, total body water, haematocrit (Hct), plasma electrolytes, urine output, fluid balance, mean corpuscular volume and mean corpuscular haemoglobin concentration were each recorded at two or more of the following intervals in order to assess any changes in these variables; Induction of anaesthesia, on-bypass, off-bypass, 20mins after CPB, four hours after CPB. Specific points of investigation for each variable are described in the report.

In this original description of MUF, the ultrafilter was placed with the inlet connected to the arterial line and the outlet connected to the venous line with filtration beginning immediately after the end of CPB. In order to achieve this, the ultrafilter was a part of the bypass circuit throughout CPB but was isolated from use by clamping its inlet until the patient was deemed sufficiently haemodynamically stable post-bypass. At this point the clamp at the ultrafilter inlet was removed and instead the venous line was clamped between the venous reservoir inlet and the ultrafilter outlet. Thus blood was redirected from arterial line (both from the patient and from the venous reservoir) through the ultrafilter to the patient's venous line. Blood was accelerated through the ultrafilter by a secondary pump at approximately 200-300mlmin^{-1} to achieve a filtration rate of approximately 100-150mlmin^{-1}. The blood being pumped from the venous reservoir is done so at a rate which would maintain a constant preload. This rate was assessed using the mean arterial and right atrial pressures. In order to keep the circuit primed, blood removed from the venous reservoir was replaced by clear fluid which also allowed all the available red cells within the circuit to be passed through the ultrafilter and back to the patient. Additionally, by keeping the circuit primed, CPB could be restarted at any time if resumption was required in an emergency situation. MUF was continued until Hct reached 0.4 or there was no more blood within the bypass circuit.

Attempts to make the CUF and MUF procedures as similar as possible were made. The same Gambro 66 ultrafilter was used for both 'conventional' and 'modified' ultrafiltration. The same transmembrane pressure gradient of -125mmHg was applied across the ultrafilter using a DeVilbliss Vacu-Aide 721 vacuum pump although during CUF a secondary roller pump at the ultrafilter inlet was not used. Figure 4 shows modified positioning of the ultrafilter during CPB and MUF.

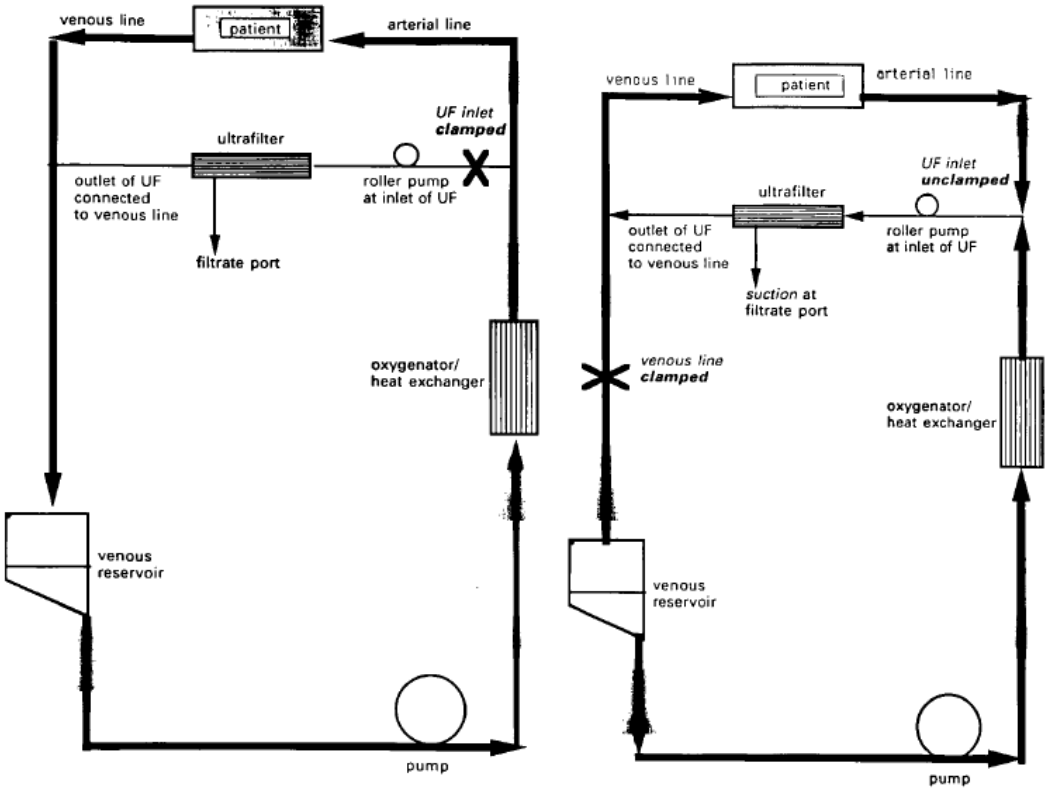


Figure 2a Modified placement of the ultrafilter in the CPB circuit during CPB

Figure 2b Modified placement of the ultrafilter in the CPB circuit after CPB, during ultrafiltration

Figure 4: Placement of ultrafilter in MUF as shown in Naik, Knight and Elliott's original description (Naik et al. 1991a)

The results of Naik, Knight and Elliott's study show that all patients within the pilot study survived the surgery and left hospital in good condition with no complications being attributed to the ultrafiltration process. The study showed that MUF resulted in a significantly larger filtrate volume, CUF produced a median filtrate volume of 37.3mlkg^{-1} (range: 23.5-65.4 mlkg^{-1}) whereas MUF produced a median filtrate volume of 107.2mlkg^{-1} (range: 53.3-133.0mlkg^{-1}).

There was a significantly smaller percentage rise in total body water (TBW) between the induction of anaesthesia and four hours postoperatively in the MUF group compared with the control group and the 'conventional' ultrafiltration group.

In terms of Hct, no significant difference between the three groups was found preoperatively, on bypass (recorded as the lowest Hct measured during CPB) or immediately after CPB. This is despite the fact that in the MUF group, patients do not begin ultrafiltration until after CPB whereas in the CUF group, patients' blood was passing through the ultrafilter throughout the procedure. Naik, Knight and Elliott also report that there was *"a marked increase in Hct in the modified ultrafiltration group following filtration to an Hct of 0.41 (0.35-0.50)"*.

In each of the remaining variables measured in this study (osmolality, plasma electrolytes, urine output, mean corpuscular volume or mean corpuscular haemoglobin concentration) no significant difference was found between the control, CUF or MUF groups. However, only the plasma osmolality data is presented.

When discussing their findings Naik, Knight and Elliott describe the beneficial attributes of MUF. Firstly it overcomes a major flaw in CUF in that it is not limited by the volume of the venous reservoir. They explain that in CUF the venous reservoir operates at a volume very close to the lowest safe acceptable level in an attempt to minimise priming volumes. This means that any reduction in fluid volume in the venous reservoir to below this lowest safe acceptable level must be corrected by the addition of clear fluid, thus negating the beneficial work of the ultrafilter. Secondly, as mentioned earlier (*1.1.2.2. Controlling Colloid Osmotic Pressure (pCOP and tCOP) and Haemodilution*), the entire CPB system is kept primed meaning that the patient can be returned to CPB in an emergency situation but also that almost all of the patient's blood passes through the ultrafilter at some point and so is reconcentrated and returned to the body. Thirdly, since MUF is based on the separation of the CPB and ultrafiltration procedures, perfusionists are able to dedicate their attention to each in turn, rather than overseeing a single, more complicated combined procedure.

In addition to these benefits, Naik, Knight and Elliott list the potential shortcomings of the MUF procedure. Firstly, performing ultrafiltration in the 10 minutes after CPB is complete means that the surgeon must wait that much longer before decannulation. This could be potentially problematic if the cannulae are causing an obstruction but otherwise it is possible to make use of this period for achieving haemostasis. There is also the risk of haemodynamic instability resulting from an inadequate rate of return of venous reservoir fluid through the ultrafilter to the patient but this can be easily avoid by maintaining a constant preload. Secondly, the path of blood from the arterial line to the venous line via the ultrafilter is equivalent to an 'arteriovenous shunt'. Naik, Knight and Elliott claim to have not found any adverse effects of this and in fact suggest that it may be advantageous in preventing pulmonary hypertensive crises by delivering warm, oxygenated blood to the pulmonary circulatory system. Thirdly, they introduce the possibility of intercompartmental fluid shifts in response to the way MUF rapidly extracts large volumes of blood but suggest that since the osmolality, mean corpuscular volume and mean venous concentration did not change in this study that the majority of fluid movement may in fact be from the interstitial fluid compartment rather than the intracellular or intravascular. They propose on-line measurements of intra- and extracellular volumes by bio-electrical impedance techniques in order to confirm this.

Further implications for CPB management in children are detailed by Naik, Knight and Elliott including the possible reduction of water accumulated in the patient after CPB which is associated with tissue edema. Additionally they suggest that, barring any unexpected and significant blood loss, the Hct increase following CPB that MUF provides would greatly reduce the need for donor blood transfusions to maintain adequate oxygen delivery. Naik, Knight and Elliott suggest that this may be the first step towards surgeries with no transfusions necessary, or *"total bloodless cardiac surgery"*, in all patients. They also suggest that since MUF demonstrated the ability to readily change Hct it will be possible to investigate success of CPB at different Hct levels in order to determine if there is an optimum or lowest possible Hct level to be worked to. Finally they propose the possibility that MUF may remove vasoactive substances and 'toxic peptides' present in the blood and thus may alter the course of capillary leak and thus possibly reduce the need for inotropic agent and ventilator support interventions.

Later that same year Naik, Knight and Elliott published "A prospective randomized study of a modified technique of ultrafiltration during pediatric open-heart surgery" designed to confirm the findings of the initial pilot study with statistically robust results from a prospective and randomised study (Naik et al. 1991b). The study was on 50 patients undergoing open-heart surgery. It compared MUF (n=24) against a control group (n=24) who did not undergo any filtration at all; there was one death in each group. There was no 'conventional' ultrafiltration group unlike in the pilot study. The MUF procedure was performed as in the pilot study, starting 10 minutes after the finishing point of CPB until the patient reached a Hct of 36-42. The results of this second trial reflected the findings of the first. MUF resulted in significantly less blood loss, a significantly bigger percentage rise in total body water, significantly less transfusion requirements for blood and colloid, (Naik et al. 1991b). Moreover, there was a significantly higher percentage rise in both systolic and diastolic blood pressure.

CPB has also been shown to have a detrimental effect on cardiovascular function; affecting myocardial thickness and contractility (Raja et al. 2006), pulmonary function; resulting in poor alveolar gas exchange, pulmonary compliance, pulmonary hypertension sometimes culminating in death (Butler et al. 1993; Asimakopoulos et al. 1999; Huang et al. 2003), neurological function (Skaryak et al. 1995; Elliott 1999b) and coagulation (Andrew et al. 1988; Kern et al. 1992; Tempe et al. 2002).

Since Naik, Knight and Elliott's original study, MUF has been the subject of many further investigations to assess its efficacy in terms of a large range of patient outcomes, including; cardiovascular function, pulmonary function, neurological function, total body water, transfusion requirements, coagulation and length of intensive care unit (ICU) stay.

1.3.2. A note on Arterio-venous and Veno-Venous configurations

There are two widely used configurations of MUF which can be employed. The first, the original configuration as described by Naik, Knight and Elliott, is the Arterio-Venous (A-V) configuration. In this configuration, blood leaves the patient via the arterial line positioned in the aorta, passes through the ultrafilter, and is returned to the patient via the venous line at the right atrium (Naik et al. 1991a; Onoe et al. 2001). An illustration of the A-V MUF configuration can be seen in Figure 5.

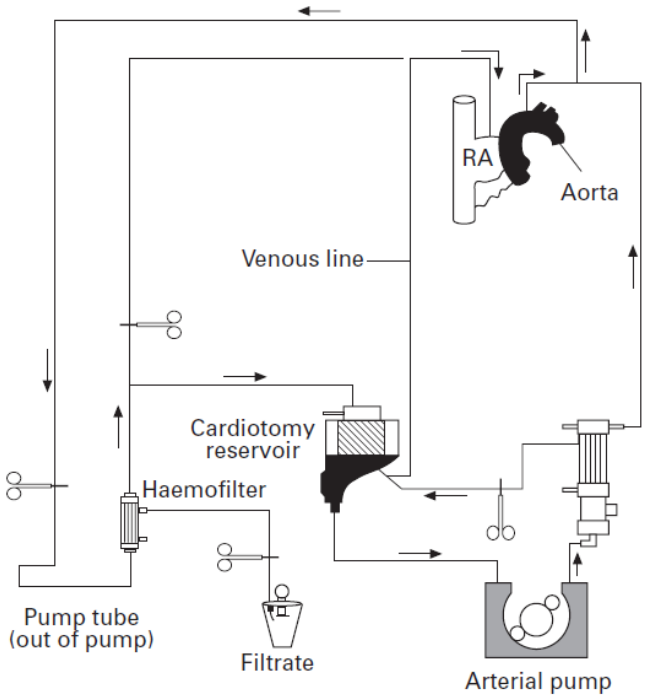


Figure 5: A-V MUF configuration (Elliott 1999a)

This configuration can be viewed as an 'arteriovenous shunt', though no evidence of this being problematic has been found (Naik et al. 1991a).

The second configuration is the Veno-Venous (V-V) configuration. V-V MUF avoids the use of the arterial line and instead blood leaves the patient via the venous cannula positioned in the inferior vena cava, passes through the ultrafilter and, as with A-V MUF, is returned to the patient at the right atrium (Bando et al. 1998a; Kiziltepe et al. 2001). An illustration of the A-V MUF configuration can be seen in Figure 6.

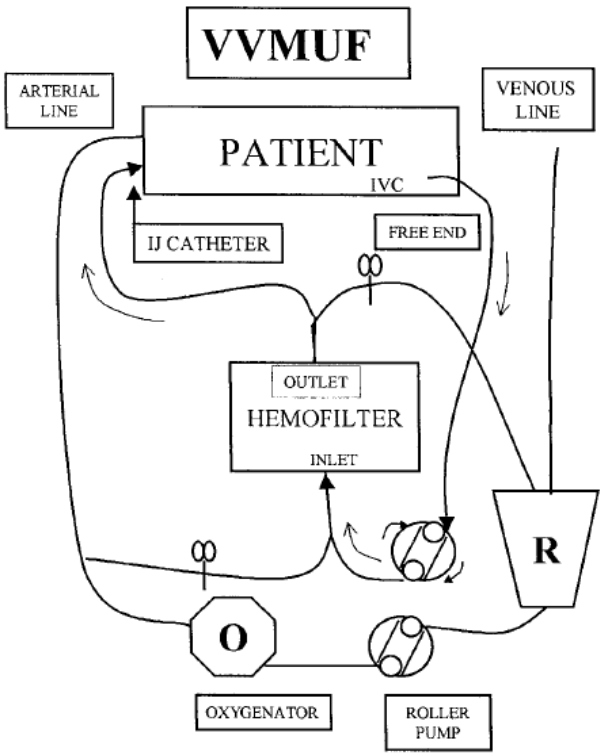


Figure 6: V-V MUF configuration (Kiziltepe et al. 2001)

Theoretically, there should be no reason for either technique to perform significantly better than the other in terms of efficiency or safety (Kiziltepe et al. 2001), however A-V MUF is the more commonly utilised technique, Darling et al reported it being used in 86% of centres in North America (Darling et al. 1998).

There is a large body of research into the efficacy and impact of MUF pertaining to various clinical and patient factors including; cardiovascular function, pulmonary function, neurological function, total body water, transfusion requirements, coagulation and length of intensive care unit (ICU) stay. What follows is summary and discussion of the research evidence available regarding the impact of MUF on these factors. For each factor, the nature and outcomes of various research papers are first tabulated and then the corroborating or conflicting evidence is discussed.

1.3.3. Impact of MUF on Cardiovascular Function and Haemodynamics

Author (Year)	Patient type	Study Type	Intervention	Impact on CV Function and Haemodynamics
Hodges (1994)	Paediatric	Prospective Single arm	A-V MUF (n=10)	↑ cardiac index ↑ systemic arterial pressure
Davies (1998)	Infant	Prospective randomised	A-V MUF (n=11) vs no UF (n=10)	↓ left-ventricular posterior wall thickness ↓ myocardial cross-sectional area ↓end-diastolic pressure seen ↓ inotropic drug use ↑ end-diastolic length ↑mean ejection pressure
Daggett (1998)	Neonatal piglet	Prospective randomised	MUF (n=6) vs CUF (n=6) vs no UF (n=6)	↑ mean arterial pressure ↑ contractility ↓ myocardial edema (wet/dry ratio)
Rivera (1998)	Paediatric (over 4kg)	Prospective randomised	immediate post-CPB V-V MUF (n=10) vs 10 min delayed V-V MUF (n=11)	↑ contractility ↑ Diastolic BP ↑ Systolic BP ↑ end-diastolic BP ↓ LV wall thickness
Aeba (1998)	Paediatric	Prospective single arm	Combined CUF & speed-controlled V-V MUF (n=46)	↑ Diastolic BP ↑ Systolic BP ↓mean right atrial pressure
Schlunzen (1998)	Paediatric	Retrospective review	A-V MUF (n=138)	↑ Systolic BP ↓ Heart rate
Chaturvedi (1999)	Paediatric	Prospective non-randomised	A-V MUF (n=16) vs CUF (n=6)	↑ global left ventricular systolic function
Tassani (1999)	Adult	Prospective randomised	Combined ZBUF & A-V MUF (n=21) vs no UF (n=22)	↑ colloid osmotic pressure ↑ mean arterial pressure
Boga (2000)	Adult	Prospective randomised	MUF (n=20) vs no UF (n=20)	↑ Cardiac Output (transient) ↑ Cardiac index (transient) ↑ pulmonary vascular resistance ↑ systemic vascular resistance (transient)
Aeba (2000)	Infant (<12 months)	Retrospective review	CUF (n=15) vs combined CUF & speed-controlled V-V MUF (n=14)	↑ systolic blood pressure ↑ pulmonary to systemic pressure ratio (Pp/Ps) ↔ Mean right atrial pressure ↔ Systolic pulmonary arterial pressure
Onoe (2001)	Adult	Prospective randomised	Combined MUF & CUF (n=9) vs CUF (n=9)	↑ systolic pressure
Kiziltepe (2001)	Adult	Prospective randomised	Combined V-V MUF & CUF (n=20) vs no UF (n=20)	↑ Cardiac Index ↑ mean arterial pressure ↑ contractility (observed only) ↓ Pulmonary Capillary Wedge Pressure (PCWP) (p=NS) ↓ Central Venous Pressure (CVP) (p=NS) ↓ systemic vascular resistance ↓ pulmonary vascular resistance ↓ Size of heart (observed only)
Kotani (2008)	Neonatal	Retrospective review	Combined CUF & MUF (n=15) vs CUF (n=21)	↑ Diastolic BP ↑ Systolic BP
Yokoyama (2009)	Paediatric	Prospective nonrandomised single arm	A-V MUF (n=35)	↑ Systolic BP ↓ Serum Prostaglandin E_2
Atkins (2010)	Neonatal Piglet	Prospective randomised	A-V MUF (n=12) vs no UF (n=12)	↑ Mean arterial pressure ↑ pulmonary vascular resistance
Honjo (2010)	Paediatric	Prospective single arm	A-V MUF (n=38)	↑ Myocardial Performance Index ↑ Ventricular Systolic function ↑ Ejection Time ↑ Fractional Shortening
Ricci (2013)	Paediatric	Prospective single arm	MUF (n=40)	↑ Mean arterial pressure ↑ Diastolic BP ↑ Systolic BP ↑ Stroke volume index ↑ Cardiac index

N.B. ZBUF = Zero-balanced ultrafiltration. ZBUF is similar to the CUF procedure except a high filtration rate is used and any fluid removed is replaced by an equal volume of balanced electrolyte solution (Tassani et al. 1999).

Table 1: Impact of MUF on cardiovascular function and haemodynamics.

The nature of the MUF procedure, removal of water and plasma from the blood, means there is likely to be a profound impact upon haemodynamics and cardiovascular function.

Elliott et al describe how in the advent of the MUF technique there was some trepidation that rapid removal of fluid from the aortic cannula and subsequent reinfusion of concentrated blood at the right atrium may result in a significant fall in intravascular volume leading to reduced tissue perfusion which in turn would manifest as a decrease in blood pressure (Elliott 1993). In fact, Naik, Knight and Elliott found the opposite to be true and instead observed a significant increase in blood pressure (Naik et al. 1991a). Subsequently, there has been a vast amount of evidence to support this finding (Aeba et al. 1998; Daggett et al. 1998; Davies et al. 1998; Rivera et al. 1998; Schlunzen et al. 1998; Tassani et al. 1999; Aeba et al. 2000; Kiziltepe et al. 2001; Onoe et al. 2001; Hodges et al. 2007; Kotani et al. 2008; Yokoyama et al. 2009; Atkins et al. 2010; Ricci et al. 2013). Yokoyama 2009 et al found that removal of prostaglandin E_2, an intrinsic vasodilator, was one reason for increased blood pressure with MUF (Yokoyama et al. 2009).

An increase in blood pressure can be seen as both a positive and a negative outcome of MUF; firstly, the rise in Hct seen after MUF would likely be coupled with a rise in blood viscosity which could increase vascular resistance and the demands on the heart, and secondly, the possibility of MUF removing surplus myocardial and pulmonary fluid could improve the functionality of the heart and thus, when coupled with improved blood pressure, oxygen delivery to the body (Elliott 1993).

As it is, the evidence suggests that cardiac function does improve after MUF. Hodges et al observed significantly increased cardiac index (Hodges et al. 1994), Davies et al saw significantly increased mean ejection pressure and end diastolic length coupled with significant reductions in myocardial cross sectional area, left-ventricular posterior wall thickness, end diastolic pressure and inotropic drug use (Davies et al. 1998), Chaturvedi et al found significantly improved global left ventricular systolic function (Chaturvedi et al. 1999), Boga et al observed transient significant improvements in both cardiac output and cardiac index (Boga et al. 2000), Kiziltepe et al saw significantly increase cardiac index and also anecdotally reported observing increased contractility and a reduction in the size of the heart (Kiziltepe et al. 2001), Honjo et al found significantly increased myocardial performance index, ventricular systolic function, ejection time and fractional shortening (Honjo et al. 2010), and Ricci et al observed significantly increased stroke volume and cardiac index after MUF (Ricci et al. 2013).

In support of the proposition that these improvements are associated with MUF reducing excess myocardial water, Daggett et al showed increased mean arterial pressure and contractility in conjunction with reduced myocardial edema (Daggett et al. 1998).

Additionally, contrary to the notion that increased blood viscosity from increased Hct may increase vascular resistance and the demands on the heart, Kiziltepe found that systemic and

pulmonary vascular resistance significantly reduced after MUF (Kiziltepe et al. 2001) however both Boga et al and Atkins et al found a significant increase in pulmonary vascular resistance (Boga et al. 2000; Atkins et al. 2010). Additionally, Boga et al found a transient significant increase in systemic vascular resistance (Boga et al. 2000). The evidence is contradictory and in addition, none of these trials involved a cohort of paediatric patients so conclusions with regard to this group are not possible. It can be suggested however that changes in vascular resistance are likely small enough so as not to diminish the benefits in cardiovascular function and haemodynamics observed.

1.3.4. Impact of MUF on Pulmonary Function

Author (Year)	Patient type	Study Type	Intervention	Impact on Pulmonary Function
POSITIVE				
Bando (1998)	Paediatric	Prospective randomised	Combined DUF & V-V MUF (n=50) vs CUF (n=50)	↑ (improvement) alveolar-arterial O_2 tension gradient (in neonates & preoperative pulmonary hypertension patients) ↓ Ventilatory support time
Bando (1998)	Infant	Prospective randomised	Combined DUF & V-V MUF (n=12) vs CUF (n=12)	↓ postoperative pulmonary hypertensive crises ↓ systolic pulmonary to systemic pressure ratio (Pp/Ps) ↓ requirement for ventilator support
Schlunzen (1998)	Paediatric	Retrospective review	A-V MUF (n=138)	↑ Arterial O_2 tension ↑ Arterial CO_2 tension
Aeba (2000)	Infant (<12 months)	Retrospective review	CUF (n=15) vs combined CUF & speed-controlled V-V MUF (n=14)	↓ carbon dioxide tension ($PaCO_2$)
Keenan (2000)	Infant (<1year)	Prospective randomised	V-V MUF (n=19) vs no UF (n=19)	↑ Static lung compliance (transient) ↑ Dynamic lung compliance (transient)
Kiziltepe (2001)	Adult	Prospective randomised	Combined V-V MUF & CUF (n=20) vs no UF (n=20)	↓ alveolar-arterial PO2 gradient (a-A PO_2) ↓ alveolar-arterial PO2 ratio (a/A O2)
Luciani (2001)	Adult (>16 years)	Prospective randomised	A-V MUF (n=284) against no UF (n=289)	↓ Morbidity (both high and low risk patients) ↓ Respiratory distress syndrome ↓ Respiratory failure ↓ Ventilation duration (p=NS)
Liu (2002)	Infants (<7kg)	Prospective randomised	MUF (n=15) vs CUF (n=15)	↓ Ventilation duration ↓ alveolar-arterial O_2 gradient (a-ADO_2) ↑ Respiratory Index
Chew (2002)	Infants (12-24 months)*	Prospective randomised	A-V MUF (n=10) vs no UF (n=8)	↓ alveolar-arterial (A-a) oxygen gradient
Huang (2003)	Paediatric	Prospective randomised	Combined ZBUF & MUF (n=15) vs no UF (n=15)	↑ Pulmonary static compliance ↓ Airway resistance ↓ alveolar-arterial (A-a) oxygen difference
Mahmoud (2005)	Paediatric (5-10kg)	Prospective randomised	Combined CUF & A-V MUF (n=20) vs CUF (n=20)	↑ Static lung compliance ↑ Dynamic lung compliance ↑ Gas exchange capacity (transient)
Kotani (2008)	Neonatal	Retrospective review	Combined CUF & MUF (n=15) vs CUF (n=21)	↑ gas exchange capacity (arterial oxygen tension fraction inspired oxygen ratio (PaO_2/FiO_2))
Torina (2013)	Adult	Prospective randomised	MUF (n=20) vs No UF (n=17)	↓ Airway resistance
NEUTRAL				
Wei Wang (1998)	Paediatric (0.6-6 years)	Prospective randomised	A-V MUF (n=20) vs no UF (n=20)	↔ Time on the ventilator.
Davies (1998)	Infant	Prospective randomised	A-V MUF (n=11) vs no UF (n=10)	↔ Ventilation time.
Grunenfelder (2000)	Adults	Prospective randomised	Hypothermic (26-28C) CPB with A-V MUF (n=30) vs hypothermic CPB with no UF (n=17) Normothermic (37C) CPB with A-V MUF (n=30) vs hypothermic CPB with no UF (n=20).	↔ pulmonary-alveolar oxygen gradient (PAaO2) *(any of the groups/subgroups)*
Maluf (2003)	Infant	Prospective Non-Randomised	Combined CUF & A-V MUF (n=20) vs CUF (n=20)	↔ duration of ventilator support. ↓ Mortality (p=NS)
Perez-Vela (2008)	Adult	Prospective Non-Randomised	A-V MUF (n=31) vs CUF (n=47) vs combined CUF & A-V MUF (n=47)	↔ oxygenation ↔ peak ventilator pressures ↔ mechanical ventilation time ↔ acute lung injury ↔ acute respiratory distress syndrome ↓ Static compliance in combined group (pre-existing at baseline)

*NB <12 months excluded as MUF was routinely administered to these patients in Chew et al's institution.

Table 2: Impact of MUF on pulmonary function.

CPB can have a profoundly negative impact on pulmonary function, impairing gas exchange and increasing intrapulmonary shunting (Kopman et al. 1978; Staub 1978; Tonz et al. 1995; Keenan et al. 2000). This has always been the case and, as mentioned previously (*1.1.1. Aetiology and Pathophysiology*), was first described as pump-lung in the 1960s (Moore et al. 2000). One major reason for the deterioration in pulmonary function is interstitial edema which has been shown to reduce pulmonary compliance and efficiency of gas transfer (Asada et al. 1971; Aeba et al. 2000; Keenan et al. 2000).

As mentioned previously (*1.3.3. Impact of MUF on Cardiovascular Function and Haemodynamics*), the reduction of excess myocardial water after MUF is likely to be a factor in improvements seen in cardiovascular function. It is reasonable therefore to suggest that reduction in excess pulmonary water after MUF may be associated with improve pulmonary function. However, the evidence for MUF improving pulmonary function is more mixed compared to the evidence regarding cardiovascular function.

There is a large body of evidence to suggest that MUF significantly improves gas exchange capacity (Mahmoud et al. 2005; Kotani et al. 2008) with Bando et al, Kiziltepe et al Liu et al and Chew et al reported significant improvements in alveolar-arterial oxygen gradient (oxygen transfer from the lungs to the pulmonary circulation (Martin 1999)) (Bando et al. 1998a; Kiziltepe et al. 2001; Chew et al. 2002; Liu et al. 2007). Moreover Mahmoud et al and Kotani et al both use alveolar-arterial oxygen gradient as one of many measures of 'gas exchange capacity' (Mahmoud et al. 2005; Kotani et al. 2008). Conversely, Grunenfelder et al did not find any significant improvements in pulmonary-alveolar oxygen gradient, however their study involved a cohort of adult patients who are less likely to suffer severe edema after CPB and may not exhibit improvements to the same magnitude as paediatric patients (Grunenfelder et al. 2000; Jones et al. 2006).

Additionally, Mahmoud et al and Keenan et al both report improved static and dynamic lung compliance, albeit transiently in the case of Keenan et al (Keenan et al. 2000; Mahmoud et al. 2005). As mentioned earlier (*1.3.4. Impact of MUF on Pulmonary Function*), pulmonary edema has been shown to negatively impact pulmonary compliance and thus it is a reasonable assumption that the removal of excess fluid by MUF would lead to the improved compliance.

In addition to these improvements Schlunzen et al found increased arterial O_2 tension with MUF, although it was coupled with increased arterial CO_2 tension which Schlunzen et al suggest this is likely to be caused by the sudden increase in tissue perfusion and thus CO_2 release rather than a pulmonary cause (Schlunzen et al. 1998). Moreover Aeba et al measured CO_2 tension over a

longer study period and found it increased immediately after MUF but decreased thereafter (Aeba et al. 2000).

However these improvements in pulmonary function do not appear to translate to reduced ventilation times post-MUF with Wei Wang et al, Davies et al, Maluf et al and Perez-Vela et al all reporting no difference in ventilation times when MUF was used or not (Davies et al. 1998; Wang et al. 1998; Maluf 2003; Perez-Vela et al. 2008). Conversely, Bando et al and Liu et al did find MUF resulted in reduced ventilation times and Luciani et al note a tendency for shorter ventilatory support but their findings did not reach significance (Bando et al. 1998a; Luciani et al. 2001; Liu et al. 2007).

Bando et al found that MUF decreased systolic pulmonary to systemic pressure ratio (Pp/Ps) and reduced postoperative pulmonary hypertensive crises (Bando et al. 1998b). Furthermore, Luciani et al found MUF reduced incidences of respiratory failure, respiratory distress syndrome and morbidity in both high and low risk patients within their study (Luciani et al. 2001).

Despite patients in the MUF group exhibiting significantly fewer incidences of complications, Luciani et al were unable to show any significant difference in postoperative clinical treatment between the two groups. They subsequently suggest that this may be a result of standard practices masking any improvements in patient condition by treating the patients according to procedure rather than their specific needs (Luciani et al. 2001). This could also be an explanation for why there appears to be strong evidence for improvements in pulmonary function but more mixed evidence for reduction in ventilation times.

1.3.5. Impact of MUF on Neurological Function

Author (Year)	Patient type	Study Type	Intervention	Impact on Neurological Function
POSITIVE				
Skaryak (1995)	Piglets (1 week old)	Prospective randomised	A-V MUF (n=9) vs non-leukocyte depleted haemoconcentrated blood transfusion to maintain Hct>40% (n=7) vs no intervention (n=10)	↑ cerebral oxygen delivery ↑ metabolic rate of oxygen consumption
Luciani (2001)	Adult (>16 years)	Prospective randomised	A-V MUF (n=284) against no UF (n=289)	↓ neurological complications ↓ postoperative coma
NEUTRAL				
Myung (2003)	Neonatal piglets	Prospective randomised	MUF (n=8) vs no UF (n=8)	↔ Central Nervous System injury
Perez-Vela (2008)	Adult	Prospective Non-Randomised	A-V MUF (n=31) vs CUF (n=47) vs combined CUF & A-V MUF (n=47)	↔ incidence of neurological morbidities (stroke, delirium, encephalopathy, coma)

Table 3: Impact of MUF on neurological function

The incidence of neurological damage after CPB has been reported at anywhere between 1% and 25% (Elliott 1999b). Deep Hypothermic Cardiac Arrest (DHCA) has been heavily implicated in neurological dysfunction after CPB and has been shown to decrease cerebral metabolic rate of oxygen consumption with increased cerebral vascular resistance and cerebral hypoperfusion contributing to impaired cerebral oxygen delivery (Greeley et al. 1991a; Greeley et al. 1991b; Skaryak et al. 1995). Additionally, organ edema, hypoxemia, decreased cardiac output, increased pulmonary vascular resistance and decreased haemoglobin have also been implicated in causing neurological injury post-CPB (Skaryak et al. 1995). Higher Hct has also been demonstrated to improve cerebral outcome after DHCA (Shin'oka et al. 1996). The ability of MUF to improve these factors would suggest that MUF may result in improved neurological function.

There is a limited amount of evidence regarding improvements in neurological function and what little evidence there is, is mixed in outlook, thus it is difficult to draw any strong conclusions. Additionally, none of the studies involved a cohort of paediatric patients. Skaryak et al found MUF improved cerebral oxygen delivery and metabolic rate of oxygen consumption in piglets (Skaryak et al. 1995), whereas Myung et al were unable to find any reduction in CNS injury after MUF in piglets (Myung et al. 2003). Myung state that behaviour and histological injury scores were lower for MUF patients, but that the differences did not reach significance. Consequently, Myung et al do not rule out that MUF may result in CNS benefits in children undergoing CPB and suggest that *"With more sensitive testing, it might be possible to detect a difference between groups" (Myung et al. 2003)*.

In a study involving a large cohort of adult patients, Luciani et al found reduced incidences of neurological complications and coma with MUF (Luciani et al. 2001). However Perez-Vela et al were not able to find any significant reduction in incidence of neurological morbidities (Perez-Vela et al. 2008). Perez-Vela et al state that although they did not have a "no UF control group", the amount of ultrafiltrate differed in the three groups but was not mirrored by difference in neurological morbidities as Luciani et al had suggested. However, Perez-Vela et al's study is also limited by the fact that it was not randomised.

None of the aforementioned studies involve a cohort of paediatric patients, the demographic we are most concerned with, and this combined with the small number of studies with conflicting findings make it impossible to draw meaningful conclusions about the impact of MUF on neurological function. It may be most prudent to suggest that improvements after MUF are possible, perhaps even rationally expected, but that the evidence is yet to confirm this.

As an addition to the above evidence and a precursor to the following evidence, Harris and Taylor et al observed clear cerebral swelling in patients after CPB as is shown in Figure 7 (Harris et al. 1993; Harris et al. 1998).

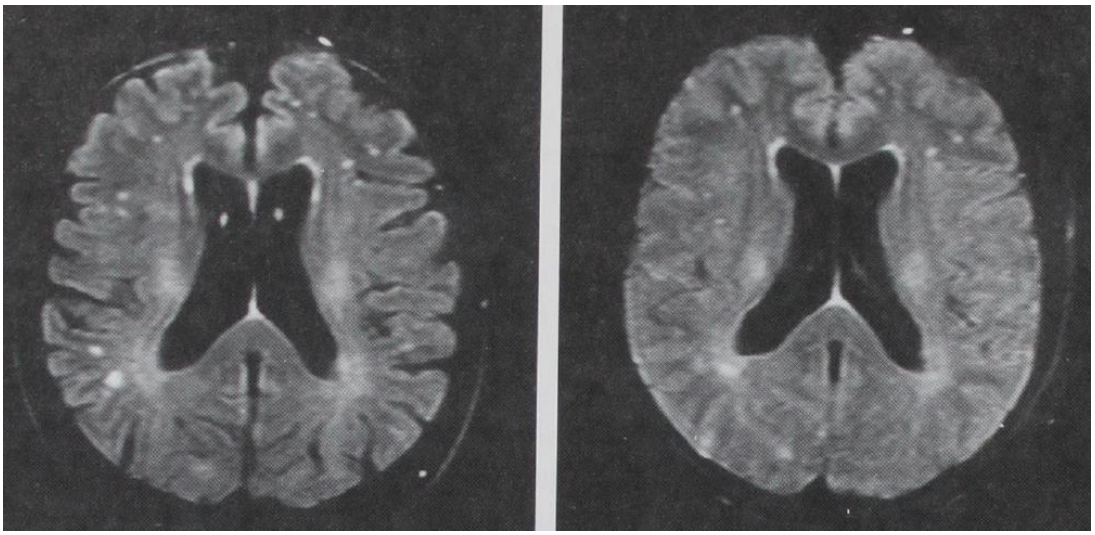


Figure 7: Fluid attenuated inversion recovery images showing cerebral swelling after normothermic CPB. Sulci are clearly visible before cerebral swelling (left) but are much less pronounced after cerebral swelling (right) (Harris et al. 1998)

1.3.6. Impact of MUF on Total Body Water

Author (Year)	Patient type	Study Type	Intervention	Impact on Total Body Water
POSITIVE				
Daggett (1998)	Neonatal piglet	Prospective randomised	MUF (n=6) vs CUF (n=6) vs no UF (n=6)	↓ Body weight gain(vs CUF and no UF)
Schlunzen (1998)	Paediatric	Retrospective review	A-V MUF (n=138)	↓ Postoperative fluid overload ↓Volume gain
Hennein (2000)	Paediatric	Prospective randomised	V-V MUF (n=13) vs CUF (n=13) vs no UF (n=13)	↓ Total body water gain
NEUTRAL				
Keenan (2000)	Infant (<1year)	Prospective randomised	V-V MUF (n=19) vs no UF (n=19)	↔ total weight gain
Perez-Vela (2008)	Adult	Prospective Non-Randomised	A-V MUF (n=31) vs CUF (n=47) vs combined CUF & A-V MUF (n=47)	↔ immediate postoperative fluid balance

Table 4: Impact of MUF on total body water

Total body water increases between 11% and 18% after CPB (Naik et al. 1991a). The most important function of MUF is to remove excess fluid from the bypass circuit and Naik, Knight and Elliott were able to reduce the rise in total body water by 50-75% (Naik et al. 1991a). As mentioned before, theoretically, as filtrate is extracted from the bypass circuit, accumulated fluid from the "third space" (areas of physiologically non-functional fluid accumulation such as the interstitium (Seifter et al. 2005)) should replace it so that effectively the fluid is being

removed from potentially problematic areas (Naik et al. 1993). In this way, it is reasonable to suggest that MUF would result in a reduction in total body water after CPB.

Few studies directly measure total body water with most content to assess ultrafiltrate volume and Hct as indicators of fluid levels. However, Daggett et al found a significant reduction in body weight gain with MUF in neonatal piglets (Daggett et al. 1998), Hennein et al found a reduction in total body water gain (Hennein 2000), and Schlunzen et al saw significantly lower fluid gain and a reduction in fluid overload when MUF was utilised in a cohort of paediatric patients (Schlunzen et al. 1998).

Conversely, Perez-Vela et al saw no improvement in fluid balance in the immediate postoperative period (Perez-Vela et al. 2008) and Keenan et al did not find any reduction in total weight gain with MUF (Keenan et al. 2000). Keenan et al suggest that MUF starts after initiation of the inflammatory cascade and thus removal of water may not be able to overcome the effects of ongoing capillary leak. They state this reflects the initial findings of Naik, Knight and Elliott in that haemodynamics improved but inotropic requirements and urine output did not change (Keenan et al. 2000).

Keenan et al's stance is somewhat supported by Schlunzen et al who, despite observing a significant rise in Hct, found no correlation between ultrafiltrate volume and volume gain or ultrafiltration volume and rise in Hct (Schlunzen et al. 1998). Consequently they suggest that within certain limits, ultrafiltration volume may influence decreases in interleukins and complement but may not be of vital importance for TBW reduction (Schlunzen et al. 1998).

However, when you consider the vast number of studies that demonstrate a significant increase in Hct after MUF (Naik et al. 1991a; Ad et al. 1996; Friesen et al. 1997; Koutlas et al. 1997; Williams et al. 1997; Daggett et al. 1998; Davies et al. 1998; Schlunzen et al. 1998) it is clear that MUF is effectively removing fluid from the patient, although reductions in total body weight may be somewhat tempered by ongoing capillary leak.

1.3.7. Impact of MUF on Transfusion Requirements

Author (Year)	Patient type	Study Type	Intervention	Impact on Transfusion Requirements
POSITIVE				
Ad (1996)	Paediatric	Prospective randomised	MUF (n=40) vs no UF (n=40)	↓ requirement for transfused blood ↓ blood loss
Draaisma (1997)	Paediatric	Retrospective review	A-V MUF(n=99) vs no UF (n=99)	↓ Red blood cell transfusion requirements ↓ mean postoperative chest drain losses ↑ mean haemoglobin
Koultas (1997)	Paediatric	Retrospective review	A-V MUF (n=41) vs no UF (n=79)	↓ Postoperative blood transfusion requirement
Gurbuz (1998)	Paediatric	Retrospective review	A-V MUF(n=13) vs no UF (n=26)	↓ requirement for transfused blood
Bando (1998)	Paediatric	Prospective randomised	Combined DUF & V-V MUF (n=50) vs CUF (n=50)	↓ RBC transfusion requirements ↓ coagulation factor transfusion requirements (fresh frozen plasma, platelets and cryoprecipitates) ↓ blood loss (preoperative pulmonary hypertension patients)
Wei Wang (1998)	Paediatric (0.6-6 years)	Prospective randomised	A-V MUF (n=20) vs no UF (n=20)	↓ Postoperative RBC transfusion requirements ↓ plasma therapy requirements
Kiziltepe (2001)	Adult	Prospective randomised	Combined V-V MUF & CUF (n=20) vs no UF (n=20)	↓ fresh frozen plasma transfusion requirements ↓ postoperative bleeding ↑ platelet levels
Leyh (2001)	Adult	Prospective randomised	A-V MUF (n=16) vs CUF (n=16) vs no UF (n=16)	↓ postoperative blood loss (vs CUF & no UF) ↓ packed RBCs requirement (vs CUF & no UF) ↔ colloids given postoperative
Liu (2002)	Infants (<7kg)	Prospective randomised	MUF (n=15) vs CUF (n=15)	↓ Transfusion requirements
Torina (2013)	Adult	Prospective randomised	MUF (n=20) vs No UF (n=17)	↓ RBC transfusion requirements ↓ postoperative drain loss
NEUTRAL				
Perez-Vela (2008)	Adult	Prospective Non-Randomised	A-V MUF (n=31) vs CUF (n=47) vs combined CUF & A-V MUF (n=47)	↔ Haemoglobin levels ↔ packed RBC transfusion requirements ↔ endothoracic chest drain volume ↔ blood loss ↑ platelet count (p=NS)

Table 5: Impact of MUF on transfusion requirements

Increases in Hct seen after MUF mean there are more blood cells within the circulating volume. Theoretically this should reduce the requirement for transfusions which are used to address cell deficiencies during and after CPB.

There is a large amount of evidence to suggest that MUF significantly reduces transfusion requirements (Ad et al. 1996; Draaisma et al. 1997; Koutlas et al. 1997; Gurbuz et al. 1998; Wang et al. 1998; Bando et al. 1998a; Kiziltepe et al. 2001; Leyh et al. 2001; Liu et al. 2007; Torina et al. 2010). Reduction in red blood cell requirement is the most strongly supported and considering the consistent improvement Hct seen after MUF it is perhaps unsurprising.

Lehy et al suggest that since MUF reduced postoperative blood loss but there was no difference between the amount of colloids given postoperatively, the associated reduced requirement for

RBCs may be linked to the higher haematocrit levels observed in the MUF group (Leyh et al. 2001).

Ad et al, Draaisma et al, Bando et al, Kiziltepe et al and Torina et al also found that MUF resulted in reduced blood loss (Ad et al. 1996; Draaisma et al. 1997; Bando et al. 1998a; Kiziltepe et al. 2001; Torina et al. 2010). Additionally, Bando et al, Wei Wang et al and Kiziltepe et al found that plasma transfusions, often administered in response to clotting deficiencies, were reduced when MUF was utilised (Wang et al. 1998; Bando et al. 1998a; Kiziltepe et al. 2001).

1.3.8. Impact of MUF on Coagulation

Author (Year)	Patient type	Study Type	Intervention	Impact on Coagulation
POSITIVE				
Friesen (1997)	Paediatric	Prospective single arm	A-V MUF (n=20)	↑ fibrinogen ↑ plasma proteins (including coagulation factors) ↔ platelet count
Bando (1998)	Paediatric	Prospective randomised	Combined DUF & V-V MUF (n=50) vs CUF (n=50)	↓ coagulation factor transfusion requirements (fresh frozen plasma, platelets and cryoprecipitates)
Ootaki (2002)	Paediatric	Prospective single arm	Combined A-V MUF & CUF (n=7)	↑ platelets ↑ albumin ↑ total plasma proteins ↑ Fibrinogen (↑ corrected for albumin) ↑ Prothrombin (↑ corrected for albumin) ↑ Factor VII (↑ corrected for albumin) ↔ Factor IX (↔ corrected for albumin) ↔ Factor X (↔ corrected for albumin)
Perez-Vela (2008)	Adult	Prospective Non-Randomised	A-V MUF (n=31) vs CUF (n=47) vs combined CUF & A-V MUF (n=47)	↑ platelet count (p=NS)
NEUTRAL				
Leyh (2001)	Adult	Prospective randomised	A-V MUF (n=16) vs CUF (n=16) vs no UF (n=16)	↔ Thrombin formation ↔ Active clotting time (ACT) ↔ Fibrinogen ↔ Clotting parameters (PT, aPTT, TT) ↔ platelet counts ↔ PF4 levels ↑ ATIII activity (vs CUF and no UF)
Chew (2002)	Infants (12-24 months)*	Prospective randomised	A-V MUF (n=10) vs no UF (n=8)	↔ F1+2 ↔ ATIII

Table 6: Impact of MUF on coagulation

Naik, Knight and Elliott originally speculated that coagulation may be improved after MUF by concentration of coagulation factors (Naik et al. 1991b). Leyh et al state that since inflammatory mediators are essential for initiating and maintaining the coagulation disturbances and increased fibrinolysis that are associated with CPB, methods aiming to reduce mediator formation such as MUF may, in theory, impact upon the coagulation and fibrinolytic systems (Leyh et al. 2001). Most ultrafilters have a pore diameter of 1-3.5nm and so in theory, any substance with a molecular weight below 20kDa should undergo ultrafiltration (Moore et al. 2000). The majority of coagulation factors have molecular weight far above this threshold; fibrinogen = 340kDa, prothrombin = 70kDa, Factor VII = 63kDa, Factor IX = 55kDa, Factor X =

16-38kDa, and so should theoretically be concentrated by MUF, although molecule conformations might play a role in determining this (Grunenfelder et al. 2000; Ootaki et al. 2002). This is discussed in more detail in *1.3.10. Impact of MUF on Inflammatory Mediators* and *2.1. Principles of Ultrafiltration*.

There is some evidence to suggest that MUF improves patients' blood's ability to clot (Friesen et al. 1997; Bando et al. 1998a; Ootaki et al. 2002). Both Friesen et al's and Ootaki et al's reports are specifically focussed on assessing the impact of MUF on coagulation, and both demonstrate increases in coagulation factors, particularly fibrinogen (Friesen et al. 1997; Ootaki et al. 2002). However, the strength of their evidence is somewhat tempered by the nature of the studies, namely the lack of control and relatively small study groups which make it difficult attribute benefits seen to the MUF technique itself.

Friesen et al make comparisons between their results and those of Journois et al (Journois et al. 1994) and Nakamura et al (Nakamura et al. 1990) who investigated CUF, and suggest that since their results relating to MUF show superior improvements in fibrinogen and plasma proteins concentrations compared to CUF, MUF may be a superior intervention (Friesen et al. 1997). These comparisons are of little value since the results stem from studies that differ in several important ways and thus direct comparisons should be received cautiously, particularly when no reliable test of statistical significance between the MUF and CUF results from these separate studies could be made. Based on the evidence of their results, and other supporting reports, that MUF increases the concentration of fibrinogen and other plasma protein coagulation factors, Friesen et al speculate that changes in fibrinogen concentrations may be an indicator as to the extent to which blood dilution has impaired the natural clotting of the blood, known as dilutional coagulopathy (Friesen et al. 1997).

Perez-Vela et al observed an improvement in platelet count when MUF was applied compared with CUF or combined CUF and MUF, although they argue that this is not likely to be clinically significant since there was no difference in amount of bleeding between the three groups in their study (Perez-Vela et al. 2008). Moreover, the study was not randomised, with MUF only being utilised if deemed safe by the surgeon, which could potentially diminish the ability for MUF to demonstrate its potential benefits in high risk patients and restricted its application to patients for whom immediate post-CPB outcomes were good and thus the potential for significant increases in platelet count or coagulation factor levels were low. Additionally the study involved a cohort of adult patients who generally are at reduced risk of severe edema and its associated complications compared to paediatric patients, and thus may not exhibit improvements to the same magnitude (Jones et al. 2006).

Bando et al did demonstrate a reduction in transfusion requirements of coagulation factors in a prospective randomised controlled trial in a large cohort of paediatric patients, which would suggest improvements in patient's natural coagulation ability, however again the result is somewhat tempered by the fact that there was no direct measurement of platelets or coagulation factors and that MUF was used in combination with DUF (Bando et al. 1998a). Bando et al's findings are supported by the findings of Wei Wang et al and Kiziltepe et al who, as shown earlier (*1.3.7. Impact of MUF on Transfusion Requirements*), saw a reduction in transfusion requirements for plasma, which is mainly administered in response to clotting deficiencies (Wang et al. 1998; Kiziltepe et al. 2001).

Leyh et al found that Antithrombin-III (ATIII) activity was significantly higher after MUF compared to CUF and no UF but found that MUF had no significant impact on the coagulation or fibrinolytic systems and performed no better than CUF or no UF in altering platelet counts, ACT or clotting factor levels (Leyh et al. 2001). Since Leyh et al found that MUF did reduce blood loss but did not induce changes in the coagulation or fibrinolytic systems, Leyh et al suggest there is no causal relationship (Leyh et al. 2001).

Friesen et al suggest that platelet concentrations may not be seen to increase (as was the case in their study) because platelets may aggregate to the apparatus, thus negating the expected increases in platelet count (Friesen et al. 1997). Harig et al found that coating the extracorporeal circuit with heparin led to a reduction in platelet activation (Harig et al. 2006).

Chew et al state that the increases seen in Prothrombin Fragment (F1+2) concentrations, peaking after CPB before returning to baseline, in conjunction with a simultaneous transient decrease in ATIII levels, indicates activation of the coagulation cascade (Chew et al. 2002). Activation of coagulation cascade is thought to be a cause of increased requirements for transfusions by increasing the risk of postoperative bleeding. However, the MUF and CUF groups differ in terms of extent of coagulation system activation meaning that MUF had no significant impact on concentrations of F1+2, or ATIII (Chew et al. 2002).

Notably, Ootaki et al assessed albumin concentration and used this measurement to correct the absolute levels of coagulation factors found in the blood. Correction for albumin was done using the formula:

$$\textit{Filtration ratio} = (\textit{coagulation factor after MUF}/\textit{coagulation factor before MUF}) \times (\textit{albumin concentration before MUF}/\textit{albumin concentration after MUF})$$

This ratio is the ratio of each coagulation factor level to that of albumin. That this was done in order to assess effect of concentrating the coagulation factors whilst minimising the effect of filtration or adhesion to the ultrafilter membrane; albumin is chosen since it does not do either (supported by that fact that no albumin was found in the ultrafiltrate) (Ootaki et al. 2002). In other words, correction for albumin is performed in order to account for increasing Hct (concentration of the blood) leading to a larger fraction of a sample volume being made up from cytokines and thus giving the appearance of increasing absolute levels of cytokines, whereas in fact the cytokines levels are not being selectively altered (Onoe et al. 2001).

Ootaki et al state that since the 'corrected for albumin concentrations' of platelets and coagulation factors were significantly lower than the absolute concentration there is reason to suspect that platelets and coagulation factors might adhere to the ultrafilter membrane or cardiotomy site. Moreover, since the 'corrected for albumin' levels of Hct were slightly, but not significantly, lower than the absolute levels Ootaki et al suggest that there may be some incidence of red blood cell damage. They state that their results show that MUF concentrates many coagulation factors whilst avoiding the risks associated with transfusions

1.3.9. Impact of MUF on Length of Intensive Care Unit/Hospital Stay

Author (Year)	Patient type	Study Type	Intervention	Impact on Length of ICU/hospital stay
POSITIVE				
Koultas (1997)	Paediatric	Retrospective review	A-V MUF (n=41) vs no UF (n=79)	↓ Hospital stay duration
Bando (1998)	Paediatric	Prospective randomised	Combined DUF & V-V MUF (n=50) vs CUF (n=50)	↓ ICU stay duration
Liu (2002)	Infants (<7kg)	Prospective randomised	MUF (n=15) vs CUF (n=15)	↓ ICU stay duration
Kotani (2008)	Neonatal	Retrospective review	Combined CUF & MUF (n=15) vs CUF (n=21)	↓ ICU stay duration
NEUTRAL				
Ming-Juih Wang (1995)	Infant & Paediatric	Prospective randomised	A-V MUF (n=25) vs CUF (n=27)	↔ ICU stay duration
Wei Wang (1998)	Paediatric (0.6-6 years)	Prospective randomised	A-V MUF (n=20) vs no UF (n=20)	↔ ICU stay duration
Gurbuz (1998)	Paediatric	Retrospective review	A-V MUF(n=13) vs no UF (n=26)	↔ Hospital stay duration
Davies (1998)	Infant	Prospective randomised	A-V MUF (n=11) vs no UF (n=10)	↔ ICU stay duration
Grunenfelder (2000)	Adults	Prospective randomised	Hypothermic (26-28C) CPB with A-V MUF (n=30) vs hypothermic CPB with no UF (n=17) Normothermic (37C) CPB with A-V MUF (n=30) vs hypothermic CPB with no UF (n=20).	↔ ICU stay duration ↔ Hospital stay duration
Keenan (2000)	Infant (<1year)	Prospective randomised	V-V MUF (n=19) vs no UF (n=19)	↔ ICU stay duration
Maluf (2003)	Infant	Prospective Non-Randomised	Combined CUF & A-V MUF (n=20) vs CUF (n=20)	↔ PICU stay duration ↔ Hospital stay duration

Table 7: Impact of MUF on length of intensive care unit/hospital stay

The ultimate result of improvements in cardiovascular function, haemodynamics, pulmonary function, neurological function would ideally be better clinical outcomes, characterised by an expedited recovery period and return to normal functioning. One measure of this is the length of time a patient spends in ICU and/or hospital.

There is some evidence to suggest that ICU stays are reduced when MUF is employed (Koutlas et al. 1997; Bando et al. 1998a; Liu et al. 2007; Kotani et al. 2008). However the majority of evidence suggests that there is no difference in either ICU or hospital stay duration whether MUF is applied or not (Wang et al. 1996; Davies et al. 1998; Gurbuz et al. 1998; Wang et al. 1998; Grunenfelder et al. 2000; Keenan et al. 2000; Maluf 2003). Kotani et al suggest that the reason for the reduction in time spent in ICU may be the cumulative effect of several small improvements in postoperative parameters (Kotani et al. 2008). As mentioned earlier (*1.3.4. Impact of MUF on Pulmonary Function*), Luciani et al had previously suggested standardised postoperative clinical treatment may mask improvements in patient condition by treating patients according to procedure rather than their specific needs (Luciani et al. 2001). This could well be the case for duration of ICU and hospital stay as it can easily be envisioned how the combination of successive rigid clinical, nursing and bureaucratic procedures would delay discharge.

1.3.10. Impact of MUF on Inflammatory Mediators

Author (Year)	Patient type	Study Type	Intervention	Impact on Inflammatory Mediators
Ming-Juih Wang (1995)	Infant & Paediatric	Prospective randomised	A-V MUF (n=25) vs CUF (n=27)	↑ plasma elastase (↔ vs CUF) ↑ plasma IL-6 (↔ vs CUF) ↑ plasma IL-8 (↔ vs CUF) ↔ plasma TNF-α (vs CUF) ↔ ultrafiltrate elastase (vs CUF) ↔ ultrafiltrate TNF-α (vs CUF) ↔ ultrafiltrate IL-6 (↑ vs CUF) ↔ ultrafiltrate IL-8 (vs CUF)
Saatvedt (1996)	Paediatric	Prospective randomised	A-V MUF (n=9) vs no UF (n=9)	↔ TNF- α ↔ IL-6 ↔ C3a ↔ C3bc
Wei Wang (1998)	Paediatric (0.6-6 years)	Prospective randomised	A-V MUF (n=20) vs no UF (n=20)	↑ TNF-α (↔ corrected for albumin) ↔ ET-1 (↓ corrected for albumin) ↔ IL-8 (↓ corrected for albumin)
Pearl (1999)	Paediatric	Prospective non-randomised	Combined CUF & MUF (n=22) vs CUF (n=12)	↔ TXB2 ↔ ET-1 ↔ LTB4
Portela (1999)	Paediatric	Prospective single arm	Combined CUF & MUF (n=22)	↓ ICAM-1 ↓ VCAM-1 ↓ IL-6
Tassani (1999)	Adult	Prospective randomised	Combined ZBUF & A-V MUF (n=21) vs no UF (n=22)	↓ IL-6 (transient) ↓ IL-8 (transient) ↔ IL-1 ↔ IL-10
Yndgaard (2000)	Paediatric	Prospective single arm	A-V MUF (n=20)	↓ endotoxin load
Boga (2000)	Adult	Prospective randomised	MUF (n=20) vs no UF (n=20)	↔ IL-6 ↔ IL-8 ↔ neopterin
Grunenfelder (2000)	Adults	Prospective randomised	Hypothermic (26-28C) CPB with A-V MUF (n=30) vs hypothermic CPB with no UF (n=17) Normothermic (37C) CPB with A-V MUF (n=30) vs hypothermic CPB with no UF (n=20).	↓ sI-CAM-1 (hypothermic only) ↓ sE-selectin (hypo- & normothermic) ↓ IL-6 (hypothermic only) ↓ IL-8 (hypothermic only) ↓ TNF-α (hypothermic only) ↔ IL-2R (hypo- & normothermic)
Onoe (2001)	Adult	Prospective randomised	Combined MUF & CUF (n=9) vs CUF (n=9)	↓ IL-8
Kiziltepe (2001)	Adult	Prospective randomised	Combined V-V MUF & CUF (n=20) vs no UF (n=20)	↔ ET-1 ↔ IL-6 ↔ IL-8 ↔ IL-10
Hiramatsu (2002)	Paediatric	Prospective randomised	Combined DUF & MUF (n=11) vs CUF (n=11)	↔ ET-1 (↓ vs CUF)
Ootaki (2002)	Paediatric	Prospective single arm	Combined A-V MUF & CUF (n=7)	↑ Fibrinogen (↑ corrected for albumin) ↑ Prothrombin (↑ corrected for albumin) ↑ Factor VII (↑ corrected for albumin) ↔ Factor IX (↔ corrected for albumin) ↔ Factor X (↔ corrected for albumin)
Chew (2002)	Infants (12-24 months)*	Prospective randomised	A-V MUF (n=10) vs no UF (n=8)	↔ neutrophil elastase ↔ IL-1ra ↔ TNF-α ↔ IL-6 ↔ IL-8 ↔ C3d ↔ C4d ↔ C-reactive protein (CRP) ↔ tissue injury markers
Atkins (2010)	Neonatal Piglet	Prospective randomised	A-V MUF (n=12) vs no UF (n=12)	↔ plasma IL-6 ↔ plasma IL-8 ↔ plasma TNF-α ↑ ultrafiltrate TNF-α ↑ ultrafiltrate IL-6 ↑ ultrafiltrate IL-8 ↓ bronchoalveolar lavage IL-6 ↓ bronchoalveolar lavage IL-8 ↓ transpulmonary TxB2 gradient

Table 8: Impact of MUF on inflammatory mediators

As mentioned earlier (*1.3.8. Impact of MUF on Coagulation*), the nature of ultrafiltration means that in theory, any solute with a molecular weight below a given threshold (the molecular weight cut-off) should be freely removed from blood. Ultrafiltration thresholds would theoretically be determined by pore size of the membrane and the molecular weight of the cytokines but the experimental findings do not always reflect this. Some groups believe molecular shape and conformation are also important (Wang et al. 1996; Tassani et al. 1999; Grunenfelder et al. 2000). The physics of ultrafiltration will be discussed in more detail later (*2.1. Principles of Ultrafiltration*), but the fact that inflammatory mediators have low molecular weights relative to other formed elements of the blood (erythrocytes, thrombocytes etc), typically in the range of 10-50kD (Grunenfelder et al. 2000), has led several groups to postulate and investigate the possibility that MUF will significantly impact plasma concentrations of these inflammatory mediators by physically extracting them from the blood.

Regardless of whether or not it can be demonstrated that inflammatory mediators are successfully removed, there is some debate as to the beneficial effect MUF may have on ameliorating the inflammatory response. One may expect that the physical removal of pro-inflammatory mediators would interrupt, or at least inhibit, the inflammatory cascade. However, as is described below, although several groups observed significant changes in inflammatory mediator levels, these were not always accompanied by observations of significant clinical benefits.

Casey et al studied the prognosis of high plasma concentrations of cytokines in sepsis and demonstrated adverse effects of experimental cytokine administration (Casey 1993) yet Grunenfelder et al stress that there has yet to be any proven clinical benefit of cytokine removal or neutralisation (Grunenfelder et al. 2000) whilst Schetz et al suggest that plasma concentrations of cytokines may be merely an indication of what is in actuality a tissue level process (Schetz 1994). Chew et al state that despite MUF resulting in improved clinical outcomes of patients in their study, that fact that there was no manipulation of inflammatory mediator levels or complement and coagulation profiles means that this cannot be held as the mechanism by which the clinical improvements were achieved (Chew et al. 2002).

Pearl et al comment that since substances like Thromboxane B2 (TXB2) have a short half-life, any intervention which removes the stimulus for their production should result in rapid reduction in their detected levels, as seen in their results. Conversely, mediators such as Endothelin-1 (ET-1) which rely on gene activation with amplified translation and transcription to increase their levels will not see such rapid reductions since the gene activation mechanisms may endure beyond the end of CPB. Pearl et al state that this, in combination with their results

which show a steady increase in ET-1 levels throughout CPB including CUF and MUF, suggests that any ET-1 removed by UF is likely to occur rapidly and so is unlikely to have any role in decreases in pulmonary hypertension seen in patients. (Pearl et al. 1999). Additionally, they point to the notion that if levels of certain inflammatory mediators increase as a result of extracorporeal circulation (such as LTB4 in their study) and if production continues beyond the end of interventions, that MUF would be unable to reduce levels of such substances since it would be simultaneously stimulating their production (Pearl et al. 1999).

Some of the more prominent inflammatory mediators in CPB which feature in multiple studies of MUF are described below.

Tumor Necrosis Factor Alpha (TNF-α) (17-50kD) was one of the early substances identified as being involved in endothelial cell activation leading to hypotension and leukopenia (Grunenfelder et al. 2000) as well as being the chief pro-inflammatory cytokine activating neutrophils early in the inflammatory response (Sonntag et al. 1998). TNF-α is released by activated monocytes, lymphocytes and cupfer cells, and results in increased phagocytosis, degranulation, oxidative products, and adhesion properties of leukocytes (Boga et al. 2000).

Interleukin-6 (IL-6) (20-30kD) is produced during the inflammatory response including from the myocardium during postischemic reperfusion (Ikeda et al. 1992). Serum IL-6 levels significantly increase in myocardial infarction (Grunenfelder et al. 2000). IL-6 contributes to regulation of acute phase response, causes fever, and induces the release of adrenocorticotrophic hormone (ACTH) (Boga et al. 2000). It could have a protective role based upon its inhibition of TNF-α derived endotoxin (Boga et al. 2000).

Interleukin-8 (IL-8) (8-10kD) has been shown to be produced during CPB and be implicated in ARDS (Messent et al. 1992) and to be a powerful chemotactic factor for neutrophils and T-lymphocytes (Grunenfelder et al. 2000) stimulating movement of neutrophils from the bloodstream and into tissues and their adhesion to endothelial cells (Baggiolini et al. 1989; Mackarel et al. 2000; Paparella et al. 2002); leading to neutrophil induced endothelial injury and resulting in some postoperative organ dysfunctions (Butler et al. 1993; Finn et al. 1993).

Interleukin-10 (IL-10) (18kD) is produced during septicaemia (Marchant et al. 1994) and is a strong inhibitor of TNF-a, IL-1b, IL-6 and IL-8 (Eppinger et al. 1996), therefore is removal may actually have a negative contribution to mitigating the inflammatory response (Journois et al. 1996).

Endothelin-1 (ET-1) is a myocardial depressant involved in adhesion and activation of leukocytes (Pearl et al. 1999). ET-1 is also strong pulmonary vasoconstrictor and plays a role in the latter phases of hypoxic or endotoxin induced pulmonary hypertension (Pearl et al. 1999). After CPB, pulmonary production of ET-1 and expression of ET-1 receptors in the lungs have been seen to increase (Kirshbom et al. 1997) suggesting ET-1 removal may result in better postoperative pulmonary outcomes.

There are a large number of studies which show MUF is capable of removing some inflammatory mediator substances, however, there does not appear to be much consensus as to which mediators are removed.

For example, Ming-Juin Wang et al found that TNF-α was the inflammatory mediator most successfully removed in their study (by both MUF and CUF) (Wang et al. 1996). Whereas, Wei Wang et al found that TNF- α levels were significantly higher in the ultrafiltrate than the patients' blood and state that in their study, TNF-α could not be freely filtered (Wang et al. 1998). Grunenfelder et al found a significant reduction in TNF-α after MUF, but only in patients undergoing hypothermic CPB (Grunenfelder et al. 2000). Grunenfelder et al suggest this may simply be due to higher concentration of adhesion molecules and cytokines in the hypothermic group meaning more adhesion molecules and cytokines being available for removal within a given timeframe. (Grunenfelder et al. 2000). Whereas, Atkins et al found although plasma concentrations of TNF-α did not significantly change post-CPB, it was the inflammatory mediator most abundant in the ultrafiltrate (Atkins et al. 2010). Additionally, Chew et al and Saatvedt et al both concluded that based on their study, MUF did not significantly impact TNF-α levels (Saatvedt et al. 1996; Chew et al. 2002).

Interleukin 6 (IL-6) and Interleukin 8 (IL-8) were affected similarly by MUF in a range of experiments. Both Boga et al and Kiziltepe et al found that MUF had no significant impact on plasma levels of IL-6 and IL-8 in adult patients, although Boga et al found IL-6 and IL-8 were present in the ultrafiltrate suggesting MUF does have some capacity for removing these mediators (Boga et al. 2000; Kiziltepe et al. 2001). Grunenfelder et al compared MUF against no UF in both normothermic and hypothermic conditions in a cohort of adult patients and found that there was a significant decrease in IL-6 and IL-8 levels after MUF but again only in hypothermic patients (Grunenfelder et al. 2000). Additionally, Tassani et al compared combined ZBUF and MUF against no UF in a cohort of adult patients but only found a transient decrease in IL-6 and IL-8 levels after MUF (Tassani et al. 1999), whilst Onoe et al found that MUF resulted in a significant decrease in serum IL-8 levels in a cohort of adult patients (Onoe et al. 2001).

Chew et al found MUF had no significant impact on plasma IL-6 and IL-8 levels in a cohort of infant patients (Chew et al. 2002). Ming-Jung Wang et al compared MUF vs CUF in a cohort of paediatric and infant patients and found that plasma IL-6 and IL-8 levels significantly increased after both MUF and CUF, and that IL-6 and IL-8 were both found in the ultrafiltrate, although significantly more IL-6 was removed by MUF (Wang et al. 1996). Atkins et al found that there was no significant change in plasma IL-6 and IL-8 levels after MUF compared to no UF in their neonatal piglet study, however there was a significant decrease in IL-6 and IL-8 levels in the bronchoalveolar lavage and both IL-6 and IL-8 were found in the ultrafiltrate suggesting at least some level of successful removal from pulmonary system (Atkins et al. 2010). Atkins et al suggest that TNF-a removal may have led to reduced TNF-a neutrophil interactions after MUF which could be a potential explanation for the reduced alveolar IL-6 and IL-8 concentrations seen. (Atkins et al. 2010). Portela et al found that a significant decrease in IL-6 levels after ultrafiltration however their study was single armed and involved combined CUF and MUF (Portela et al. 1999). Whereas, Saatvedt et al found no significant change in IL-6 levels with MUF (Saatvedt et al. 1996). Journois et al suggest that one reason that IL-8 may struggle to be removed by ultrafiltration is because it is significantly bound by red cells so the free fraction that can be filtered is low (Neote et al. 1993; Journois et al. 1996).

Wei Wang et al found that when they compared MUF against no UF in paediatric patients, a significant decrease in IL-8 and ET-1 was only found after they had 'corrected for albumin' (the rational for which is explained in *1.3.10. Impact of MUF on Inflammatory Mediators*) (Wang et al. 1998).

The evidence for the impact of MUF on ET-1 is equally as varied. In addition to the findings of Wei Wang et al who stated that ET-1 could be freely filtered by MUF, Hiramatsu et al found that MUF led to significantly lower plasma ET-1 levels post-MUF compared to the CUF control group in a cohort of paediatric patients (Hiramatsu et al. 2002). However, both Pearl et al and Kiziltepe et al (studying infant and adult patients respectively) found that MUF had no impact on ET-1 levels (Pearl et al. 1999; Kiziltepe et al. 2001).

The impact of MUF on various other inflammatory mediators has been investigated by several groups.

In Yndgaard 2000 studied the impact of arterio-venous MUF on circulating endotoxins (described as potent inflammatory mediators that weigh approximately 5kD although often found circulating as protein molecule aggregates) in a single-arm prospective observational study in 20 paediatric patients. Yndgaard et al state that MUF had been an accepted procedure at their centre and they anecdotally report less haemodynamic problems and thus a control

group was not appropriate. MUF significantly reduced endotoxin load, a large portion of which was recovered in the ultrafiltrate. Yndgaard et al theorise that some of the major morbidities found in paediatric patients as a result of CPB including capillary leak, myocardial depression and systemic inflammatory response are caused by endotoxins. Despite the fact that MUF does not remove endotoxins already bound to cells and that it is acting after the endotoxins have already risen significantly, Yndgaard et al conclude that MUF may be beneficial (Yndgaard et al. 2000).

Ootaki et al found that MUF significantly increases fibrinogen, prothrombin and factor VII (Ootaki et al. 2002) whilst Portela et al found the MUF resulted in a significant decrease in ICAM-1 and VCAM-1 (Portela et al. 1999). Grunenfelder et al saw significant decrease in sI-CAM-1 for hypothermic patients and significant decrease in sE-selectin in all patients but not change in IL-2R levels in any group (Grunenfelder et al. 2000). Grunenfelder et al concede that despite the fact that they were able to demonstrate significant reductions in cytokines and adhesion molecules, these were not translated into significant clinical benefits for the patients in the study but suggest MUF may be more beneficial to high risk patients (Grunenfelder et al. 2000). Whilst Atkins et al found a significant reduction in transpulmonary TXB2 gradient after MUF (Atkins et al. 2010).

Pearl et al found that MUF did not result in a significant change in levels of LBT4, and despite the fact there was a significant yet transient decrease in TXB2 after MUF, this was no better than what was achieved with CUF (Pearl et al. 1999). Pearl et al conclude that any benefits seen as a result of MUF are likely to be due to reductions in haemodilution and total body water and that any improvements in cardiopulmonary function are not as a result of cytokine removal. However, their study is limited by a lack of properly analogous measurement intervals between the two groups and no randomisation leading to significantly older and heavier patients in the MUF group.

Ming Juih Wang et al found significantly increased plasma elastase after MUF and elastase present in the ultrafiltrate, but the increase was not significantly higher than that seen in the CUF group. Tassani et al found no significant change in IL-1 or IL-10 levels after MUF (Tassani et al. 1999) whilst Boga et al saw no significant change in neopterin after MUF (Boga et al. 2000) Similarly, Kiziltepe et al found no significant change in IL-10 levels after MUF suggest that clinical benefits related to the use of MUF are by some other mechanism than the removal of inflammatory mediators (Kiziltepe et al. 2001). Whilst Chew et al state that MUF had no significant impact on levels of C-reactive protein (CRP), complements C3d and C4d, nor on the concentrations of IL-1ra or neutrophil elastase (Chew et al. 2002) and Saatvedt et al found no significant change in C3a or C3bc with MUF (Saatvedt et al. 1996).

Several other groups have postulated why a large molecule like TNF-alpha appears to be successfully filtered whilst smaller molecules such as IL-6 and IL-8 are not.

Ming-Juih Wang et al explain that the reason that, in their study, TNF-α was the inflammatory mediator most successfully removed by both UF procedures of the four being measured cannot be explained by their respective molecular weights alone. The ultrafilter being used in their study was only able to remove molecules below 17kD, TNF-α has a molecular weight in the range of 17-50kD whilst IL-6 is in the range 20-30kD and IL-8 is far smaller at 8-10kD (Wang et al. 1996).

Tassani et al noted that the molecular weights of the cytokines in their study were far lower than the cut-off point of the ultrafilter used and thus molecular weight could not be an explanation for why some of these cytokines were more successfully removed than others (Tassani et al. 1999). Grunenefelder et al suggest that, based on the results of their study, the filtration capabilities of ultrafilters used in MUF are not determined by molecular weight alone and that the shape, conformation and configuration of molecules may be more important.

Absolute measurements from Wei Wang et al's study would have suggested that neither IL-8, endothelin nor TNF-alpha could be filtered whilst somehow TNF-alpha levels in fact increased (probably due to the systemic inflammatory response). Once Wei Wang corrected for albumin the story was quite different. The smaller molecules IL-8 and endothelin levels in fact significantly decrease whereas the TNF-alpha levels remain the same which would suggest that in fact the smaller molecules ARE able to be filtered (this was corroborated by the fact that ultrafiltrate levels of TNF-alpha were significantly lower than the blood levels whilst IL-8 and endothelin levels were similar) which would make much more sense based on the size of the respective molecules and the molecular weight filterability cut off of the ultrafilters being used.

Furthermore, as seen earlier (*1.3.8. Impact of MUF on Coagulation*), Ootaki et al found that 'corrected for albumin concentrations' of platelets and coagulation factors were significantly lower than the absolute concentrations, lending weight to the impact such corrections can have (Ootaki et al. 2002). Correction for albumin is performed in order to account for increasing Hct (concentration of the blood) leading to a larger fraction of a sample volume being made up from cytokines and thus giving the appearance of increasing absolute levels of cytokines, whereas in fact the cytokines levels are not being selectively altered (Onoe et al. 2001). No other studies explicitly described correcting for albumin in this way and it would be interesting to see if their finding would be similarly altered if they had done so.

Even for given inflammatory mediators there is variation in the reported efficacy of removal by MUF between groups. This variation could be explained by differences in method, particularly in regards to the ultrafilters used.

1.3.11. MUF related complications

Theoretically, passing blood through an ultrafilter may lead to an increase in red blood cell damage and haemolysis caused by high transmembrane pressure applied across the control membrane and high shear forces at the boundary wall (Holt et al. 1982; Moore et al. 2000) along with adhesion to the control membrane (Wang et al. 1998). However, if this were the case one might expect to see significant rises in serum free haemoglobin, released from the damaged cells, in patients receiving UF. Increases in free haemoglobin during CPB do occur yet rises specifically attributable to the UF procedure have not been observed (Karliczek et al. 1986; Nakamura et al. 1990) and moreover, Wang et al found significantly lower free haemoglobin in the blood 24hrs post-MUF compared to the control group (Wang et al. 1998). Additionally one might expect the free haemoglobin to have an adverse affect on renal function (Wang et al. 1998) but renal function has not been shown to be adversely affected by MUF (Naik et al. 1991a; Wang et al. 1996). Nevertheless, design of ultrafilters must bear in mind the potential for haemolysis and act to minimise it, whether by reducing flow induced shear forces or transmembrane pressure induced shear forces.

Another potential concern with MUF is that the additional exposure to foreign surfaces within the ultrafilter may invoke or perpetuate an inflammatory response (Grunenfelder et al. 2000; Moore et al. 2000). When the ultrafilter material used includes nylon, cuprophane or cellulose acetate, significant immunologic reactivity can occur, however this is lessened when polyacrylonitrite or polysulfone materials are used (Journois et al. 1996; Moore et al. 2000). Based on the evidence presented in this chapter it is clear than the beneficial effects of MUF more than outweigh any detrimental effect brought about by additional exposure to foreign surfaces, though amplification of these benefits might be achievable with circuit design which considers this drawback.

The major source of disquiet related to the inflammatory response when using MUF would be its possible indiscriminate filtration of both pro-inflammatory and anti-inflammatory mediators. The group highlight the evidence from Marchant and Eppinger (Marchant et al. 1994; Eppinger et al. 1996) showing the potent inhibitory effect IL-10 exerts on TNF-α production in tissue macrophages as well as that of other cytokines including IL-1b, IL-6 and IL-8. Removal of anti-inflammatory mediators would seemingly be counter-productive to the purpose of MUF but

Tassani et al and Kiziltepe et al found no significant reduction in IL-10 after MUF. The possibility of MUF removing beneficial substances from the blood remains however.

Nakamura et al noted that ultrafiltration resulted in some loss of platelets, fibrinogen and immunoglobulin (Nakamura et al. 1990), the loss of platelets is likely to be caused by their adhesion to the ultrafilter membrane (Wang et al. 1998). Additionally there has been concern over the loss of administered heparin as it can be freely filtered (Holt et al. 1982), however, Williams et al found that plasma concentrations of heparin increased after V-V MUF (Williams et al. 1997). For these reasons, perfusionists must closely monitor anticoagulation status of the patient when MUF is employed.

As mentioned before, the path through the ultrafilter in an A-V MUF configuration is equivalent to an arteriovenous shunt. No adverse effects of this have been found thus far and in fact Naik, Knight and Elliott suggest that it may be advantageous in preventing pulmonary hypertensive crises by delivering warm, oxygenated blood to the pulmonary circulatory system (Naik et al. 1991a).

There is a theoretical possibility of intercompartmental fluid shifts and/or hypotension in response to the rapid rate of fluid extraction via MUF possibly affecting coronary blood flow and myocardial perfusion (Naik et al. 1991a; Grunenfelder et al. 2000). However, it is possible that the majority of fluid movement may in fact be from the interstitial fluid compartment rather than the intracellular or intravascular (Naik et al. 1991a).

Due to the fact that MUF takes place after CPB is complete, there is an inherent delay to decannulation if the technique is utilised. This could be problematic if the cannulae are obstructive and there is some hindrance to myocardial perfusion and blood flow (Naik et al. 1991a; Grunenfelder et al. 2000), however the time is well spent if used to achieve haemostasis and if MUF results in significant improvements in patient condition, which based on the evidence seems to be the case. Naturally though, any reduction in MUF time whilst still achieving these improvements would be welcomed.

Perhaps the most significant consideration when discussing the use of ultrafiltration would be the extra cost in providing this intervention. Luciani et al made a cost analysis of MUF based on their study from 2001. The MUF procedure was responsible for an additional $85 in surgery cost (entirely comprised by the cost of the ultrafilter) but was more than covered by the associated reduction in postoperative care which was calculated based on estimates of $1000/day ICU stay, $500/day ward stay and packed RBC transfusion ($500/unit) and totalled

$6994 for control patients and $6378 for MUF patients, a difference of $616, resulting in an overall saving of $531 (Luciani et al. 2001).

1.3.12. Summary

In summary, since the emergence of MUF in 1991, the technique has been the subject of a vast amount of research into its efficacy in improving various biological and clinical outcomes. There is a clear body of evidence to show that MUF improves both cardiovascular and pulmonary function whilst reducing total body water increase after CPB, improving coagulation and reducing transfusion requirements. Additionally there is emerging evidence to suggest that MUF also improves neurological function. Studies into the impact of MUF on the duration ICU and hospital stays tend to find little reduction; however, as mentioned previously (*1.3.9. Impact of MUF on Length of Intensive Care Unit/Hospital Stay*) it is possible that rigid standardised clinical, nursing and bureaucratic procedures could result in patients being treated according to the procedure they received rather than their individual condition, thus delaying discharge. Evidence regarding the impact of MUF on inflammatory mediators is mixed, there are a large number of studies which show MUF is capable of removing some inflammatory mediator substances, however, there does not appear to be much consensus as to which mediators are removed. More studies into this area are required before any benefit can be claimed. Although MUF has become more widely used in recent years, there is still space for development, including development which will broaden its availability.

1.4. Thesis Objective

This thesis will detail the process and outcomes of the development of a super-adsorber based haemofiltration technology for use in paediatric cardiopulmonary bypass. More specifically the device will involve the integration of the existing technique of modified ultrafiltration with a new superabsorber technology with the aim to create a novel device which has the ability to extract excess fluid from flowing blood, suitable for clinical use within a cardiopulmonary bypass circuit.

1. The device is anticipated to achieve passive, chemically driven extraction of this excess fluid at a fixed blood flow rate, with little to no adjustment of other necessary inputs.

2. Haemoconcentration should occur in a timely manner, comparable to current modified ultrafiltration procedures, measured by achieving a suitably rapid rise in haematocrit.

3. The haemoconcentrated blood should be of good quality with minimal damage to the constituent parts of the blood, specifically maintaining as much as possible the populations of red blood cells and platelets. In addition, demonstration of removal of some pro-inflammatory mediators would be advantageous.

4. Development of the device should always proceed with consideration to the aim that the device will be deployed in a clinical setting and thus the procedures required to achieve the aforementioned objectives make reasonable expectations of, or where possible remove, input from clinical staff.

Chapter 2

Device concept

2. Device Concept

The new device being proposed is based on a combination of the existing MUF technique and emerging superabsorber technology. This chapter will look at the fundamental principles of both elements with a view to their combination and at relevant considerations needed to develop a device suitable for this function.

2.1. Principles of Ultrafiltration

The basic principle of ultrafiltration is the separation of low molecular weight solutes from a fluid with the aim of retaining either the filtered fluid now free of the solutes or, in the case of MUF, a fluid containing a higher concentration of those solutes.

Conventionally the driving force for the separation of the solutes from the fluid is a hydrostatic pressure difference, (generally between 0.1 and 0.7MPa (Nilsson et al. 2007)) applied across a control membrane (Moore et al. 2000). By increasing the pressure of the unfiltered fluid or, more commonly, applying a negative pressure to the effluent side of the control membrane, a transmembrane pressure (TMP) is generated, the magnitude of which is determined by the formula:

$$TMP = \frac{P_A + P_V}{2} + P_s$$

Where P_A is the pressure at the inlet of the ultrafilter, P_V is the pressure at the outlet of the ultrafilter and P_S is the additional negative pressure applied to the effluent side of the control membrane (Moore et al. 2000). The subscripts 'A' and 'V' have been selected since, for the case of A-V MUF, P_A would represent the arterial pressure at the inlet and P_V would represent the venous pressure at the outlet. Arterial blood pressure is typically between 60mmHg and 90mmHg, whilst venous pressure is generally 10mmHg (Iaizzo 2010). Applied pressure can vary widely and has been reported at between 100mmHg and 200mmHg (Groom et al. 1994; Mahmoud et al. 2005).

As the driving force for fluid extraction, it is clear that the rate of ultrafiltration is highly dependent upon TMP. In the case of MUF, TMP in turn is dependent not only upon the applied negative pressure P_S but also upon the patient's blood pressure. As we have seen earlier (*1.3.3. Impact of MUF on Cardiovascular Function and Haemodynamics*), blood pressure can alter significantly with MUF and thus the TMP may fluctuate during the procedure without changes to the applied negative pressure P_S.

Pousille's Equation tells us that there is an interrelationship between blood pressure, blood flow rate and viscosity.

$$Q \propto \frac{\Delta P . r^4}{\mu . L}$$

Where Q is flow rate, P is blood pressure, μ is viscosity and L and r are the length and radius of the blood vessel respectively (Viswanath et al. 2007).

In this way, for a fixed flow rate and vessel dimensions, blood pressure (and in turn TMP and ultrafiltration rate) can be significantly impacted upon by blood viscosity (defined as resistance to flow (Cinar et al. 1999)) which can be altered in several important ways related to CPB and MUF. Firstly, blood viscosity decreases with increased shear, therefore in high flow scenarios (when shear forces are high) resistance to flow is disproportionately lower than it would be in low flow scenarios. Secondly, blood viscosity will increase with increases in Hct (de Simone et al. 1990; Cinar et al. 1999) (concentration of formed elements in the blood can be said to 'thicken' the blood) and thus as the MUF procedure progresses and Hct rises, the increasing blood viscosity will lead to increased flow resistance. If a constant flow rate is maintained, blood pressure must be increased too. Thirdly, increased serum protein concentrations will result in higher viscosity (de Simone et al. 1990). Fourth, cooling of the blood (such as in hypothermic CPB) leads to significant increases in viscosity (Wheeldon et al. 1986). Fifth, Leukocyte activation can also lead to increased blood viscosity (Levy et al. 2008), as we have seen activation of leukocytes occurs in response to contact with the foreign surfaces of the CPB and MUF circuits.

The impact which these changes in viscosity ultimately have on ultrafiltration rate has been demonstrated, and is shown in Figure 8 (Paganini 1986; Moore et al. 2000).

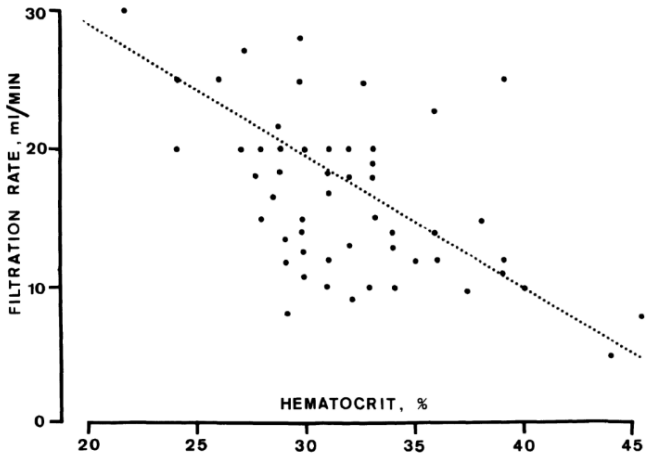


Figure 8: Relationship between filtration rate and haematocrit (Paganini 1986)

TMP is the major factor in determining the ultrafiltration rate but it is not the only factor, the physical parameters of the membrane itself will also have an important impact. Membrane pore diameter, pore density (the number of pores in a given area) and the pore length (equal to the membrane thickness) will all contribute to determining the ultrafiltration rate (Moore et al. 2000).

Figure 9 is a graph showing the generalised relationship between TMP and ultrafiltration rate (Jacobs 1996).

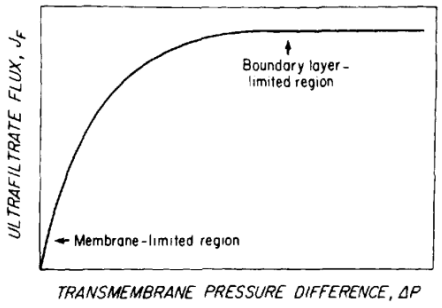


Figure 9: Relationship between TMP and ultrafiltration rate (Jacobs 1996)

Is can be seen that for a given ultrafilter, i.e. a fixed set of physical membrane characteristics, there is a relatively linear relationship between TMP and the ultrafiltration rate at low transmembrane pressures (Jacobs 1996). In this region of the graph, the ultrafiltration rate is said to be 'membrane limited' that is, the ultrafiltration rate is determined by the TMP and hydraulic permeability of the membrane (Jacobs 1996). Hydraulic permeability refers to the volumetric flow rate of a fluid per unit area of the membrane per unit pressure gradient (Jacobs 1996), and is determined by the physical characteristic of the membrane, pore dimensions etc.

However, at high TMP the ultrafiltration rate is no longer 'membrane limited' i.e. TMP and hydraulic permeability no longer influence ultrafiltration rate and instead ultrafiltration rate is 'boundary layer limited' (Jacobs 1996). This change occurs because there is a fixed number of pores available for ultrafiltration and there is an accumulation of serum proteins at the membrane surface, hence the term 'boundary layer limited' (Darup et al. 1979).

In the 'boundary layer limited' region of the graph the ultrafiltration rate is now determined by the blood flow rate, the serum protein levels and the device geometry (Jacobs 1996). Increased ultrafiltration rate can be achieved by increasing the blood flow rate, reducing serum protein levels and narrowing the channel diameter (Jacobs 1996). These changes will result in more rapid removal of the accumulated serum protein on the membrane surface and thus more available pores for fluid transfer (Darup et al. 1979).

Pore size is obviously an important factor in determining which solutes will be removed from the plasma. Theoretically, for ultrafilters with a pore diameter of 0.001-0.0035μm, molecules up to 20kDa should be filtered (Moore et al. 2000). However, as detailed earlier (*1.3.8. Impact of MUF on Coagulation*), several studies have questioned the use of molecular weight alone in determining which substances are filtered, suggesting other factors such as molecular conformation are important too (Wang et al. 1996; Tassani et al. 1999; Grunenfelder et al. 2000; Ootaki et al. 2002). Nevertheless, the efficiency with which an ultrafilter removes a given substance is generally considered related to molecular weight and is known as the sieving coefficient (Solem et al. 1987).

For a given membrane, the larger a given molecule, the less efficiently it will be removed from the blood (Solem et al. 1987). A measure of this is known as the sieving coefficient. Sieving coefficients range from 0 to 1 where 1 represents unrestricted transport of a substance across the membrane and 0 represents no transport across the membrane (Moore et al. 2000). Sieving coefficients are calculated by

$$S_O = \frac{C_F}{C_P}$$

Where S_O is the sieving coefficient, C_F is the concentration of a given solute in the ultrafiltrate, and C_P which is the concentration of the same solute in the bulk plasma water (Jacobs 1996). Therefore, we can see that a sieving coefficient of 1 can also be described as a substance which is found in equal concentrations in the bulk plasma as in the ultrafiltrate, and thus must be freely filtered.

Figure 10 is a graph showing the sieving coefficient curve for a range of membranes which depicts the efficiency at which substances of different molecular weight will be filtered by those membranes.

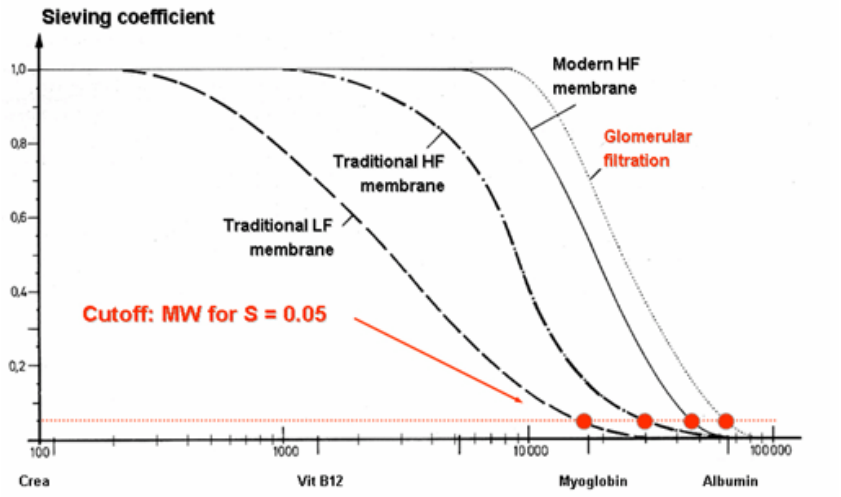


Figure 10: Sieving coefficient for various membrane types (Nederlof et al. 1984)

With many ultrafilters (and membranes in general) a molecular weight cut-off (MWCO) is provided as a 'dividing line' between the size of molecule which will cross the membrane, and those which will not. Generally this will refer to the lowest molecular weight solute which will be 90% retained by the membrane (i.e. a sieving coefficient of 0.1) although there is no set standard for this percentage retention figure (for example it could just as easily be defined as 95% retention) (Li et al. 2011). Care must also be taken when comparing MWCOs since the sieving coefficient will vary with TMP. Sieving behaviour can also be affected by whether or not there is interaction between the solute and the membrane (van Eijndhoven et al. 1995), and protein sieving coefficients in particular are impacted by pH and ionic strength (Saksena et al. 1994).

When talking about post-dilution haemofiltration (which MUF is an example of) it is important to note the impact of the distribution of a given solute in blood and consider that whilst ultrafiltration acts by removing solutes from the plasma water, the solutes can also be present in the red blood cells (Jacobs 1996). Clearance of solutes from the whole blood is more important that clearance from the plasma water when characterising the efficacy of an ultrafilter (Jacobs 1996). The graph in Figure 11 shows the relationship between whole blood clearance and sieving coefficients for two scenarios of solute distribution. The first scenario K=1 depicts a

solute evenly distributed between the plasma and the red blood cells; the second scenario depicts a solute which is wholly contained within the plasma.

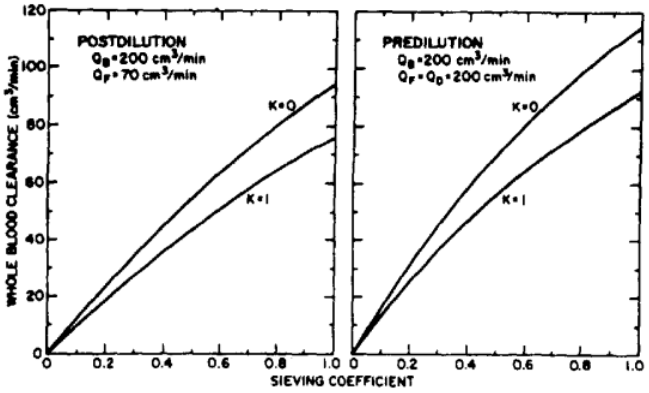


Figure 11: Relationship between whole blood clearance and sieving coefficient in different solute distributions (Jacobs 1996)

We can see that the higher the sieving coefficient, the more whole blood clearance is affected by solute distribution (Jacobs 1996). Removal of smaller substances within the blood cannot be as easily modelled or predicted by the same rudimentary principles that govern concentration of larger formed elements; for example the case of red blood cells and the measurement of increased Hct. Expectations of certain substances being removed of concentrated after MUF could easily fail to be met unless proper consideration is given to solute distribution within the blood and its impact on sieving coefficients and filtration. This may go some way to explaining the conflicting evidence surrounding the removal of inflammatory mediators from the blood.

2.2. Ultrafilters

2.2.1. Classification

Ultrafilters can be subdivided in a number of ways; according to the flow type (either dead-end flow or cross flow), according to the membrane element (either flat sheet, tubular or hollow fibre), or according to the membrane module (either stirred cell, plate and frame, spiral wound, tubular or hollow fibre) (Matsuura 1993; Nath 2008; Li et al. 2011; Foley 2013).

Flat sheet membranes are used in both dead-end flow (stirred cell) and cross flow systems (plate and frame, spiral wound), whilst tubular and hollow fibre membranes are used in cross flow systems (Li et al. 2011; Foley 2013).

2.2.2. Dead-end flow filtration

In dead-end filtration, flow is perpendicular to the membrane surface and those particles too large to pass through are stopped at the membrane surface (Seneviratne 2007). In this scenario there are two streams present; the feed stream (fluid entering the system) and the permeate stream (fluid which has successfully passed through the membrane) (Seneviratne 2007). The retained particles build up at the membrane surface and form a 'filter cake' which obstructs further transport (Seneviratne 2007). The higher the solid concentration of the feed fluid, the faster the filter cake accumulates (Foley 2013) and consequently dead-end filtration is limited to low solid applications (Seneviratne 2007). Increasing TMP will increase the filtration rate but since the resistance to cake removal is linked to TMP it is not a linear relationship (Foley 2013).

At this point, the complex issue of filter cake should be briefly addressed.

2.2.2.1. Filter Cake

The rate at which fluid crosses a membrane per unit area can be thought of as a flux (J) with units m/s (Foley 2013). If we consider pure water (viscosity μ) moving across a membrane, the flux will be given by: (Foley 2013)

$$J = \frac{TMP}{\mu R_M}$$

Where TMP is the transmembrane pressure and R_M is the resistance of the membrane, a term derived from the properties of the membrane (membrane thickness, pore size, pore density, charge etc) (Foley 2013). If we then consider the additional impact filter cake has on resisting fluid transfer we must add to the resistance term giving: (Nath 2008; Foley 2013)

$$J = \frac{TMP}{\eta(R_M + R_c)}$$

where R_C is the cake resistance; a complex function of particle and fluid properties, TMP and 'cake compressibility' (Foley 2013). Just as R_M is related to the properties of the membrane, R_C is related to the properties of the cake. We can write R_C as (Foley 2013)

$$R_C = \alpha M$$

Where M is the mass of solid deposited per unit area of the membrane, a product of cake thickness (L_c), cake particle density (ρ_s), and cake particle volume fraction (φ_c) by (Foley 2013)

$$M = \rho_s \varphi_c L_c$$

and α is the mean specific cake resistance a highly complex term which can be derived from particle morphology using the Kozeny-Carman equation

$$\alpha = \frac{K {a_v}^2}{\rho_s} \frac{1 - \varepsilon_c}{{\varepsilon_c}^3}$$

where K is the Kozeny constant, ρ_s is particle density, a_v^2 is particle surface area per unit volume, and ε_c is the cake porosity (Foley 2013). If we assume the particles in the suspension are spherical and have diameter *d*, we can estimate mean specific cake resistance as:

$$\alpha = \frac{36K}{\rho_s d^2} \frac{1 - \varepsilon_c}{{\varepsilon_c}^3}$$

and, if we assume that K=5 (e.g. in gas absorption or distillation (Foley 2013)) we can estimate cake resistance as (Nath 2008):

$$R_C = \frac{180(1 - \varepsilon_c)L_c}{{\varepsilon_c}^3 d^2}$$

Clearly, the role of cake in the filtration process is highly complex, and this complexity is hugely increased when we consider that for MUF the feed fluid is blood, which is comprised of a variety of active biological formed elements of varying sizes, shapes, densities which will not only passively obstruct the membrane surface but in the case of proteins actively adhere to it (Wang et al. 1998; Ootaki et al. 2002). In the context of this thesis it will be sufficient to note the negative impact cake and other obstructive phenomena have on ultrafiltration rates and understand the ways in which different flow configurations and ultrafilter designs aim to prevent obstruction of the membrane pores.

2.2.2.2. Stirred cell modules

An example of dead-end flow filtration is the stirred cell system. In stirred cell systems fluid is fed into a pressurised cell which is divided laterally by a flat sheet membrane, the other side of which the filtrate is collected (Li et al. 2011; Foley 2013). A stirrer (typically magnetically controlled) is positioned very close to the membrane surface to generate tangential flow and

prevent cake build up and concentration polarisation (discussed further in *3.2.3.6. Concentration Polarisation and Membrane Fouling*) (Nath 2008; Li et al. 2011; Foley 2013). The feed fluid can be processed in several batches without adjusting the membrane (Nath 2008; Foley 2013).

2.2.3. Cross flow (tangential flow) filtration

Cross flow filtration (sometimes called tangential flow filtration) involves fluid moving tangentially to the membrane surface rather than perpendicularly, as was the case for dead-end filtration. Unlike in dead-end flow filtration where we had two streams (feed and permeate), in cross flow filtration there are three streams present; the feed stream moving parallel to the membrane surface, the *permeate stream* moving across the membrane surface as a result of the TMP generated by the *feed stream*, and the *reject stream* (sometimes called the *concentrate stream*) which is comprised of the solids which fail to cross the membrane and instead move tangentially to the membrane surface scouring it of deposits in the process (Seneviratne 2007). The movement of the reject stream acts to reduce cake built up and mitigates concentration polarisation and consequently maintains good conditions for fluid transfer over longer periods (Seneviratne 2007; Nath 2008; Li et al. 2011). Maintaining good ultrafiltration rates is advantageous when uninterrupted operation is required and prevention of membrane adhesion is particularly important. The streams in a cross-flow filtration system are shown in Figure 12.

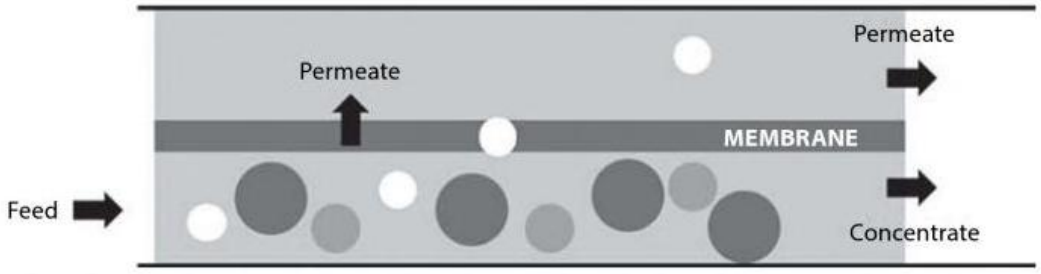


Figure 12: Cross flow or tangential filtration streams (Kucera 2015)

In addition to the reject stream scouring the membrane surface, cross flow filtration systems can employ 'back pulsing' which involves a short period of reverse flow tangential to the membrane surface to scour it from a different direction (Nath 2008). A similar procedure to back pulsing which also aims to clean the membrane surface is 'back flushing' in which movement of fluid across the membrane is periodically reversed removing cake and some particles which have penetrated the membrane (Foley 2013). Figure 13 illustrates the change in flux with backflushing:

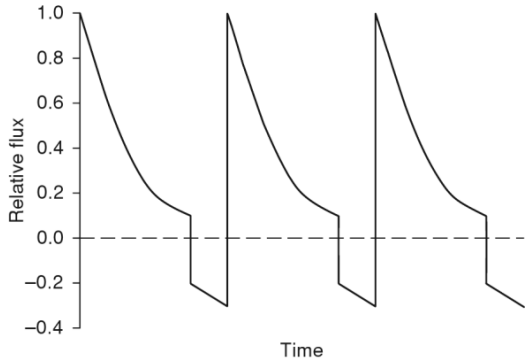


Figure 13: change in flux with backflushing (Foley 2013)

In cross flow filtration, the ultrafiltration rate is often assisted by applying a negative pressure to the filtrate side of the membrane (Li et al. 2011). Cross flow filtration systems are used in combination with flat sheet based membrane modules (plate and frame modules and spiral wound modules) as well as tubular based modules and hollow fibre based modules.

2.2.3.1. Flat sheet based membrane modules

Flat sheet cross flow modules consist of a shallow channel with rectangular flat sheet membranes, often accompanied by supporting structures, on one or both sides of this channel (Li et al. 2011). The feed fluid enters the channel at one end and the retained fluid leaves the channel at the other whilst the flow of fluid across is usually assisted by applying a negative pressure to the effluent side of the membrane (Li et al. 2011). Large scale flat sheet based cross flow membrane modules often utilise a cassette system whereby several flat membrane units are connected in series or parallel (Li et al. 2011). Flat sheet based cross flow membrane modules are advantageous as they are easy to clean or replace membrane elements and they perform well with viscous feed fluids, however they are limited by a low membrane area to module volume ratio and high holdup volume (amount of fluid remaining in filter after filtration) (Li et al. 2011).

Plate and frame modules involve a series of alternately stacked membrane plates and spacer plates around and through which the feed fluid passes with the filtrate passing through the membrane and being collected at each membrane plate (See Figure 14) (Matsuura 1993). The motion of the feed fluid, coupled with the fact that the spacer plate surfaces are intentionally uneven, generates turbulence at the membrane surface to minimise concentration polarisation and cake build up and improve ultrafiltration rates (Matsuura 1993).

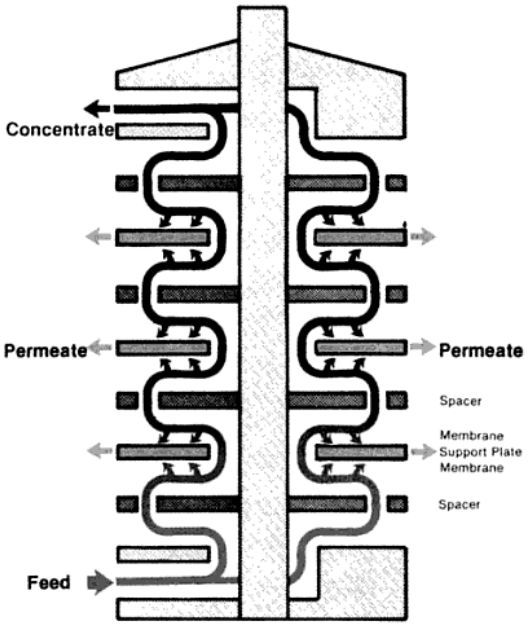


Figure 14: Plate and frame membrane module (Matsuura 1993)

The fact that the filtrate is collected at each individual membrane plate means that identification and replacement of faulty elements is straightforward (Matsuura 1993). Additionally the plate and frame design permits backflushing and thus is suitable for high fouling scenarios (Seneviratne 2007).

In spiral wound systems there is a single or series of membrane envelope(s) branching off from a central filtrate duct which form a filtrate channel (Matsuura 1993; Seneviratne 2007). Figure 15 shows the structure of a spiral wound system when unwound. Within the filtrate channel there is often a filtrate collector fabric (Seneviratne 2007). This assembly is rolled up into a spiral shape together with plastic mesh (which forms spacer channels for feed flow between the filtrate channels) and placed inside a tubular pressure vessel. The feed fluid flows down the spacer channels along the length of the tube and the filtrate is able to pass through the membrane and spiral towards a central filtrate duct where it is extracted (Matsuura 1993; Seneviratne 2007; Foley 2013). Spiral wound systems are compact which makes them susceptible to membrane fouling, however they are able to withstand high pressures and have a low cost per membrane area (Matsuura 1993; Seneviratne 2007).

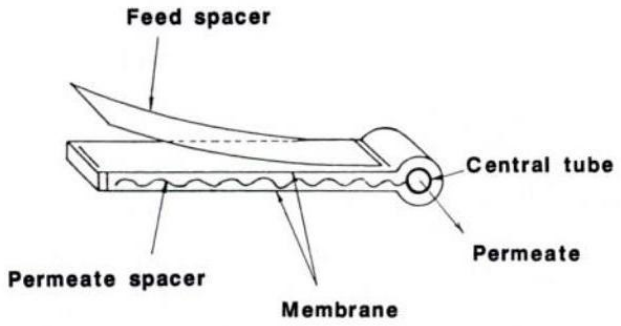


Figure 15: Spiral wound membrane module assembly(Matsuura 1993)

2.2.3.2. Tubular based membrane modules

In the tubular system, several membranes which are tubular in shape are encased in a container or 'shell'. The feed liquid flows inside these tubes and the filtrate moves radially through the membrane and out of the tubes and is retained by the module shell before exiting the system via an outlet port (Matsuura 1993; Foley 2013). In some scenarios the situation is reverse with the feed fluid moving on the outside of the membrane tubes and the filtrate passing to the inside. The major advantage of tubular systems is the high turbulence which reduces fouling and leads to high filtration rates (Foley 2013). Additionally, tubular systems are more robust and can operate at high pressures, and have the ability to be backflushed (Matsuura 1993; Seneviratne 2007). However they are disadvantaged by their large size, high cost, low membrane area to module volume ratio, difficulty in changing membranes, high cleaning costs and susceptibility to particle plugging (Matsuura 1993; Seneviratne 2007; Foley 2013).

2.2.3.3. Hollow fibre membrane based modules

Hollow fibre membrane based modules work by a similar principle to tubular modules but consist of large number of flexible narrow bore (<200μm) fibres bundled together in parallel to form a single unit with the open ends 'potted' together in a head plate (Matsuura 1993; Seneviratne 2007; Li et al. 2011). Like tubular membrane systems they can operate in both the inside-out and outside-in configurations (Li et al. 2011). Hollow fibre membrane based modules are advantageous in that they have a very large membrane surface area to module volume ratio making the overall unit small, moreover extra surface area for transfer can be added by simply adding more fibres in parallel (Matsuura 1993; Seneviratne 2007; Li et al. 2011). Additionally, hollow fibre membrane based modules have a small priming volume and the design allows for backflushing, however they are prone to fouling and particle blockages and the fibres are delicate and are very difficult to regenerate once contaminated or degenerated (Matsuura 1993;

Seneviratne 2007; Li et al. 2011). Figure 16 shows the structure of a typical hollow fibre membrane module.

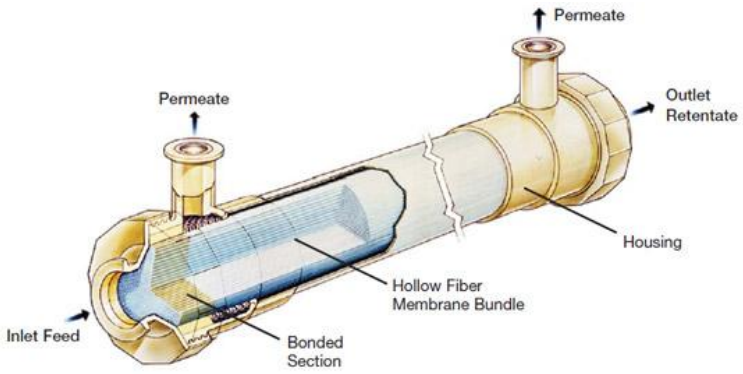


Figure 16: Hollow Fibre membrane module (Pall Corporation 2014)

2.2.4. Membrane materials

Traditionally, ultrafilter membranes were made from cellulose based materials which were preferable largely due to the fact that they are hydrophilic and are less prone to membrane fouling (Li et al. 2011), membrane fouling is discussed in more detail later (*3.2.3. Membrane fouling*). However, cellulose based membranes can only be used within tight pH boundaries and can degrade under sterilisation, therefore synthetic polymers became more widely used (Li et al. 2011). The most common synthetic polymers used in ultrafilters are; polysulfone (PS), polyethersulfone (PES), polyvinylidine fluoride (PVDF), polyamide (PA) and Polyacrylonitrile (PAN) (Li et al. 2011). Synthetic polymer membranes are generally hydrophobic which makes them more susceptible to fouling, although surface treatments exist to achieve hydrophilicity (Li et al. 2011).

If a membrane is to be used in a biomedical setting it must be able to withstand sterilisation. The most common forms of sterilisation for membranes in the biomedical industry use steam and high temperatures, chemical agents, organic solvents, whilst fouled membranes are generally cleaned with alkali detergents, thus membranes must be robust enough to withstand these treatments (Li et al. 2011).

2.2.5. Current Ultrafilters for MUF

Darling et al polled paediatric cardiac surgery centres across North America and found that for MUF all ultrafilters used were of the hollow fibre membrane type with the Hemocor HPH400 haemoconcentrator (*Minntech Corp., Minneapolis, MN, USA*) being by far the most popular followed by similar haemoconcentrators from COBE Cardiovascular (*Arvada, CO, USA*) and Dideco (*Mirandola, Italy*) (both now part of *Sorin Group, Milan, Italy*), and Gambro AB (*Lund, Sweden*) (Darling et al. 1998).

Recent studies involving MUF also use hollow fibre membrane ultrafilters. Table 9 shows the various ultrafilters which have been used in recent MUF related studies, plus the most widely used from Darling 1998, and specifications where known.

Study	Patient Type	Ultrafilter	Company	Membrane Material	Surface area	Priming Volume	MWCO
Zhou (2013)	Paediatric	BC20plus	Maquet Cardiopulmonary AG (Hirrlingen, Germany)	Polyarylethersulfone	0.22m²	17ml	65kDa
Papadopoulos (2013)	Adult	BC140plus	Maquet Cardiopulmonary AG (Hirrlingen, Germany)	Polyarylethersulfone	1.35m²	102ml	-
Atkins (2010)	Piglet	COBE HC 700 Midi	COBE Cardiovascular (Arvada, CO, USA)	Polyarylethersulfone	0.7m²	65ml	69kDa
Torina (2010)	Adult	H500	Braile Biomédica (São José do Rio Preto, Brazil),	Polyethersulfone	0.5m²	34ml	20kDa
Honjo (2010), Yokoyama (2009), Kotani (2008)	Paediatric	Aquastream 04	JMS Co Ltd (Tokyo, Japan)	Polyethersulfone	0.4m²	29ml	-
Perez-Vela (2008)	Adult	D30-NR	Minntech BV (Heerlen, The Netherlands)	Polysulfone	0.66m²	65ml	-
Darling (1998)	Paediatric	Hemocor HPH400	Minntech Corp. (Minneapolis, MN, USA)	Polysulfone	0.3m²	34ml	65kDa

Table 9: Specifications of current ultrafilters for MUF

The ultrafilters currently in use for MUF are very similar in design, all being hollow fibre ultrafilters using some variant of a polysulfone membrane material with priming volumes for paediatric patients in the range of 15-35ml and surface areas in the range of 0.2-0.4m².

We will now look in detail at two specific ultrafilters used for MUF to assess in more detail the expectations of performance and design which a new device might have to meet to compete directly with currently used systems. Since we are concerned with paediatric CPB we will look at two haemoconcentration devices specifically designed for use in MUF after paediatric CPB;

the Hemocor HPH Junior from Minntech Corp. (*Minneapolis, MN, USA*) and the Dideco DHF0.2 from Sorin (*Milan, Italy*). Table 10 shows a specification comparison of the two devices.

Specification	Hemocor HPH Junior	Dideco DHF0.2
Surface Area (m^2)	0.09	0.25
Fibre Material	Polysulfone	Polyethersulfone
Inner Diameter (µm)	200	200
Wall thickness (µm)	-	30
Priming Volume (ml)	8	30
Max blood flow rate (ml/min)	-	500
Min Pressure Drop (mmHg)	55	50-150 (min-max)
Maximum TMP (mmHg)	500	500
Unit Dimensions		
Length (mm)	150	145
Diameter (mm)	25 (internal)	55
Weight (g)	-	98
MWCO	65kDa	-

Table 10: Specifications for Hemocor HPH Junior and Dideco DHF.02 haemoconcentrator (Sorin Group USA 2009; Minntech Therapeutic Technologies 2014)

Figure 17 and Figure 18 show the sieving coefficients and ultrafiltration rates for the Hemocor HPH Junior and Dideco DHF0.2 respectively.

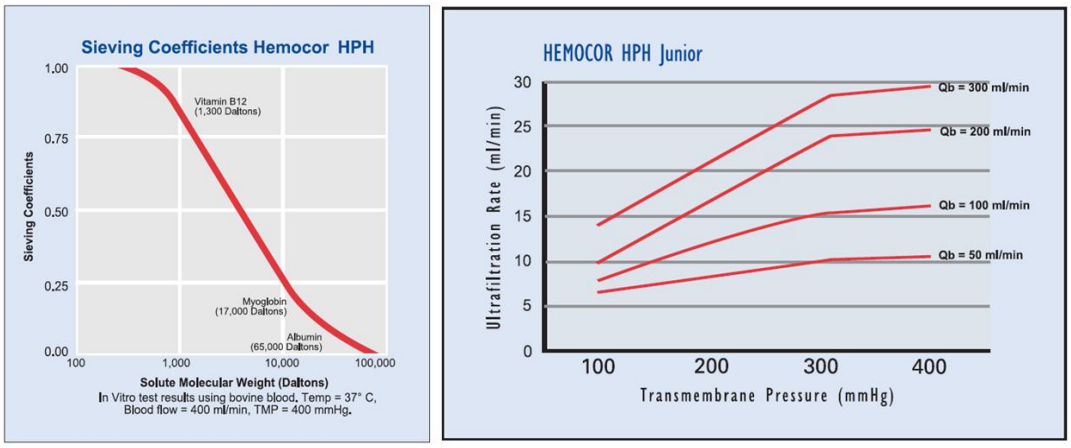


Figure 17: Sieving Coefficient and Ultrafiltration Rate for Hemocor HPH Junior (Minntech Therapeutic Technologies 2014)

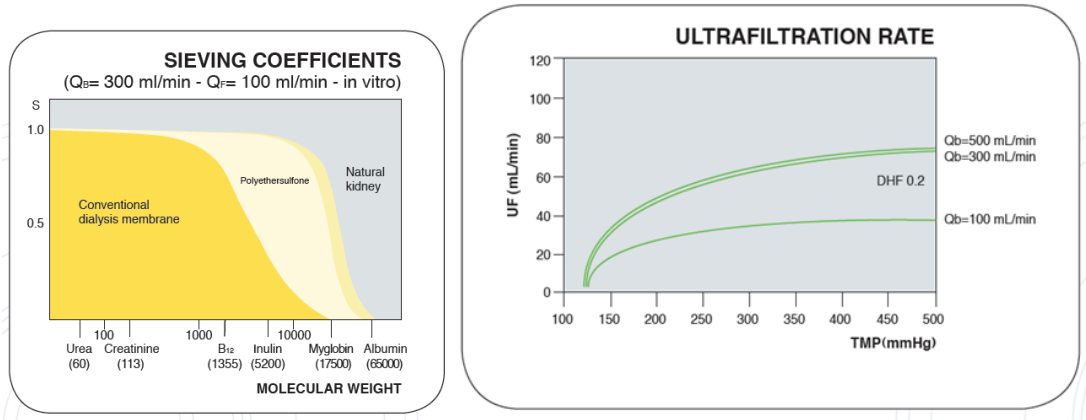


Figure 18: Sieving Coefficient and Ultrafiltration Rate for Dideco DHF0.2 (Sorin Group USA 2009)

Both Minntech and Sorin state that the HPH Hemocor Junior and Dideco DHF0.2 respectively are beneficial due to the fact that they do not require rinsing and thus can be placed in the extracorporeal circuit at any time before MUF. Minntech state that the HPH Hemocor Junior fibres are made from highly biocompatible glycerine-free polysulfone, whilst Sorin claim polyethersulfone is designed for blood contact.

A new device would be expected to compete on some level with the performance of these devices if it were to be adopted for MUF. It is important to bear in mind the capabilities of these devices when developing the proposed device and if possible attempt to replicate or surpass their performance. Both devices have very low priming volumes and are able to filter particles of high molecular weights in the region of albumin at 65kDa. Additionally at a flow rate of 100ml/min and TMP of 200mmHg the ultrafiltration rate is approximately 10-30ml/min.

Now we have assessed the current available options for MUF procedures in paediatric CPB, we have a better understanding of the expectations of a new MUF device. We are yet to determine which of the aforementioned ultrafiltration systems/modules would be most suitable for the proposed device. In order to decide this we must consider the filtration options in combination with the other major element of the proposed device, the superabsorber.

2.3. Superabsorber and Hemosep

2.3.1. Superabsorbent Polymers

As the name suggests, superabsorbent polymers (SAPs) are materials which are able to retain large volumes of aqueous solutions. In some cases these polymers are able to absorb up to 1500g of distilled water per gram of SAP (Mechtcherine et al. 2012). For technical purposes this swelling capacity is defined as the amount of 0.9 wt% saline solution that an SAP can absorb under unrestricted swelling conditions and after surface water has been removed by centrifugation (Elliott 2004). Chemically speaking, SAPs can be defined as cross-linked polyelectrolytes which swell and form hydrogels in the presence of aqueous solutions (Mechtcherine et al. 2012). Cross-linking is a method of joining molecules, and in the case of SAPs the most common type is the covalent cross-link in which covalent bonds between individual polymer chains form a three dimensional network (Elliott 2004; Brannon-Peppas et al. 2012).

The fact that SAPs are made of cross-linked chains means that they do not dissolve in water, but instead hold together and absorb it in a diffusion process (Masoodi et al. 2012). SAPs come as hard granule powders with particle sizes in the range of 45-850μm but form a soft rubbery gel when in contact with aqueous solutions (Masoodi et al. 2012). Aside from their large capacity for absorption of aqueous solutions, there are a number of important characteristics that define SAPs. SAPs must retain their original identity, that is, the swollen particles must have the physical integrity to resist flow and fusion with neighbouring particles (Brannon-Peppas et al. 2012). In SAPs this is achieved by the high osmotic pressures preventing fluid loss under load (Chatterjee et al. 2002). A further requirement of SAPs is when an excess of fluid is encountered, the particles must swell to their full capacity but not degrade or dissolve beyond that (Brannon-Peppas et al. 2012).

When there is limited space between the SAP particles the swelling process can lead to a 'gel blocking' phenomenon in which the swollen particles form a barrier between the fluid and the still dry particles (Chatterjee et al. 2002; Masoodi et al. 2012). This phenomenon is desirable in applications such as cable sheathing when the SAP is designed to prevent fluid propagation, but in applications such as nappies, where the intention is to retain as much fluid is possible, steps must be taken to avoid gel blocking, specifically the introduction of non-swelling elements (also known as fluff) which distribute fluid evenly to the dry particles (Chatterjee et al. 2002; Masoodi et al. 2012).

These swelling and non-swelling elements are representative of the two general mechanisms of fluid retention in absorbent products; capillarity and osmosis. Capillary suction pressure resulting from intermolecular forces draws fluid into the porous structure of the fluff, and is almost completely determined by the surface energy and pore dimensions of the absorbent, as well as the fluid surface tension (Chatterjee et al. 2002). Osmosis in SAPs (driven by the same thermodynamics as dissolution) involves the free energy of mixing driving fluid into the polymer, leading to the dissipation of polymeric chains throughout the solvent which is resisted by the cross-linking of those chains, resulting in a swollen element (Chatterjee et al. 2002). A more detailed description of the swelling phenomena specific to one particular SAP is described later (*2.3.2.1. Swelling*).

Osmotic pressure is the main driving force of SAP swelling and is proportional to the concentration of ions in the aqueous solution (which will be discussed in more detail later in *2.3.2.3. Other factors which influence SAP capacity*) (Mechtcherine et al. 2012). The ions in the SAP polymer network are close together and as such there is a high osmotic pressure inside (Mechtcherine et al. 2012). As water is absorbed this osmotic pressure is reduced by diluting the charges and the driving force for water uptake is diminished, reducing absorption rate

(Mechtcherine et al. 2012). As mentioned earlier in this section, the dissolution of the polymer as it absorbs water, driven by the swelling force, is counterbalanced by the retraction force induced by the cross-links in the network (Peppas et al. 2000; Chatterjee et al. 2002). The point at which these forces are equal is the swelling equilibrium, and no more absorption takes place (Peppas et al. 2000). The retraction forces are accompanied by a reduction in entropy of the chains as they become stiffer compared to their originally coiled state (Elliott 2004). The thermodynamics of the swelling process have been studied by other groups, most prominently Flory and Rehner (Flory et al. 1943), but due to the highly nonideal thermodynamic behaviour of the polymer networks, particularly in the case of electrolyte solutions (which, as it will emerge, will be applicable to the proposed device), the prediction of swelling behaviour is highly complex and not completely successfully (Peppas et al. 2000). In this thesis we are concerned with developing a device based on current SAP technology and not SAP technology development itself and thus an understanding of the thermodynamics of SAP swelling is not imperative.

An important principle concerning SAP swelling is that the degree of cross-linking in the system has a direct effect on the absorption capacity, that is the higher the cross-link density (average number of monomer repeating units between cross-links (Brannon-Peppas et al. 2012)), the higher the gel strength, but the lower the swelling capacity (Chatterjee et al. 2002; Elliott 2004). This is true for a fixed degree of neutralisation (amount of salts formed from the acid-base reactions that are pivotal in SAP production) but changing the degree of neutralisation has an impact on this relationship (Elliott 2004). Generally speaking, for a higher degree of neutralisation, there is a higher degree of swelling (Elliott 2004), this will be explained in more detail later (*2.3.2.2. Production of Polyacrylate Gels*).

There are three models based on the relationship between SAP capacity and the degree of neutralisation. The first model, Flory's theory, predicts capacity always increases with increased neutralisation (Elliott 2004). The second model, Hasa's theory, predicts a plateau in swelling is reached at a certain level of neutralisation and is based on the counter-ion condensation theory that beyond a certain neutralisation level free ions will condense on the polymer chains and thus will not contribute to osmotic pressure (Elliott 2004). The third model, Konak's theory, predicts a maximum capacity is reached at a certain neutralisation level, after which capacity reduces with further increased neutralisation (Elliott 2004). The relationship between capacity and neutralisation according to the three models is shown in Figure 19.

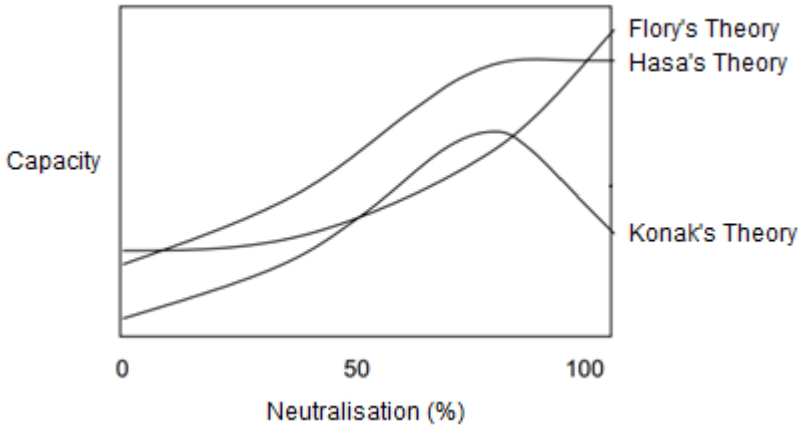


Figure 19: Three models of the relationship between SAP capacity and the degree of neutralisation (Elliott 2004)

The chemistry explaining this relationship is best explained through an example and is done so in 2.3.2. Sodium Polyacrylate.

As we have seen, reducing the cross-link density of an SAP leads to an increase in absorption capacity (Chatterjee et al. 2002; Elliott 2004). However, the caveat of this was a reduction in gel strength with reduced cross-link density. Reducing the gel strength means that under load, there is a reduction in absorption capacity and, to further exacerbate the problem an increase in 'gel blocking' (Elliott 2004; Jockusch et al. 2009). To improve the resilience under pressure of SAPs cross-link density can be increased on the surface only, creating a resistive shell, whilst enabling good absorption capacity (Elliott 2004; Jockusch et al. 2009). This process is called surface cross-linking and is shown in Figure 20.

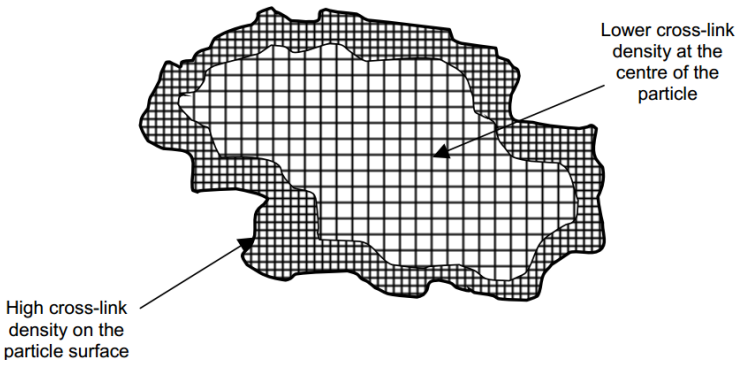


Figure 20: Surface cross-linking (Elliott 2004)

There are a wide range of applications for superabsorbent polymers including fire fighting, landscaping, cable sheathing, and food packaging, but their most common use is the hygiene industry which accounts for 98% of consumption of superabsorbers (Bullinger et al. 2009;

Masoodi et al. 2012; Mechtcherine et al. 2012). In the past, superabsorbers were made from hydrophilic materials such as chemically modified starch and cellulose and other polymers such as polyvinyl alcohol and polyethylene oxide, however modern superabsorbers are made from partially neutralised, lightly cross-linked polyacrylic acid which has been shown to have the best performance-cost ratio (Elliott 2004).

2.3.2. Sodium Polyacrylate

The superabsorber to be used in the proposed device is sodium polyacrylate based. Sodium polyacrylate has the ability to absorb up to 800 times its own weight in distilled water (Kotz et al. 2009; Gooch 2010). When dry, sodium polyacrylate takes the form of a coiled polymer chain on which there are two important groups; the carbonyl carboxylic acid (COOH) and carboxylate associated with sodium (COO^-Na^+) (Kotz et al. 2009; Gooch 2010). Sodium polyacrylate's ability to absorb water and swell has four contributing factors; hydrophilic chains, charge repulsion, osmosis and cross-links between chains (Gooch 2010).

2.3.2.1. Swelling

In the presence of an aqueous solution, sodium ions (Na^+) are disassociated from the carboxylate (COO^-) as shown in Figure 21 (Peppas et al. 2010).

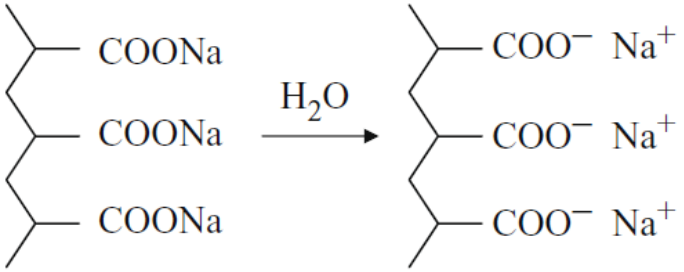


Figure 21: Disassociation of Sodium Polyacrylate (Peppas et al. 2010)

The polar water molecules are attracted to the positive sodium ions and the negative carboxylate groups by a hydration interaction (shown in Figure 22); this attraction is what makes SAPs hydrophilic (Elliott 2004; Kotz et al. 2009; Gooch 2010).

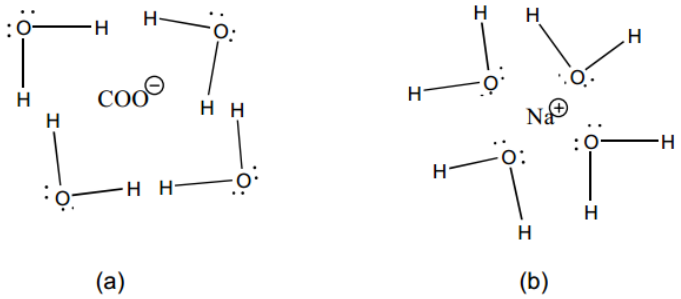


Figure 22:Polar water molecules are attracted to the positive sodium ions and the negative carboxylate groups by a hydration interaction (Elliott 2004)

At the same time, electrostatic charge repulsion between the negatively charged carboxylate group strands ($R\text{-}COO^-$) forces the polymer to unwind and uncoil and the expansion of the network allows more water molecules to enter the cavities between the molecules by capillary action and associate with the polymer chain (Bullinger et al. 2009; Kotz et al. 2009; Gooch 2010).

The association with the polymer chains is by hydrogen bonding (electrostatic interaction between molecules which have hydrogen attached to small electronegative atoms e.g. with oxygen in H_2O, in which the hydrogen atoms are attracted to the non-binding pairs on neighbouring oxygen atoms (Elliott 2004)) which hold the water molecules tightly within the network (Gooch 2010). Additionally the hydrogen bond dipole between the water molecules (the electronegative oxygen atom draws the hydrogen electron towards itself and the hydrogen atoms are attracted to the non-binding electron pair on other water molecules) decreases the energy and increase the entropy of the system further assisting uptake (Elliott 2004). This hydrogen bond dipole is shown in Figure 23.

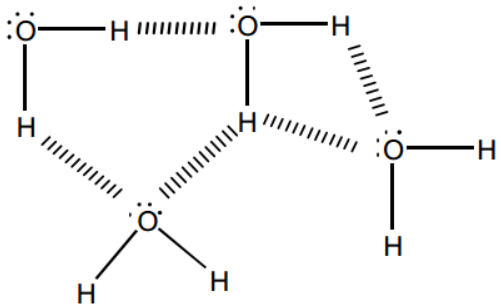


Figure 23: Hydrogen bond dipole between the water molecules (Elliott 2004)

As water enters the polymer network there is an osmotic pressure generated by the high ion concentration in the polymer from the sodium neutralised carboxylate (COO^-Na^+), which causes

yet more water molecules to enter the polymer along the diffusion gradient (Kotz et al. 2009; Gooch 2010).

The expansion of the network is what we see as the swelling of the sodium polyacrylate particles to form a gel.

2.3.2.2. Production of Polyacrylate Gels

Polyacrylate gels are produced from an aqueous monomer mix solution which is 25-40% acrylic acid by mass (Chatterjee et al. 2002; Mechtcherine et al. 2012). The solution is cooled to between 0°C and 10°C and transferred to a reactor where polymerisation is initiated by generating free radicals, usually through decomposition of peroxides (Chatterjee et al. 2002; Mechtcherine et al. 2012). Due to the inhibitory effect of oxygen on polymerisation, the reaction must take place in an inert environment (Chatterjee et al. 2002). This polymerisation reaction generates a large amount of heat and increases the viscosity of the solution to form a gel which is subsequently cut into pieces, dried, and ground to a powder (Chatterjee et al. 2002; Mechtcherine et al. 2012).

Chemically speaking, sodium polyacrylate (the salt) is produced when small acrylic acid (the weak acid) monomers are linked together by polymerisation, after which a reaction with sodium hydroxide (the strong alkali) produces negative charges on the polymer chains which are neutralised by positively charged sodium ions (Bullinger et al. 2009; Peppas et al. 2010). In this reaction, some of the ionised groups formed ($R\text{-}COO^-$) are able to combine with hydrogen atoms (H^+), produced by the self-ionisation of water, to produce a carboxylic acid group ($R\text{-}COO^- + H^+ \rightarrow R\text{-}COOH$) (Peppas et al. 2010). Consequently, a portion of the functional groups of sodium polyacrylate are deionised, and this is what leads to the differing degrees of neutralisation of SAPs that were described earlier (*2.3.1. Superabsorbent Polymers*) (Peppas et al. 2010). It was stated earlier (*2.3.1. Superabsorbent Polymers*) that, generally speaking, a higher degree of neutralisation leads to a higher degree of absorption capacity and swelling (Elliott 2004). It can now be seen clearly that the more neutralised groups (COO^-Na^+) on the polymer network the more overall capability there is for sodium disassociation and the absorption of water by the process described above. It was seen earlier in Hasa and Konak's theories on the relationship between neutralisation and absorption capacity (*2.3.1. Superabsorbent Polymers*) that at approximately 75% neutralisation a peak or plateau in capacity was reached (Elliott 2004). This peak occurs in some SAPs (such as sodium polyacrylate) because beyond this point, there is an excessive concentration of disassociated ions (e.g. Na^+) which will condense on the polymer chains (according to Manning's counterion condensation theory (Manning 1969)) and thus not contribute to osmotic pressure (Elliott 2004; Peppas et al. 2010).

2.3.2.3. Other factors which influence SAP capacity

Thus far, we have concerned ourselves with sodium polyacrylate and SAPs in general with regard to their interactions with pure water. SAPs can absorb 1000 times their own weight in distilled water, 300 times their own weight in tap water, and 50 times their own weight in saline (Bullinger et al. 2009). This huge reduction in capacity with saline is important to our application of SAPs since we are concerned with absorption of fluids isotonic to saline. It is therefore important to understand the reason for this capacity disparity.

Sodium polyacrylate is formed from a weak acid (polyacrylic acid) and consequently is a polyelectrolyte (seen by the ionisation of $R\text{-}COO^{-}Na^{+}$ in water) (Chatterjee et al. 2002). As we have seen, in order to maintain charge neutrality in the vicinity of the polymer chains after disassociation, the anion groups ($R\text{-}COO^{-}$) along the polymer chains in sodium polyacrylate prevent the counterions [Na^{+}] from leaving the gel resulting in a high concentration of ions in the polymer network (Chatterjee et al. 2002). This high concentration of ions in the polymer network is what creates the high osmotic pressure which is the driving force for water entering and swelling the polymer network (Chatterjee et al. 2002; Mechtcherine et al. 2012).

When a polyelectrolyte such as sodium polyacrylate is placed in an aqueous salt solution, a portion of the salt is able to move into the polymer network, however, the polyion network ($R\text{-}COO^{-}$) and counterions (Na^{+}) will obstruct the remainder (Chatterjee et al. 2002). The retention in the fluid of a portion of these ions reduces the osmotic pressure driving water into the polymer network and equilibrium is reached at a lower absorption capacity (Chatterjee et al. 2002; Peppas et al. 2010). Thus the absorption capacity of SAPs is highly dependent upon the ion concentration of the fluid to be absorbed (Mechtcherine et al. 2012). The reason for this phenomena is best explained with reference to 'Donnan equilibrium' which refers not only to an ion balance, but a thermodynamic balance between the water in the polyelectrolyte solution inside the polymer network, and the water in the salt solution outside of the polymer network, and explains why the polyion network and counterions restrict salt movement (Chatterjee et al. 2002; Peppas et al. 2010).

Salts and other low molecular weight electrolytes are able to pass into the polymer network and the degree to which they can travel, in combination with the difference in total ion concentrations between the phases, determines the swelling capacity (Peppas et al. 2010).

If we consider sodium polyacrylate in a sodium chloride solution, the completely disassociated ions (Na^{+}) at the surface of the polymer network tend to leave the polymer gel phase by thermal

motion (the disorderly, apparently random, motion of molecules (Atkins et al. 2014)) and consequently the immobile anion groups ($R\text{-}COO^-$) create an electrostatic field which attracts low molecular weight ions from the solution (which would be more abundant in a solution of higher salt concentration) into the gel phase (Nobel 2009; Peppas et al. 2010). This phenomena only occurs at the surface of the sodium polyacrylate gel (the boundary between the gel phase and the solution phase) as it is only here where ions (Na^+) can leave the gel, and as such, the electrostatic field forms as a double electric layer (Peppas et al. 2010). This double electric layer acts as a boundary which restricts motion of low molecular weight ions from the gel phase and the surrounding solution phase and creates a electrical potential between the two phases known as the 'Donnan potential' (Nobel 2009; Peppas et al. 2010). The Donnan potential thus interferes with the normal balancing of chemical potential and it is only when then components of diffusion and this electrostatic attraction balance that we have equilibrium, the 'Donnan Equilibrium' (Peppas et al. 2010). In this way the solution phase is overpopulated with ions and the osmotic pressure which drives water into the polymer network is reduced, reducing the absorption capacity of the SAP (Chatterjee et al. 2002). Thus it can be seen how salt concentration of the fluid to be absorbed can greatly impact on absorption capacity of SAPs and sodium polyacrylate.

Another factor which can impact absorption capacity is pH. Low pH solutions have a high concentration of H^+ ions which will protonate the polymer network and essentially cause it to no longer be a polyelectrolyte (Chatterjee et al. 2002).

Yet another factor which can influence absorption capacity is the presence of multivalent ions. Polyelectrolytes have the ability to bind soluble di- and trivalent metal ions (e.g. Ca^{2+} and Al^{3+}) through chelation at the carboxylate groups (Chatterjee et al. 2002; Gooch 2010; Mechtcherine et al. 2012). This acts as additional cross-linking which increases the restraining force of the polymer network, thus reducing the absorption capacity of the SAP (Mechtcherine et al. 2012).

The impact of salt concentration, pH, and the presence of multivalent ions can be seen in the graphs in Figure 24 and Figure 25.

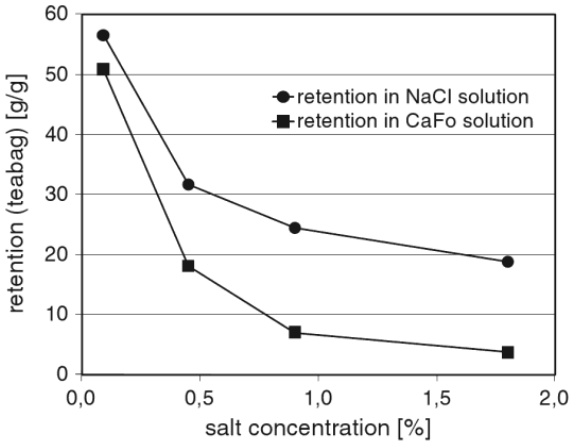


Figure 24: Absorption capacity in solutions containing sodium chloride (NaCl) and calcium formate (CaFo) (Mechtcherine et al. 2012)

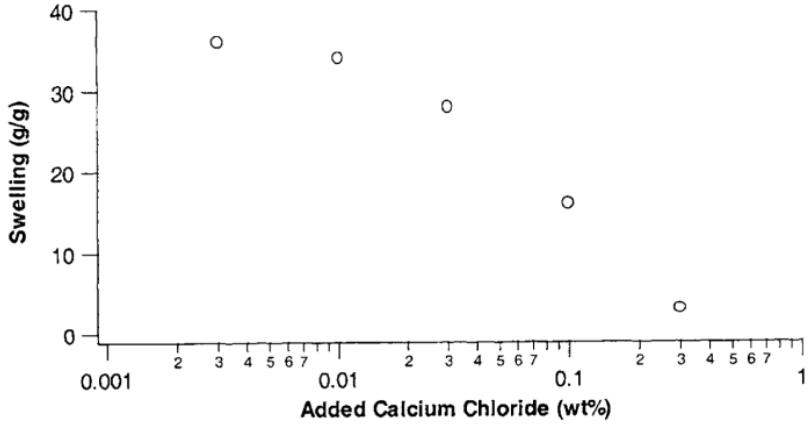


Figure 25: Impact of additional calcium ions on swelling of cross-linked sodium polyacrylate in 0.9% NaCl solution (Chatterjee et al. 2002)

2.3.3. Hemosep

The superabsorber is currently utilised in a device called Hemosep. Hemosep was developed at the University of Strathclyde Department of Biomedical Engineering by Prof Terry Gourlay and is designed to recover, concentrate blood spilled during cardiac and trauma surgeries allowing it to be transfused back to the patient (Gunaydin et al. 2013).

The device consists of a sodium polyacrylate superabsorber entirely encompassed within a control polyethersulfone hydrophilic membrane which, in turn, is encompassed within a polymer bag. The polymer bag is filled with the diluted blood which is gently agitated and processed for a period of 40 minutes. During this time, blood plasma is free to cross the control membrane and be absorbed by the superabsorber, where it is held separately from the

remainder of the blood. The concentrated blood can then be collected and returned to the patient by transfusion. In Hemosep, the sodium polyacrylate superabsorber is impregnated within a cellulose support material. Use of this support material (also known as 'fluff') is a commonly employed technique to assure good distribution of fluid to dry particles of SAP, intended to prevent the aforementioned gel-blocking phenomena (Chatterjee et al. 2002; Bullinger et al. 2009; Masoodi et al. 2012).

Hemosep was clinically trialled in over 100 open-heart surgeries and demonstrated a reduction in blood transfusion requirements and inflammatory reaction whilst preserving normal clotting function (Gunaydin et al. 2013). Hemosep is now being sold worldwide.

2.3.4. Utilising the superabsorber

In the proposed device the superabsorber technology successfully employed in Hemosep will be combined with principles of ultrafiltration to create a superabsorber-based haemofiltration technology for use in paediatric MUF. The superabsorber will be used in conjunction with a control membrane to remove excess plasma from the blood whilst retaining the erythrocytes, leukocytes and thrombocytes, thus causing haemoconcentration. As seen earlier (*2.3.1. Superabsorbent Polymers*), the ability for the superabsorber to absorb vast quantities is based on an osmotic pressure generated by concentration gradient. In the proposed device, this concentration gradient will greatly subsidise the pressure gradient which is utilised in current ultrafilters for paediatric MUF. This will remove the need for high flow rates and the application of negative pressure to the effluent side of the membrane which are currently employed to generate the driving force for ultrafiltration. By removing these factors, the proposed device should reduce the shear forces which the blood must endure which may be beneficial in reducing haemolysis. Additionally, by removing the need for the application of a negative pressure to the effluent side of the haemofilter, the proposed device will be less complex than current MUF systems as there will be no need for a vacuum pump system. This reduction in complexity means the proposed device will encompass everything needed for the MUF procedure in a single small, lightweight and simple entity.

2.3.5. Retention of different blood components

The method by which Hemosep concentrates blood is subtlety different to the way modern ultrafilters achieve haemoconcentration. Hemosep removes large volumes of plasma whilst retaining only the erythrocytes, leukocytes and thrombocytes, whereas, as was seen earlier (*2.2.5. Current Ultrafilters for MUF*), modern ultrafilters used in paediatric MUF are able to retain substances over 65kDa. When proposing the application of the Hemosep-like device to the

ultrafiltration procedure it is important to understand the nature and consequences of this distinction.

Blood plasma represents approximately 55% of the total blood volume and in turn is comprised of over 90% water, 7% plasma proteins and 2% additional solutes including; electrolytes, enzymes, gases, nutrients, hormones, and waste products (Draper et al. 2008).

Water is by far the most abundant component of plasma, making up over 90% of its volume and thus the difference in removal of a volume of plasma versus removing a volume of water alone is only based on a small fraction of plasma constituents. There are many mineral salts and ions dissolved in the plasma including sodium chloride, metal ions such as calcium and iron which are important for biological functions and are carried in plasma proteins, and buffer salts which maintain a constant pH (Schaller et al. 2008). Many types of low molecular weight components are contained within the plasma including; carbohydrates such as glucose, amino acids, nucleotides, hormones, lipids, vitamins, urea etc (Schaller et al. 2008). High molecular weight substances in the plasma include polynucleotides such as DNA and RNA, peptides and glycoproteins, polysaccharides, oligosaccharides, and oligonucleotides (Schaller et al. 2008). Gases such as oxygen and carbon dioxide are mostly bound when carried in the blood but are also found dissolved (Draper et al. 2008). 98.5% of oxygen in the blood is bound to haemoglobin but the remaining 1.5% plays an important role as it is this fraction which is detected by chemoreceptors and used by the brain as a measure of blood oxygen content (Draper et al. 2008). Similarly, 75% of carbon dioxide in the blood is carried as carbonic acid and 20% is bound to haemoglobin as carbaminohaemoglobin, but the remaining 5% is dissolved as used detected by sensory receptors as a measure of blood pH (Draper et al. 2008).

The lower molecular weight elements of the blood plasma such as those listed above and unbound mineral salts and ions and dissolved gases, are not retained in the blood by modern ultrafilters for paediatric MUF or Hemosep. The difference comes with the higher molecular weight blood plasma components such as those blood plasma proteins involved in blood coagulation and fibrinolysis, the complement system, the immune system, transport and storage, as well as hormones, cytokines and growth factors (Schaller et al. 2008). Key plasma proteins are synthesised in the liver such as; albumin which carries fat-soluble hormones and fatty acids, globulins which have an immune function, and fibrinogen which is essential to blood clotting (Draper et al. 2008). Inability to retain these products may put the device at a disadvantage when clinicians compare it against current ultrafilters for paediatric MUF.

Due to the fact that the proposed device will remove plasma, including some important substances, it may be more suitable in applications where the ability to retain these factors is

secondary to the ability to perform haemoconcentration at all, or at a reduced cost. Low resource settings are a perfect example of this.

2.4. Applications for the proposed device

Low resource settings can encompass; developing countries where clinical centres are poorly equipped due to poverty and/or remote locations, as well as emergency scenarios where it is not possible to transport the patient to an ideal clinical setting due to time and/or physical constraints e.g. disaster relief and military operations. As mentioned earlier (*2.3.4. Utilising the superabsorber*), the proposed device is also advantageous in these applications as it removes the need for a vacuum pump system thus reducing complexity and meaning the proposed device will encompass everything needed for the MUF procedure in a single small, lightweight and simple entity. More generally, MUF devices reduce the requirements for blood transfusions which are expensive, risk transmission of infectious diseases and are unavailable in a timely manner to many living in low resource settings. Special considerations for medical devices are discussed in *7. Considerations for Medical Devices in Low Resource Settings.*

2.5. Design considerations for the proposed device

Based on what has been learnt about the ultrafiltration process and the way in which the superabsorber will act to concentrate the blood, the following design consideration for the development of the proposed device have been made:

The proposed device will be cross flow system as this is most applicable to the continuous flow present in CPB circuits and will more successfully limit filter cake and protein adhesion to the membrane.

The proposed device will be a single flat bed chamber as this configuration best allows for the swelling of the superabsorber and requires only a single surface to be managed. This system allows use of the same membrane material as Hemosep which has demonstrable success in haemoconcentration. Additionally, this configuration is by far the simplest and cheapest to produce, and make modifications to, in-house and thus a rapid succession of prototypes can be developed.

The development and testing of the various prototypes of the proposed device and the impact the results had on the device design is discussed in the next chapter.

Chapter 3

Device development

3. Device Development

3.1. Experiment Design

As stated earlier, the intended purpose of the proposed device is to perform haemofiltration as part of the MUF procedure in paediatric CPB surgery, potentially in low resource settings, with a view to superseding current technologies which are not developed with this specific application in mind. In order to develop a device fit for purpose it is important to closely mimic typing operating conditions present in MUF procedures during testing. The work of several other groups was taken into consideration when designing the experiment protocol to ensure a suitable testing regimen.

3.1.1 Circuit design

In the original description of the MUF procedure by Naik, Knight and Elliott (Naik et al. 1991a), the MUF circuit was depicted as in Figure 26.

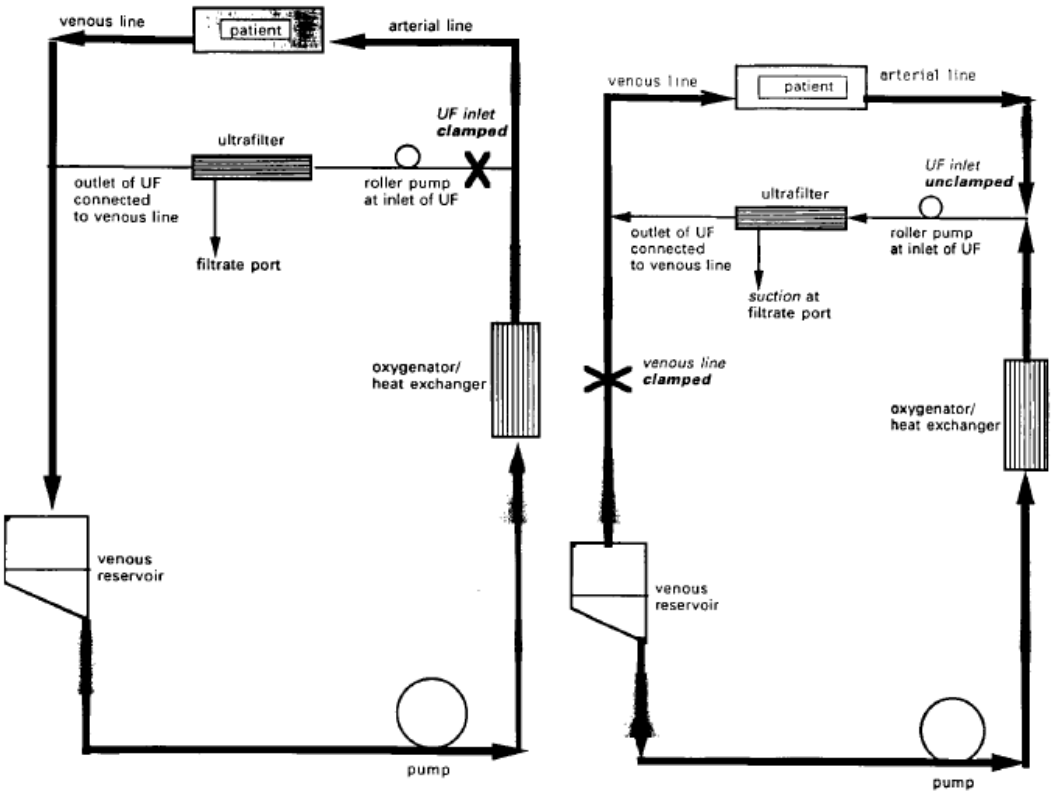


Figure 26: Original depiction of MUF circuit from Naik, Knight and Elliott, modified placement of the ultrafilter in the CPB circuit during CPB (left) and after CPB, during ultrafiltration (right) (Naik et al. 1991a)

The MUF circuit is a simple loop in which blood is first drawn through the arterial line by a roller pump before passing through the ultrafilter and returning to the patient via the venous line. This circuit is clearly very simple and is it easy to mimic this loop very closely. The one simplification which will be implemented is the removal of the venous reservoir 'feeder' line. As can be seen from Figure 26, the venous line is clamped immediately after CPB which leaves a residual volume in the venous reservoir. The venous reservoir volume is connected as a branch of the MUF loop and is thus able to feed blood into the MUF loop and replace the volume lost as ultrafiltrate. Instead of having this feeder line as part of our test circuit, the 'venous reservoir volume' will simply be combined with the 'patient blood volume' as they both feed into the ultrafilter at the same point and can, for the purposes of our initial lab testing, be considered the same blood volume. This combined volume will be represented by an appropriately sized volume of blood stored in a polypropylene beaker. Silicone tubing will be used as the arterial and venous lines and a Stockert pulsatile roller pump system *(Stockert, Munich, Germany)* without the pulsatile control system will be used to drive the flow through the haemofilter. Outflow rate will be monitored with a Transonic TS410 tubing flow module *(Transonic Systems Inc., Ithaca, NY)* In this way we have a simple lab-appropriate close approximation to the MUF circuit which will be used to test the ability of proposed device to haemoconcentrate blood. The circuit can be seen schematically and photographically in Figure 27 and Figure 28 respectively.

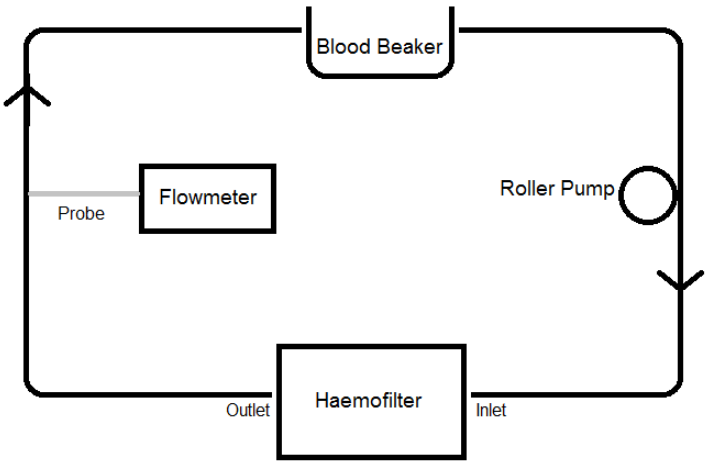


Figure 27: Schematic of MUF test circuit used in laboratory testing

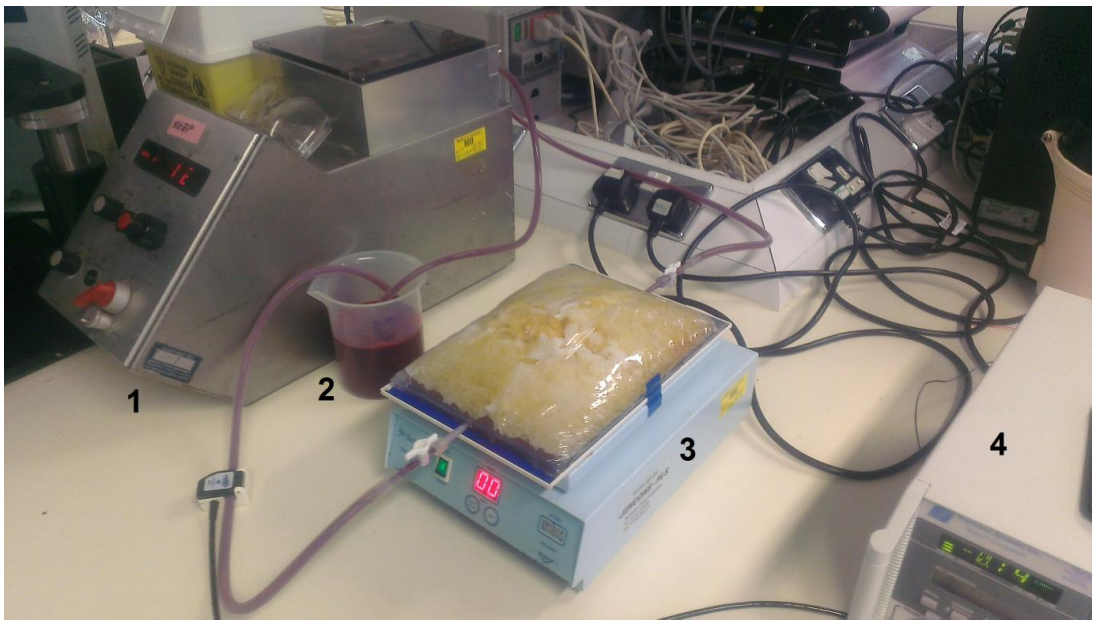


Figure 28: Photograph of MUF test circuit used in laboratory testing, 1) roller pump, 2) blood beaker, 3) haemofilter, 4) flow meter.

3.1.2 Proposed device test rig

The ultrafilter element of the test circuit comprises the blood flow channel, control membrane, and superabsorber and forms what could be termed a 'testing prototype' of the proposed device. This 'testing prototype' or 'test rig' is first designed in Pro/ENGINEER Wildfire 4.0 integrated 3D CAD/CAM software *(PTC Inc, Needham MA)* and manufactured from poly(methyl methacrylate) (acrylic glass) at the University of Strathclyde Department of Biomedical Engineering. Figure 29 shows a CAD model of an early test rig.

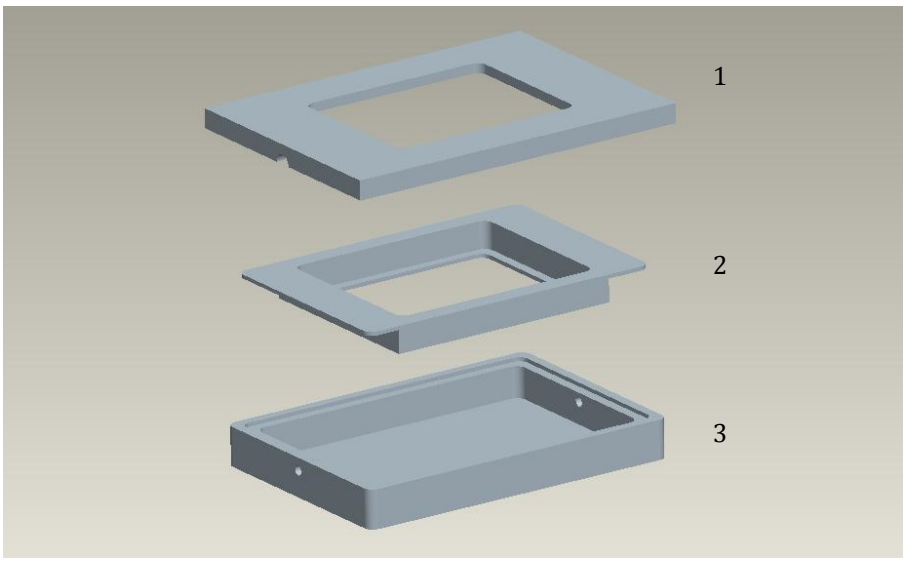


Figure 29: Exploded view of CAD model of an early test rig showing the three component parts: 1) frame which seals the tests rig 2) insert to which membrane is attached and houses superabsorber 3) base in which blood flows

In order to ensure the test rig was watertight a silicon rubber compound *(RS Components, UK)* was used to seal the unit. In order to avoid the difficulty of attaching the delicate control membrane to the test rig, it was first 'framed' with two sheets of cellulose acetate using the silicon rubber compound, allowed to dry, and subsequently attached to the test rig. Figure 30 depicts this procedure.

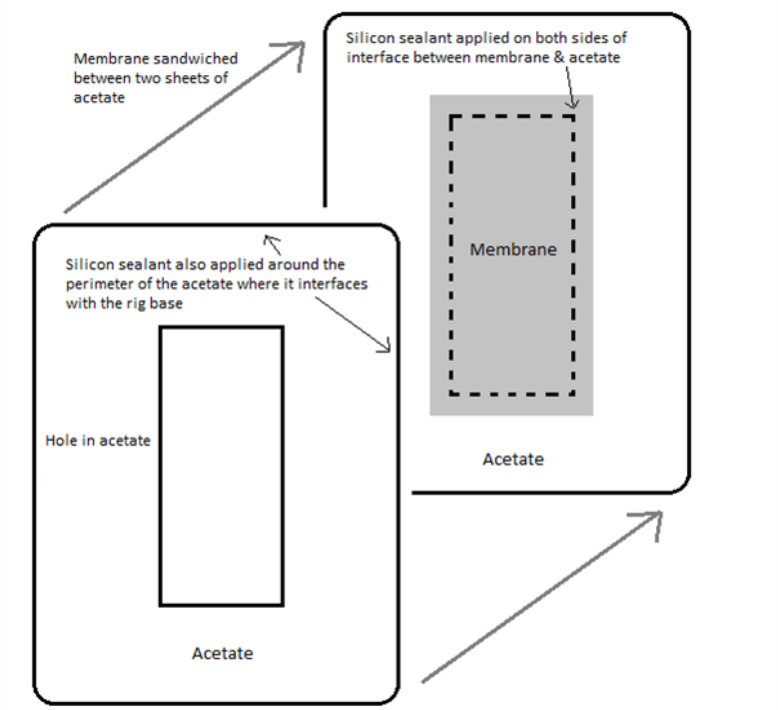


Figure 30: Membrane framing with cellulose acetate and silicon rubber compound

The testing prototype used in early experiments is depicted in Figure 30 and had an exchange surface measuring 80mm x 100mm and a priming volume of approximately 76ml.

3.1.3. Blood volume

The proposed device is intended to be used with MUF in paediatric CPB and thus it is required to process a volume of blood equal to that found in a typical paediatric patient. Consequently the device testing experiments will use a suitable volume of blood. A patient's blood volume is related to a number of different factors but reasonable estimates can be made based upon a patient's weight.

The WHO (based upon the findings of Pearson) report that the average blood volume of a child over six months old is approximately 70-80ml/kg and close to that of older children and adults (Pearson 2003; Howie 2011). This ratio is different in neonatal patients with newborns having a blood volume of 85ml/kg, premature infants having a blood volume of 90kg/ml (Pearson 2003). Additionally, in neonatal patients (as might be expected) the ratio is subject to dramatic change with blood volume rising to a ratio of 105ml/kg in the first few days and decreasing thereafter (Pearson 2003). Similar figures have been used by groups studying MUF, for example Gurbuz et al who assumed the blood volume in their paediatric cohort to be equal to 80ml/kg (Gurbuz et al. 1998).

It is therefore important to know the approximate weight of the paediatric patients the proposed device could be used in. The average weight of a newborn baby is approximately 3.4kg (Beers et al. 1999; Olds et al. 2000; Pillitteri 2010) whilst the average weight of a one year old is approximately 10kg (Durney et al. 1986; Pillitteri 2010). Using a blood volume ratio of 85ml/kg for neonates and 75ml/kg for a one year old this gives us a blood volume range of potential patients of 289-800ml. This closely correlates with the WHO estimate which states the blood volume of a 15 month old to be approximately 800ml (Howie 2011). It is important to consider that paediatric patients receiving MUF may well be at the low end of this range as the kind of heart defects requiring CPB with MUF are likely to impair their development. The most common weight limit exclusion criteria found in the paediatric MUF survey conducted by Darling et al was that patients should weigh less than 10kg (although higher limits were used by other centres) and thus our initial testing will be focussed on patients below this threshold (Darling et al. 1998).

Another important consideration when designing the experiment is the characteristics of future testing models. The next logical step after any successful laboratory testing with a fixed volume

of blood would be an animal model. An appropriate animal model for paediatric MUF would most likely be a piglet model as has been used previously (Atkins et al. 2010). Our centre has experience with use of two week old piglet models to test paediatric cardiovascular devices and it is likely that these models would be applicable to testing of the proposed device.

Linderkamp et al state that the mean weight and blood volume of a two week old piglet are 4.956kg and 95.9ml/kg respectively; giving an average total blood volume of 475.28ml (Linderkamp et al. 1980). Hansard et al report the mean weight and blood volume of two week old piglets as 4.536kg and 74ml/kg respectively; giving an average total blood volume of 335.66ml (Hansard et al. 1953). Ramirez et al state the mean weight and blood volume of two week old piglets as 5.023kg and 83ml/kg respectively giving an average total blood volume as 416.91ml. The mean of these three reported figures is 409.28ml.

It appears that on balance, using a blood volume of 400ml for initial testing of the proposed device will provide an appropriate analogy to a paediatric patient receiving MUF whilst also allowing for easy transition to a suitable animal model without the need to vastly alter circuit and prototype designs.

3.1.4. Level of dilution

Since haemodilution is the major issue addressed by the MUF procedure and associated devices it is important to simulate the diluted blood in the bypass circuit. In order to achieve this, the 400ml volume of blood to be used will be diluted to an appropriate Hct level.

In the original MUF study Naik, Knight and Elliott report the immediate post-CPB Hct to be 24% on average, ranging from 20-32% (Naik et al. 1991a), whilst Elliott (part of the same research group) suggests a typical on-bypass Hct to be 20% (Elliott 1993). Other groups report similar figures. Perez-Vela et al maintained Hct between 22 and 28% during CPB (Perez-Vela et al. 2008), Glogowski et al report a 'starting haematocrit' of 22±2% (Glogowski et al. 2001) and Boga et al maintained Hct at 20-25% during CPB (Boga et al. 2000). Furthermore, Davies, Onoe and Keenan each report increases in Hct from MUF from post-bypass values of 26.0±2.7%, 21.2±2.0% and 21.7±3.4% respectively (Davies et al. 1998; Keenan et al. 2000; Onoe et al. 2001).

Based on these reported figures, a baseline Hct of 20% will be used in the device testing procedures as an analogous post-CPB level of haemodilution. Given that typically the Hct of whole blood is approximately 40% (Burton 2000) and that 400ml of blood is being used, this will give a typical working volume of diluted blood of 800ml. Mongero et al state that the total

CPB volume for paediatric patients is generally between 500ml and 1200ml so 800ml seems like a reasonable working volume for our experiments (Mongero et al. 2008).

In order to achieve this level of haemodilution from the original blood sample, and in the interests of simplicity, blood will be diluted using a 0.9% NaCl saline solution in accordance with the calculation:

$$\%Hct_1 \times BV_1 = \%Hct_2 \times BV_2$$

where: %Hct1 = percentage Hct of the blood initially,
BV1 = blood volume initially
%Hct2 = percentage Hct of the blood after dilution with saline solution
BV2 = blood volume after dilution with saline solution

In order to measure the Hct of the blood both before and after dilution and at various stages during the experiments, blood will be collected in a capillary tube by capillary action and the end of the tube will be sealed with an inert wax. The capillary tube will then be spun in a 'Hawksley 127 haematocrit centrifuge' (*Hawksley and Sons Ltd., Brighton, UK*) for 2 minutes and then the Hct will be read by eye using a Hawksley micro-haematocrit reader (*Hawksley and Sons Ltd., Brighton, UK*). The volumes of blood collected in the capillary tubes are negligible compared to the overall working volume and thus will not have a significant affect on subsequent Hct readings. It is important to note that due to the fact that this method of measuring Hct relies on reading 'by eye' on the microhaematocrit reader, a degree of subjective human error is introduced. Figure 31 an image showing an example of Hct being read using a microhaematocrit reader.

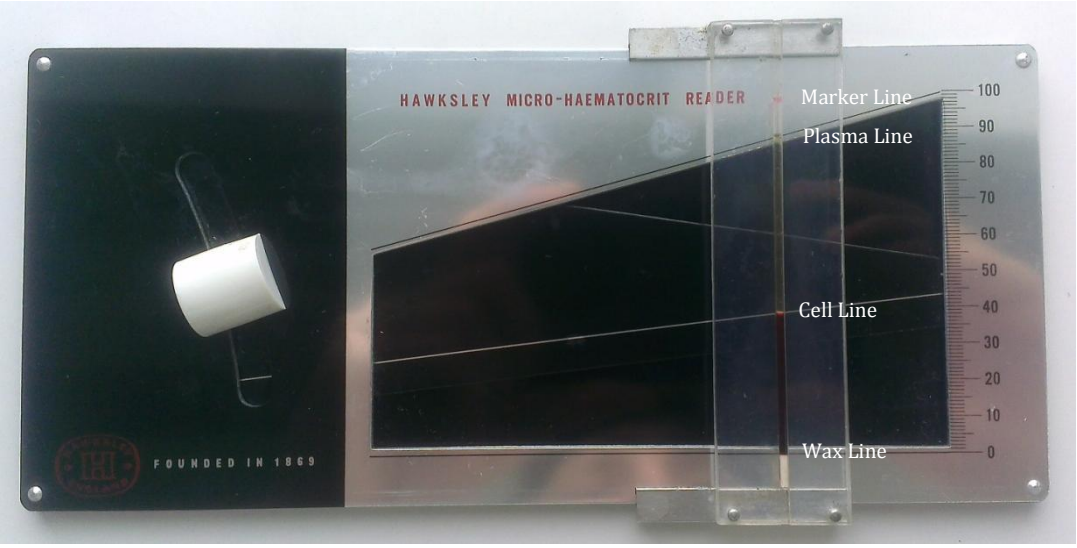


Figure 31: Microhaematocrit reader with sample showing a reading of 43.5% Hct

As can be seen, the reading method involves 'lining up' the wax line, the cell line and the plasma line with lines on the microhaematocrit reader all of which are subjective. Moreover, the scale on which the Hct level is read has only 1% intervals so at best the resolution is 0.5%. However, at this early stage it will be sufficient to demonstrate a rise in Hct at this level of accuracy using this method. The red marker line on the capillary tube is not used for reading Hct.

3.1.5. Blood source and pre-treatment

The blood used in early testing of the proposed device will be collected from a bovine source on site at a local abattoir. Bovine blood provides a satisfactory likeness to human blood, especially with regards to the formed elements which are the focal point of our early analyses. Bovine blood is abundant and subsequently is drastically less expensive than human blood. The bovine blood is collected in a sealed 5L container. In order to prevent clotting, the blood must be treated with 10,000IUs of heparin sodium from porcine mucosa (*Sigma Aldrich*). This heparinisation is achieved by mixing the appropriate amount of heparin sodium with 50ml of 0.9% NaCl saline and placing this solution in the 5L container before the blood is collected. The bovine blood will therefore be immediately heparinised on collection. The blood collection procedure is discussed further in *4.5.3 Blood quality at source*.

3.1.6. Priming

As described earlier (*1.1.2.2. Controlling Colloid Osmotic Pressure (pCOP and tCOP) and Haemodilution*), the CPB requires priming to expunge any air from the bypass circuit before commencing extracorporeal circulation. For neonatal patients this prime volume may be 200-

300% of the patient's blood volume and is generally a mixture of colloid and crystalloid solutions (Mongero et al. 2008; Mavroudis et al. 2012). In the early testing procedures of the proposed device the elimination of air from the circuit prior to the introduction of blood is not so important as there is no risk of introducing air into a patient's bloodstream and thus the MUF circuit will not be primed. The Hemosep system does include a priming period before haemoconcentration begins, however the purpose is not the same. Rather than to expunge air from the system, Hemosep requires priming to create state of continuous contact between the superabsorber and the blood through the pores in the membrane. This continuous contact allows movement of fluid from the blood phase, through the pores of the control membrane and into the gel phase of the superabsorber.

Since the proposed device absorbs fluid by the same mechanism as Hemosep, the MUF system will be primed in a similar way, either by wetting the membrane directly, or by pumping a priming fluid into the flow chamber through which blood will pass such that it comes in contact with the membrane. In the interests of simplicity priming will not use a crystalloid colloid mixture solution but, as is the case with Hemosep, will simply use a 0.9% NaCl saline solution.

Despite the fact that air expunction before blood is introduced is not necessary during early phase testing it should be remembered that ultimately complete priming of the proposed MUF device will be required and subsequently prototype designs should still aim to reduce prime volumes thereby reducing the contribution priming the system makes towards haemodilution.

3.1.7. Flow rate and pump strategy

The blood flow rate through the proposed device is an important consideration as we have seen it is known to impact on ultrafiltration rate (Jacobs 1996; Sorin Group USA 2009; Minntech Therapeutic Technologies 2014). By looking at the flow rates used by other groups researching MUF, we can get a sense of typical flow rates clinicians are currently comfortable using. Rodruigez et al report an institutional standard MUF flow rate of 70-100ml/min for patients weighing less than 10kg and 150ml/min for patients weighing more than 10kg (Rodriguez et al. 2005). In addition Rodriugez found that high flow rates transiently decrease cerebral circulation possibly as a result of blood moving from the aorta into the MUF circuit down the pressure gradient inducing flow 'stealing' from the intracranial circulation (Rodriguez et al. 2005).

Whereas, Naik, Knight and Elliott et al used a flow rate of 100-150ml/min during MUF, Kotani et al used 100ml/min, Groom et al used 125-200ml/min, Davies et al used 200ml/min, Onoe et al used 220ml/min and Glogowski et al used 250ml/min (Naik et al. 1991a; Groom et al. 1994; Davies et al. 1998; Glogowski et al. 2001; Onoe et al. 2001; Kotani et al. 2008). Other groups

determined flow rate by patient weight including Mahmoud et al and Perez-Vela who used 50ml/kg/min and 10-15ml/kg/min respectively (Mahmoud et al. 2005; Perez-Vela et al. 2008). As we have seen before, the average neonate weight is approximately 3.4kg meaning for neonates these equate to 170ml/min and 34-51ml/min respectively.

Moreover, the paediatric MUF survey report by Darling et al found that the average flow rate used by all the centres in their survey was 120-196ml/min and 12-22 ml/kg/min for those which based flow rate on patient weight (Darling et al. 1998).

It seems reasonable that a standard flow rate of 150ml/min be used in order to closely mimic many similar MUF experiments.

Darling et al found that most A-V MUF centres maintained a constant flow rate throughout MUF whilst adjusting the rate at which blood is titrated from the venous reservoir into the MUF circuit according to the filling pressure of the heart. Since our test circuit is a model of the MUF circuit only and we do not have to contend with fluctuating filling pressures, a constant flow rate will be maintained throughout the MUF procedure. However, it may be important to bear in mind this need to adjust venous reservoir titration rates in any future animal studies.

3.1.8. Agitation rate

The Hemosep device which forms the basis for the proposed MUF device uses mechanical orbital agitation to assist absorption of plasma. The improvement in haemoconcentration rate can be seen in Figure 32.

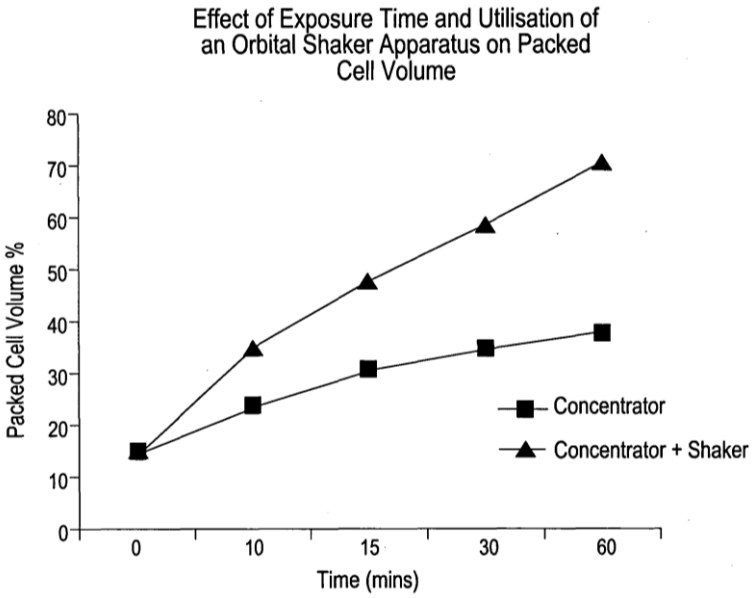


Figure 32: Impact of agitation in Hemosep (Gourlay 2011)

It is reasonable to assume that a MUF device based upon similar technology would also benefit from orbital agitation and thus it will be used as part of standard testing procedures. Hemosep uses an agitation rate of 120rpm as this leads to the most improvement in fluid exchange without inducing cell damage (Gunaydin et al. 2013) and a similar rate will be used in initial testing procedures, however, the impact of agitation of will be investigated.

3.1.9. Measurements

The primary measure of the capability of the proposed device to perform MUF is its ability to haemoconcentrate. As mentioned previously (*1.2. Conventional Ultrafiltration*), haemoconcentration is measured by haematocrit (Hct). It is the measure of the percentage of a given volume of blood which is comprised of cells, the majority of which are red blood cells (Burton 2000). Therefore Hct will be the primary measurement taken during these early testing procedures.

In the proposed MUF test circuit there are two locations from which it will be easiest to take Hct measurements. The first is the 'outlet' which is taken from the end of the tube leading from the test rig which sits in the 500ml 'patient's blood' beaker. The second is the 'pot' which is taken from the 'patient's blood' beaker itself. Due to the mixing of concentrated and unconcentrated blood, the 'pot' Hct is likely to always lag behind the 'outlet' Hct and thus is important that the measurement site is consistent throughout each experiment.

Most MUF testing procedures involve some measure of the filtrate volume. However, due to the nature of the proposed device filtrate volume is more complex. In current ultrafilter systems the filtrate is drawn away from the ultrafilter as a liquid along a separate filtrate channel and retained in a reservoir. It is therefore easy to measure the volume of fluid in this reservoir and thus know the exact filtrate volume. In the proposed device, the filtrate is 'captured' by the superabsorber material and thus it is more difficult to assess the volume of fluid removed from the blood. In the proposed test circuit filtrate volume can be measured by weighing the superabsorber before and after the experiment and/or by measuring the loss in volume in the 'patient's blood' beaker. Neither of these methods is ideal since the superabsorber will absorb some of priming saline and there will inevitably be unrecoverable volumes of blood lost in test circuit. However, a reasonable estimate of filtrate volume, obtained by the aforementioned methods, used in conjunction with the measure of Hct will provide a sufficiently robust measure of the ability of the proposed device to haemoconcentrate at this stage.

Later in the testing procedure, in addition to Hct, there is scope for measurement of 'Active Clotting Time', haemolysis by flow cytometry, platelet counts and blood differential counts. The procedures for these measurements are discussed in *4. Device Impact on Blood Quality*.

3.1.10. Termination of MUF

There are a number of different parameters used as indicators for the termination MUF. Darling et al found from their survey that the most common procedure was to wait until the circuit contents were completely salvaged (Darling et al. 1998). Aside from this, just under a quarter of the centres survey employed a fixed time limit on the MUF procedure of between 10 and 20 minutes, whilst at other centres MUF was terminated at a predetermined Hct level or predetermined filtrate volume (Darling et al. 1998). One centre continued MUF *"as long as the surgeon's patience permitted"* (Darling et al. 1998).

The findings of Darling et al are reflected when looking at other MUF studies. In the original description of MUF, Naik, Knight and Elliott used 40% Hct as a completion point and Elliott quotes a Hct based end point of 35-40% (Naik et al. 1991a; Elliott 1999a). Kotani et al used a Hct of 45% or a time limit of 10 minutes (Kotani et al. 2008). Bando et al terminated MUF when the venous reservoir was empty (Bando et al. 1998a). Friesen et al also use this indicator but describe it as "when the venous reservoir was empty and its contents had been flushed through the ultrafilter with normal saline" (Friesen et al. 1997). Aeba et al also use the emptying of the venous reservoir but planned to terminate MUF before this point if a target ultrafiltrate volume has been reached (Aeba et al. 1998). Gurbuz et al set a limit of 20ml/kg of fluid to be removed as the completion point for their MUF procedure (Gurbuz et al. 1998). However, a commentary on

this paper by Dr Ross M. Ungerleider (Durham, NC) suggests that this figure should be increased to 50ml/kg to allow for a Hct of 30% to be reached (Gurbuz et al. 1998). Tallman et al also use filtrate volume as the determining factor for the termination of MUF but set the figure at 3L/m^2 body surface area.

Given the fact that in our testing procedure Hct will be measured regularly, and additionally filtrate volume cannot be easily measured and the circuit set up does not include a venous reservoir, the most sensible termination point for MUF when testing the proposed device will be once a Hct of 40% is reached. Once reliable haemoconcentration can been achieved with a prototype device, a suitable time period will be selected as a complementary end point i.e. MUF will be terminated once 40% Hct is reached or once a given time period has passed.

3.2. Control Membranes

3.2.1. Membrane Characteristics and Options

Since the control membrane forms an integral part of the proposed device it is important to have a good understanding of the various membrane options present. Membranes can vary in a number of different ways including; material, pore size, pore architecture (whether symmetrical, where pores are the same on both sides of the membrane, or asymmetrical, where pores differ through the depth of the structure), and the membrane chemistry (whether hydrophobic or hydrophilic and whether there is a charge present) (Merck Millipore Corporation 2014). Other important characteristics which impact the performance of the membrane include the membrane thickness, wettability, compatibility (whether chemical compatibility, biocompatibility or compatibility to sterilisation techniques) and the bubble point (Merck Millipore Corporation 2014).

The wettability of a material is a surface characteristic which is unique to a material in response to a given liquid and is related to the surface tension (Yuan et al. 2013). Contact angle is used as the basic parameter for determining wettability and its defined by the angle between the membrane surface and a tangent to the liquid at the gas-liquid-solid line of contact as shown in Figure 33 (Xu et al. 2009).

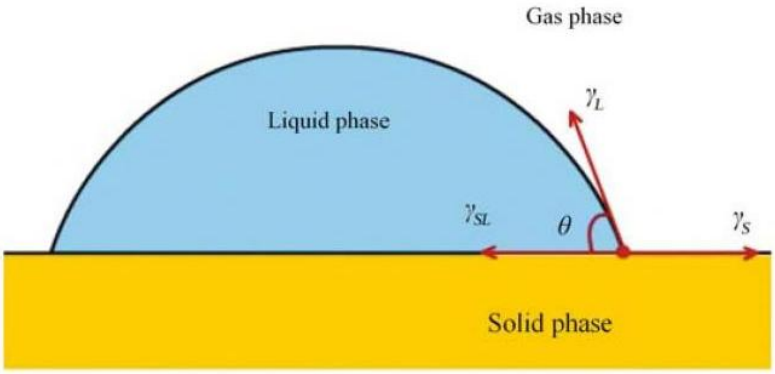


Figure 33: Depiction of contact angle, θ, as used to calculate membrane wettability (Xu et al. 2009)

The Young's Equation is used to calculate the contact angle and is shown below (Xu et al. 2009).

$$\gamma_L cos\theta = \gamma_S - \gamma_{SL}$$

Where γ_L is the liquid-vapour interfacial tension, γ_S is the solid-vapour interfacial tension and γ_{SL} is the solid-liquid interfacial tension as shown in Figure 33 (Xu et al. 2009). The Young's equation makes several assumptions including that the surface is perfectly smooth and flat, and that capillarity and gravity can be ignored (Xu et al. 2009). Much more complex models and theories of contact angle and its relation to surface tension exist involving acid-base interaction, dipole-dipole interactions and hydrogen bonding (Xu et al. 2009) but this thesis is concerned only with how membrane properties affect their suitability to use in the proposed device and thus the Young's equation as a measure of wettability and related membrane characteristics will suffice.

The fundamental importance of contact angle as it relates to membrane properties is that, generally speaking, a fluid that exhibits a low contact angle when in contact with a membrane, indicates a low surface tension, and consequently the liquid is likely to spread on the membrane. In this way contact angle is used to quantify the hydrophobicity or hydrophilicity of a membrane (Benjamin et al. 2013).

Figure 34 shows the relationship between contact angle and the hydrophobicity or hydrophilicity of a membrane as well as its correlation with short-term cell attachment from serum-containing cultures which will be of importance when discussing protein adsorption later (*3.2.3.1 Protein Adsorption*) (Hollinger 2011). A contact angle of 65° has been suggested as a possible 'dividing line' between hydrophobic and hydrophilic membranes (Hollinger 2011). The contact angle of a polycarbonate filtration membrane such as that used in Hemosep was reported by Choi et al to be 66.2 ± 2.8° (Choi et al. 2008).

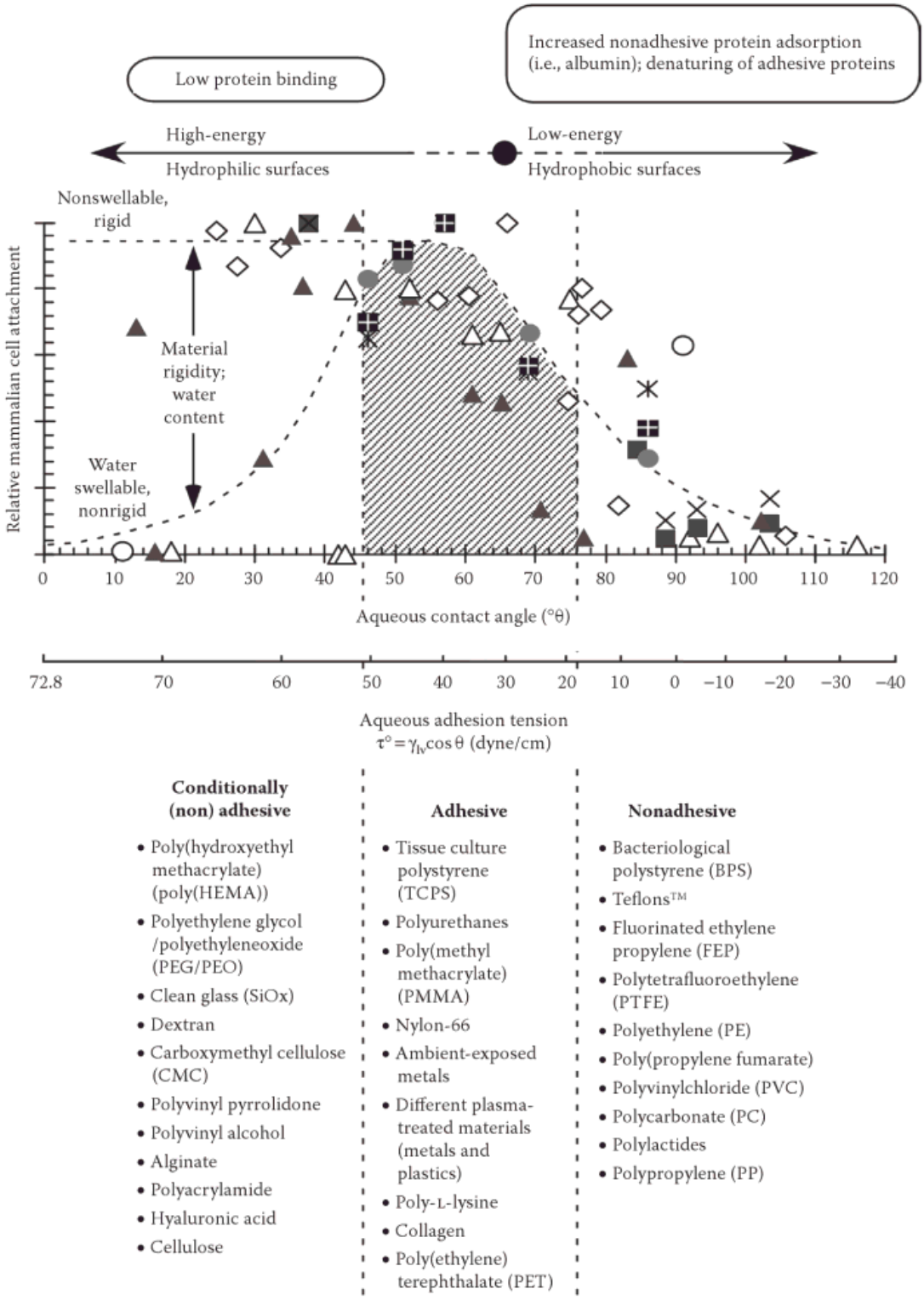


Figure 34: Relationship between contact angle, hydrophilicity/hydrophobicity and protein binding characteristics for a range different conditionally adhesive, adhesive and non-advesive membrane materials (Hollinger 2011)

Hydrophilic membranes, as the name suggests, have and affinity for water due to highly polar or ionised functional groups on their surface which facilitate water transport (Benjamin et al. 2013) These functional groups do however make hydrophilic membranes more susceptible to biodegradation and oxidant degradation (Benjamin et al. 2013). Examples of hydrophilic membranes include cellulose based membranes and polyamides (Benjamin et al. 2013). Conversely, hydrophobic membranes such as those made from polytetrafluoroethylene (PTFE) and polyacrylonitriles (PAN) lack the functional groups and thus have a low affinity for water (Benjamin et al. 2013). Hydrophobic membranes are more susceptible to membrane fouling (discussed in detail later *3.4.2.1. Membrane Surface Modifications*) as hydrophobic solutes tend to accumulate on their surface (Hilal et al. 2005; Benjamin et al. 2013). This is particularly important to the development of the proposed MUF device as many organic solutes are hydrophobic and thus hydrophilic membranes are less likely to suffer from fouling through adsorption of organic matter such as proteins (Hilal et al. 2005; Xu et al. 2009; Benjamin et al. 2013).

Therefore, in blood related applications such as MUF it is important to avoid interactions between the membrane and blood components including platelets and other blood proteins and cells (Xu et al. 2009). It is generally recognised that hydrophilic membranes exhibit less non-specific binding of proteins and cells resulting in improved biocompatibility and moreover their resistance to protein adsorption reduces bio-fouling (Xu et al. 2009; Hollinger 2011). Membrane fouling, with particular regards to blood and bio-fouling will be discussed in more detail later (*3.2.3.6. Concentration Polarisation and Membrane Fouling*).

Bubble point can be defined as the minimum pressure required to force air through a membrane which has been pre-wet with a given fluid (Advantec MFS Inc 2014). If a membrane pore is thought of as a perfect cylinder, any liquid within the pore will have a certain surface tension at the liquid-air interface and a specific pressure will be required to force the liquid column out of the pore cylinder (Merck Millipore Corporation 2014). This pressure is known as the bubble point pressure and since a smaller diameter cylinder would require a larger pressure, thus bubble point pressure can be used as a measure of pore diameter (Merck Millipore Corporation 2014). Bubble point is measured by pre-wetting the membrane with a given liquid (such as water or alcohol), placing it in a manifold and covering it with the same liquid, after which the air pressure in the chamber below the membrane is gradually increased until a steady stream of bubbles is forced through the membrane, thus giving the bubble point pressure (Merck Millipore Corporation 2014). The bubble point test is quick, efficient and non-destructive but is specific to the fluid being used, is only effective between 0.05μm and 10μm and can be affected by surface modifications (e.g. coatings which affect wetting characteristics may partially 'fill-in' pores) (Merck Millipore Corporation 2014). The bubble point for a 1μm pore diameter

Polycarbonate membrane such as the one used in Hemosep is approximately ≥0.041MPa (≥6psi, ≥300mmHg) when pre-wet with isopropylalcohol (Advantec MFS Inc 2014).

Currently, the membrane used in the Hemosep device is a polycarbonate track etched 'Cyclopore' membrane from Whatman with a pore diameter of 1µm (Fisher Scientific 2014). The polycarbonate membrane is hydrophilic but exhibits very low levels of water adsorption and non-specific protein binding and is biologically inert; consequently it is specifically listed as applicable for use in blood filtration procedures (Fisher Scientific 2014). Whatman quote a burst strength of >10psi for this membrane which is approximately equal to 69kPa or 517mmHg (Fisher Scientific 2014). The membrane has a uniform flat surface and straight through pores (Zydney et al. 1989). Zydney et al utilised a similar membrane in their own work related to plasma filtration form whole blood, choosing a pore size of 0.2µm to minimise mechanical deformation and lysis of RBCs in the pores which was found in some membranes with pore diameters between 0.4- and 0.6-µm by (Blackshear Jr et al. 1978; Zydney et al. 1989).

The term 'Track etched' refers to the method by which membranes of this type are produced. Track etching involves bombarding a thin polymeric sheet with a collimated beam of high energy heavy particles such as U^{235} fission fragments, ions or nucleons (Zeman et al. 1996; Apel 2001; Pabby et al. 2008). This bombardment create tracks in the polymer surface and a chemical etching process (typically bathing the polymer in hot NaOH or H_2O_2) is required to enlarge the tracks into cylindrical pores (Zeman et al. 1996).

Track etched membranes have well defined pore sizes determined by the energies of the particles involved and the etching chemical, temperature and time, which results in sharp cut-off characteristics for filtration (Zeman et al. 1996; Apel 2001; Pabby et al. 2008; Fisher Scientific 2014). Additionally, track etched membranes exhibit more precise pore distribution which is determined by the irradiation exposure time (Zeman et al. 1996; Apel 2001; Pabby et al. 2008). However, there is a maximum thickness (~20µm) of the polymeric material above which the particle energies are no longer sufficient to create pores (Zeman et al. 1996; Pabby et al. 2008).

Track etched membranes are structurally uncomplicated and thus easy to characterise, in fact, due to their simple and easily measurable geometry track etched membranes have been used as calibration tools for indirect pore measurements of other membranes with more complex pore structures (Zeman et al. 1996). Additionally, track etched membranes are non-hygroscopic, that is, they do not attract and retain water from the environment (Zeman et al. 1996).

Figure 35 shows an image taken of the membrane surface using a *Hitachi TM-1000* tabletop scanning electron microscope (SEM) (*Hitachi Ltd., Tokyo*). The regular pore size created by the particle bombardment can be clearly seen.

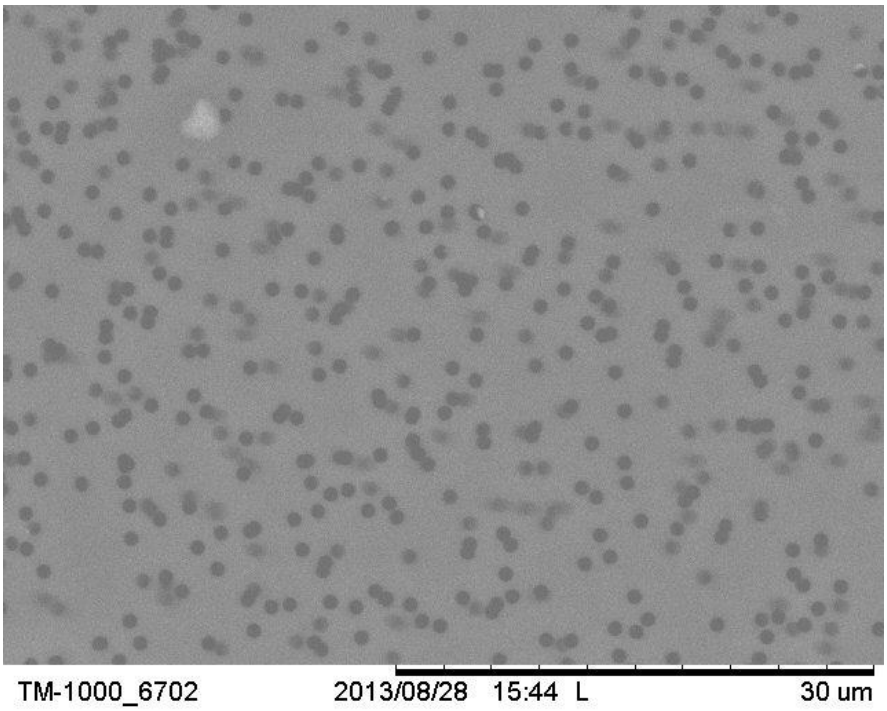


Figure 35: SEM image at x3000 magnification of unused Hemosep membrane showing regular pore size and irregular pore distribution arising from track-etching technique

The image was obtained by cutting a small sample from the Hemosep membrane (approximately 10mm x 10mm) and mounting it on a metal stub using an adhesive carbon disc. The metal stub is then screwed into place in the viewing chamber of the TM-1000 SEM. This process can be seen in Figure 36, Figure 37 and Figure 38.

Figure 36: Hitachi TM-1000 Workstation used for scanning electron microscopy

Figure 37: Membrane sample mounted on black carbon disc and metal stub for observation

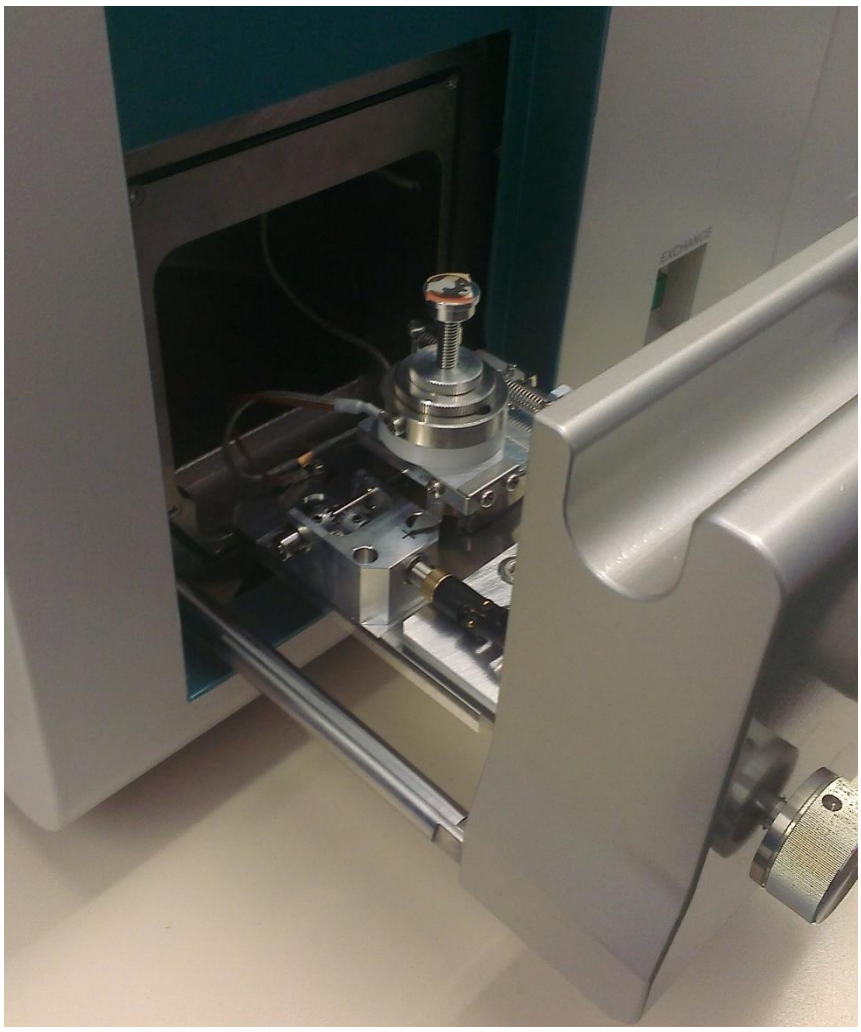


Figure 38: Mounted membrane sample placed in viewing chamber of TM-1000

In early testing, to reduce costs, the membrane was salvaged from unused Hemosep bags intended for disposal; however, at a later point a large roll of the same membrane was obtained. There was no difference between the membranes from these two sources as can be seen by comparing the SEM of the Hemosep membrane surface (Figure 35) to the SEM scan of the membrane sourced from the roll shown in Figure 39.

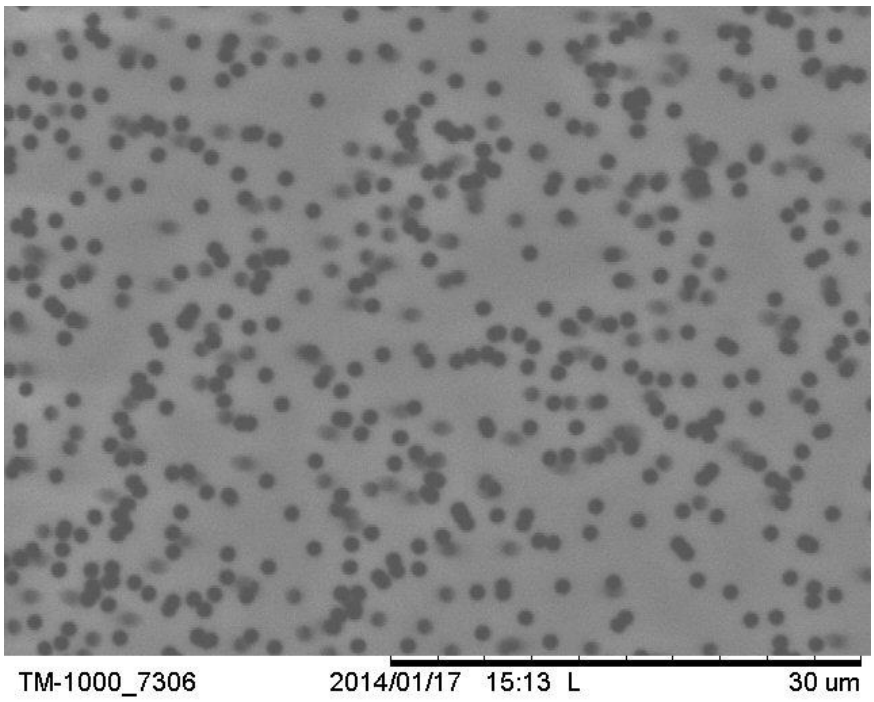


Figure 39: SEM image at x3000 magnification of Whatman Cyclopore Track Etched Membrane 1µm pore diameter

The Whatman Cyclopore track etched membrane used in Hemosep has a pore diameter of 1µm. This means that when filtering blood all formed elements with a diameter larger than this will be retained in the haemoconcentrated blood including erythrocytes (6-8µm), lymphocytes (7-10µm), monocytes (10-15µm), neutrophils (8-12µm), eosinophils (9-14µm), basophils (7-10µm) and platelets (1-4µm) (Sadler 2011). Additionally, a 1µm pore diameter should avoid the mechanical deformation and lysis of RBCs in the pores which was found in some membranes with pore diameters between 0.4- and 0.6-µm by (Blackshear Jr et al. 1978).

A number of nylon based membranes were available for use in the proposed device. Scans taken using the aforementioned TM-1000 SEM and brief descriptions of the nylon membrane samples sourced are shown in Figure 40, Figure 41 and Figure 42.

The Biodyne A membrane from Pall is an amphoteric membrane made from Nylon 6-6. The fact that the membrane is amphoteric means it comprises randomly arranged regions of positively charged and negatively charged regions in the polymer matrix allowing it to bind many kinds of macromolecules in applications where this is desirable (Pall Life Sciences 2003; Sata 2004; Pabby et al. 2008). Figure 40 shows an SEM image of the unused Pall Biodyne A membrane taken with the TM-1000 SEM.

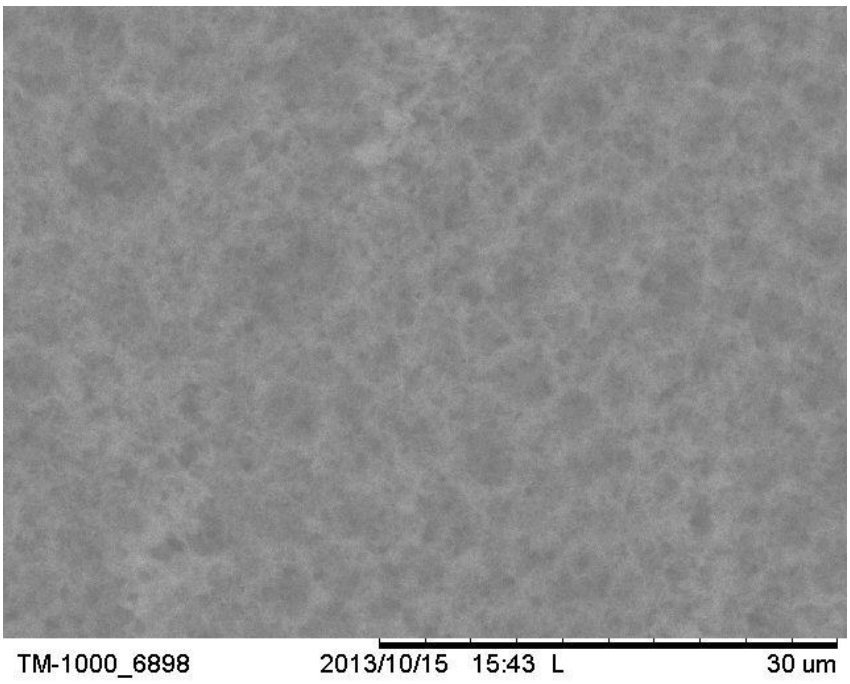


Figure 40: SEM image at x3000 magnification of unused Pall Biodyne A membrane showing mesh-like structure. It is not possible to identify positively and negatively charged regions.

The Biodyne B membrane from Pall is a positively charged membrane made from Nylon 6-6 and thus provides good binding for negatively charged molecules but also has the potential to repel positively charged molecules and prevent their passage through the membrane pores or adhesion to the membrane surface (Kiser et al. 1981; Pall Life Sciences 2003). Figure 41 shows an SEM image of the unused Pall Biodyne B membrane taken with the TM-1000 SEM.

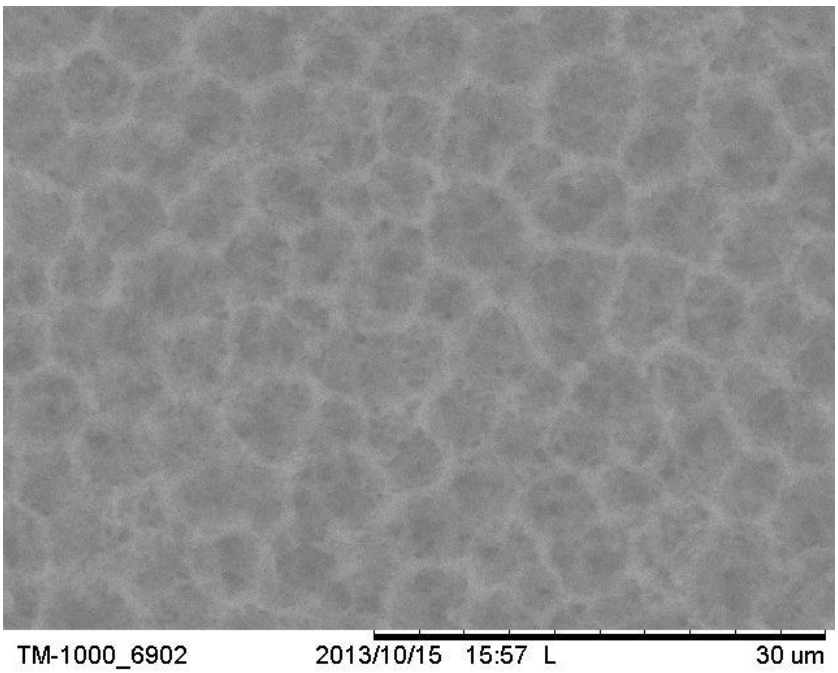


Figure 41: SEM image at x3000 magnification of unused Pall Biodyne B membrane showing mesh-like structure

The Biodyne C membrane from Pall is a negatively charged membrane made from Nylon 6-6 and thus acts in the opposite was to Biodyne B providing good binding for positively charged molecules but like Biodyne B has the potential to repel negatively charged molecules (Kiser et al. 1981; Pall Life Sciences 2003). Figure 42 shows an SEM image of the unused Pall Biodyne C membrane taken with the TM-1000 SEM.

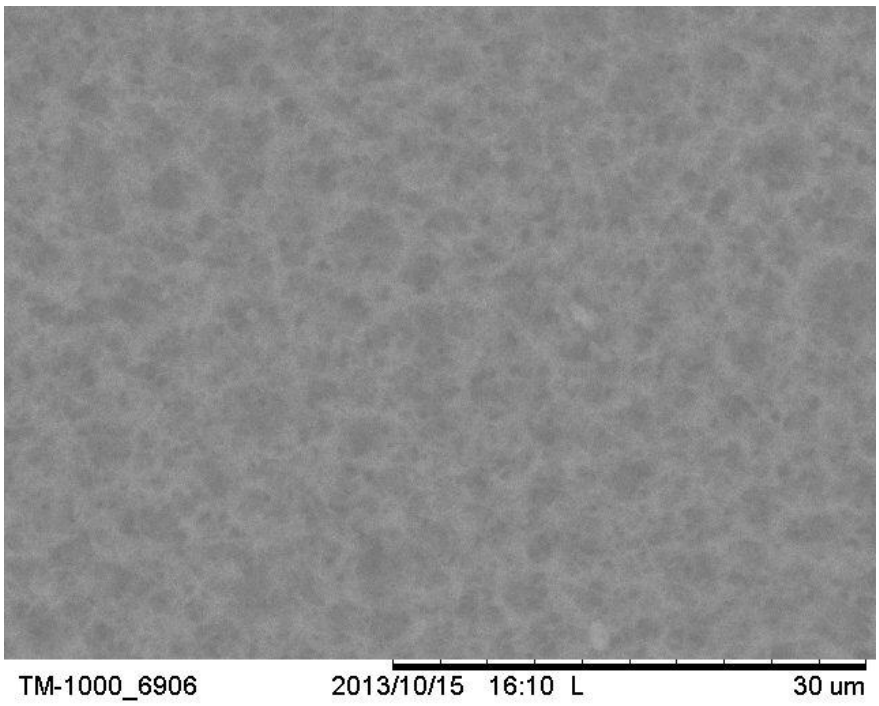


Figure 42: SEM image at x3000 magnification of unused Pall Biodyne C membrane showing mesh-like structure

Each of the Pall Biodyne membranes has a pore size of 0.4µm. Obtaining an adequatley clear image of the surface of these nylon mesh based membranes was found to be much more difficult that imaging polymeric sheet membranes. Thus, for clarity in discussing its structure, an SEM image from Advantec MFS Inc of the surface of a nylon based membrane is shown in Figure 43.

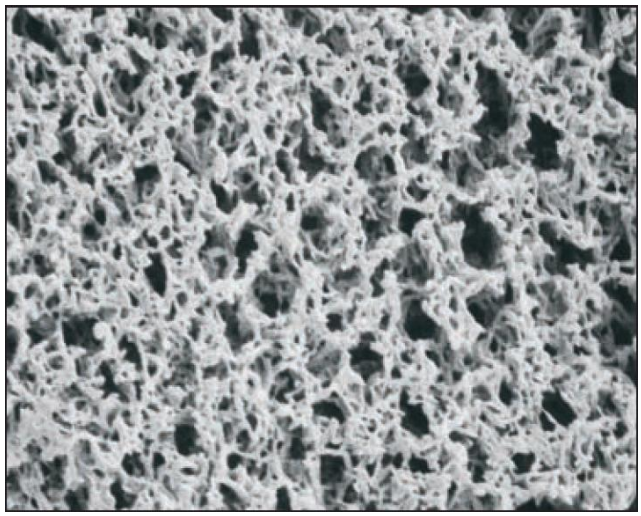


Figure 43: Typical surface topography of a nylon based membrane when vied under a SEM (Advantec MFS Inc 2014)

Unlike the track etched polycarbonate membrane seen earlier in this section which, structurally speaking, was a single sheet with smooth regular surface and well defined pores, the structure

of the nylon membrane is much more complex and mesh like. There are a number of different membranes with a similar structure as can be seen in Figure 44.

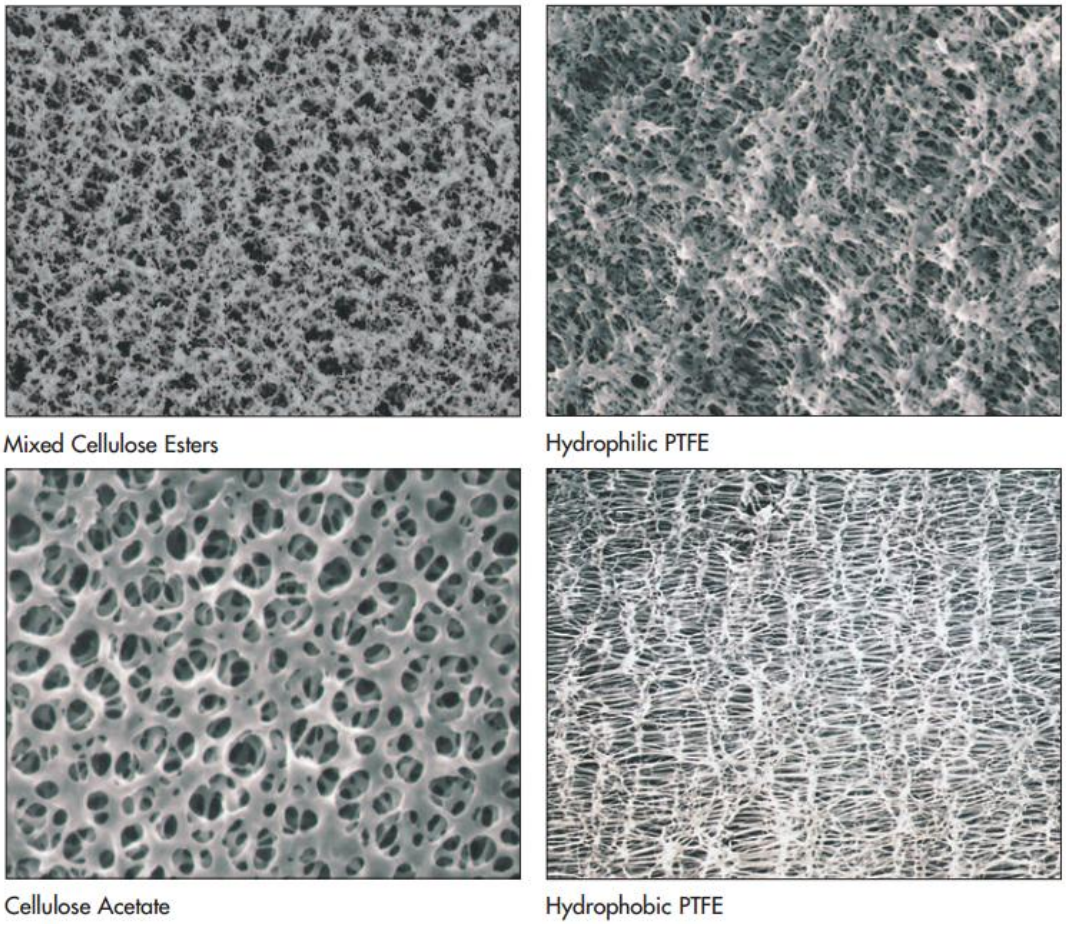


Figure 44: Typical SEM images of different membrane types (Advantec MFS Inc 2014) (Adapted)

Microporous membrane filters and fibrous filter such as those shown in Figure 44 have high pore densities but rely on particle collection based on attachment to microstructure elements (Jackson 2006). This is in contrast to 'straight-through' pore filters such as the track etched polycarbonate membrane used in Hemosep which retain particles by impaction and interception (Jackson 2006). When considering blood filtration in MUF, the ideal scenario would be removal of excess plasma water (possibly along with pro-inflammatory mediators) whilst retaining all formed elements, thus particle losses to the membrane structure are detrimental. Moreover, materials such as Nylon-66 have been associated with protein adhesion and reductions in platelet counts, both of which are problematic in blood filtration applications (Bergdahl et al. 1980; Hollinger 2011; Dubitsky et al. 2014). Additionally from experience with manipulating nylon membranes in the laboratory it was found that the nylon membranes were more brittle and fragile and consequently harder to work with when compared with the polycarbonate membrane used in Hemosep.

Due to the suitability of polycarbonate membranes for this application, coupled with the fact that the Hemosep has demonstrated good haemoconcentration performance in a static setting, early prototypes of the proposed MUF device made use of the *Whatman Cyclopore* track etched polycarbonate membrane with a pore diameter of 1μm.

3.2.2. Membrane response to pressure

As mentioned earlier (*3.2.1. Membrane Characteristics and Options*), the 1μm pore diameter Whatman Cyclopore track etched membrane used in Hemosep and to be used in the proposed device has a burst strength of >10psi or 69kPa (Fisher Scientific 2014). As the name suggests, burst strength refers to the maximum pressure a given membrane can withstand before it bursts (Tanaka 2007).

The generalised relationship between TMP and ultrafiltration rate has been described previously (*2.1. Principles of Ultrafiltration*) and was shown in Figure 9 (Jacobs 1996).

Jaffrin et al state that in cross-flow filtration of plasma from blood; for membranes with 0.6μm diameter pores the linear 'membrane limited' region occurs at TMPs below 50mmHg whilst the 'boundary layer limited' region occurs at TMPs between 70mmHg and 120mmHg (Solomon et al. 1978; Jaffrin et al. 1992). It has also been seen previously (*2.1. Principles of Ultrafiltration*) how traditional ultrafilters utilise TMP as the driving force for fluid extraction. With this in mind, early testing involved measuring the TMP seen within the system in order to better characterise the ultrafiltration procedure and allow for comparisons with current technologies. Again, as described earlier (*2.3.4. Utilising the superabsorber*), the driving force for fluid extraction with the proposed device will not be TMP but rather the concentration gradient created by use of the superabsorber material. For this reason there should be no reason to induce high TMPs in to achieve filtration (possibly to the benefit of the quality of the haemoconcentrated blood), however moderate TMPs may be assistive and, if nothing else, an interesting point of comparison with current traditional ultrafilter systems, and thus TMP was measured.

TMP was measured using the BIOPAC MP36R data acquisition system and two SS13L blood pressure probes (*BIOPAC Systems Inc., Goleta, CA, USA*). The blood pressure probes were attached to the inlet and outlet of the MUF device prototype and monitored the blood pressure at both these locations throughout each experiment using the AcqKnowledge 4.1 data acquisition and analysis software (*BIOPAC Systems Inc., Goleta, CA, USA*). Figure 45 shows the apparatus setup.

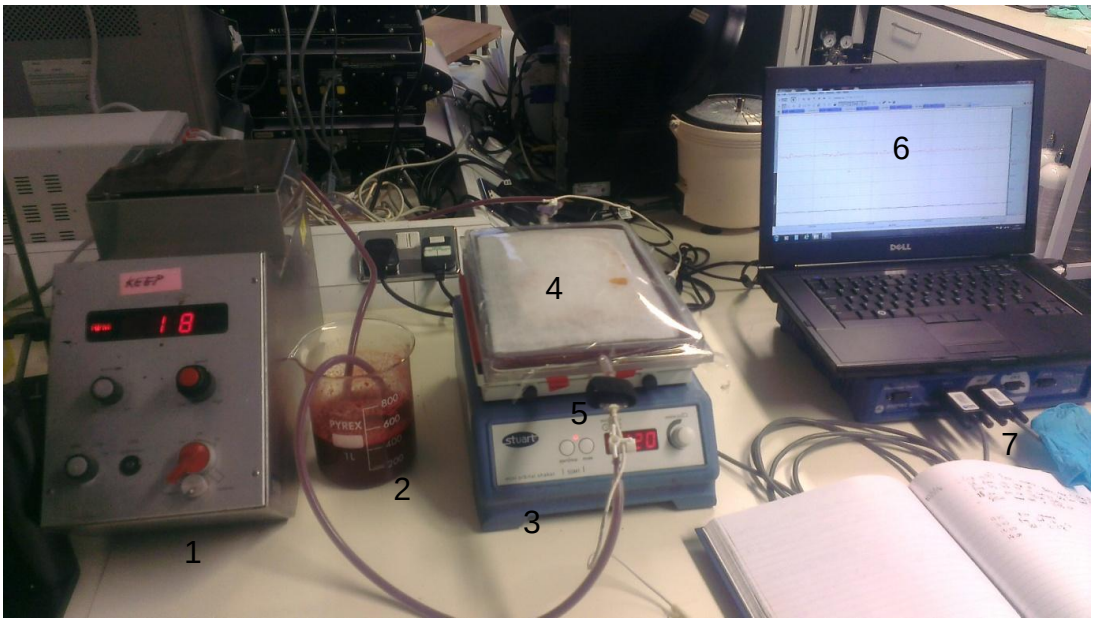


Figure 45: Photograph of BIOPAC MP36R data acquisition system attached to rig with pressure monitor probes at the inlet and outlet. 1) Roller pump 2) bovine blood 3) orbital agitator 4) Test Rig 5) Outlet pressure probe 6) PC 7) BIOPAC MP36R data acquisition system

TMP was calculated from this using the previously mentioned equation:

$$TMP = \frac{P_A + P_V}{2} + P_s$$

Where P_A is the pressure at the inlet of the ultrafilter, P_V is the pressure at the outlet of the ultrafilter and P_S is the additional negative pressure applied to the effluent side of the control membrane (Moore et al. 2000). Since there is no additional negative pressure applied to the effluent side of the control membrane, $P_S = 0$ and TMP is thus simply the mean of the inlet and outlet pressures as recorded by the blood pressure probes. Raw data acquired from the BIOPAC data acquisition system was processed using MATLAB R2012b (*Mathworks, Natick, MA, USA*).

Figure 46 is an example of a typical TMP profile produced when using the BIOPAC system in combination with MATLAB. This graph demonstrates the change in TMP over time when a fixed volume of water is pumped through the MUF circuit with the small test rig (exchange surface area = 80x100mm, internal volume based on dimensions = 76ml).

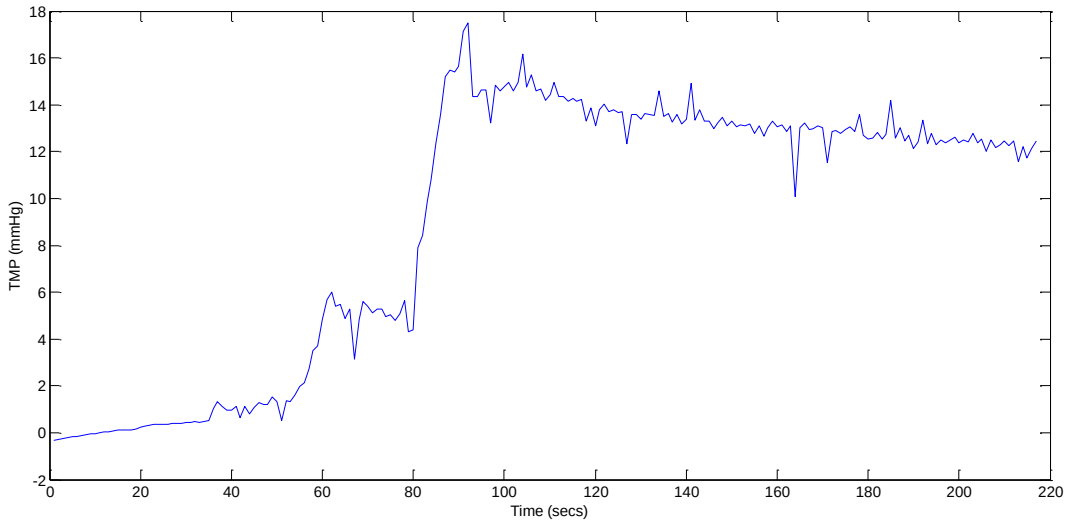


Figure 46: TMP profile over time when a fixed volume of water is pumped through the MUF circuit with the small test rig

There is a gradual rise in TMP as the rig fills, and a sharp rise in TMP (from 6mmHg to 17.68mmHg) between 80 and 90 seconds after which it is preferential for the water to cross the control membrane and a short drop in TMP can be seen followed by a steady decline as progressively more water crosses the membrane. The pulsatile flow profile naturally associated with a roller pump mechanism can be seen in the regular spikes in TMP.

In order to attempt to characterise the approximate magnitude of TMP which would be required for plasma to cross the control membrane, static tests involving no flow but rather the gradual increase of fluid volume inside the fixed volume of the test rig (thus increasing TMP) were performed whilst the impact this increasing TMP had upon plasma transfer was observed. This was done by closing the outlet of the small test rig and gradually syringing fluid into it via the inlet.

The above procedure was first performed using water to observe how a fluid whose entire molecular contents are freely able to pass through the membrane pores would transfer in relation to TMP. 80ml of water was gradually added to the small test rig, the TMP profile can be seen in Figure 47.

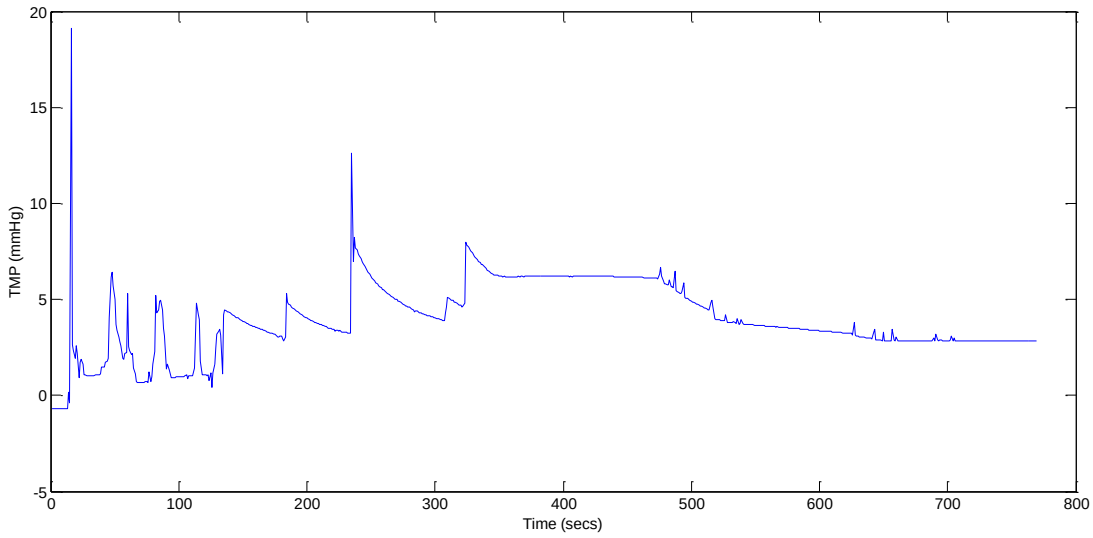


Figure 47: TMP profile as water volume in fixed volume test rig is gradually increased

The large spikes in TMP in the first 300 seconds of the experiment are the result of inlet TMP spikes when fluid was syringed into the rig in small increments. It can be seen that despite the increase in volume inside the test rig the TMP does not reach a high level as the water in the rig begins to cross the membrane, thus reducing the volume inside the test rig and reducing the TMP. The maximum TMP observed (disregarding the inlet TMP spikes) was 8.2mmHg and occurred at 322 secs. Figure 48 shows that as water was syringed into the rig below, beads of water began forming on the open air side of the membrane. These beads of water indicate the initial points of fluid transfer across the membrane and gradually increased in size and number as more water moved through the membrane pores.

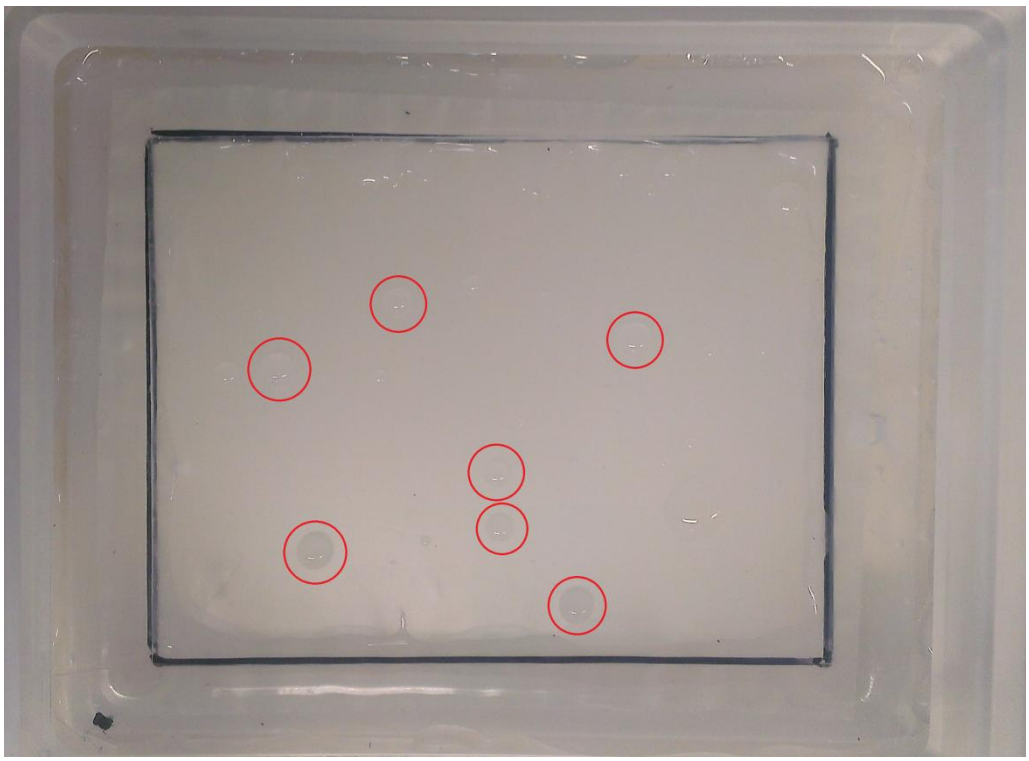


Figure 48: Beads of water on air side of membrane surface indicate fluid transfer across the membrane

After making a basic observation of water movement across the control membrane in response to gradual increases in TMP, the same procedure was performed with blood instead of water. Blood was expected to respond differently due to the fact that a large proportion of its constituents would be too large to pass through the membrane pores and thus would not contribute to reducing the volume inside the test rig which would normally relieve the rising TMP.

Figure 49 shows the TMP profile when blood was gradually syringed into the rig. Unlike the previously seen water profile (Figure 47), there is an initial period of stable TMP (0-500secs) followed by a clear spike in TMP (~500secs) when the capacity of the small test rig (~76ml) is exceeded followed by a gradual decline in TMP.

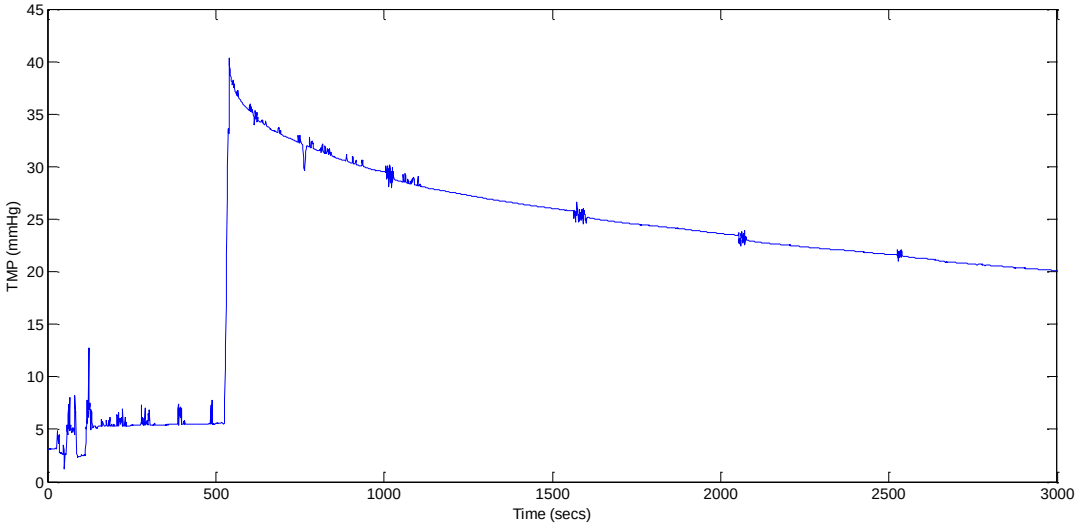


Figure 49: TMP profile as blood volume in fixed volume test rig is gradually increased past its theoretical capacity

In a similar fashion to the water bead formation seen previously (Figure 48), plasma could be seen on the surface of the membrane on the open air side as it slowly passed through the membrane pores. The slow increase in this 'sitting plasma' is illustrated in Figure 50 and Figure 51.

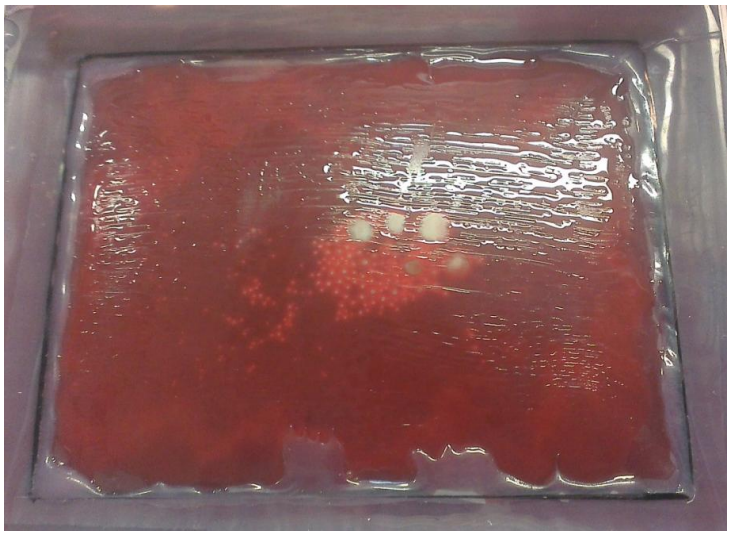


Figure 50: TMP profile as blood volume in fixed volume test rig is gradually increased past its theoretical capacity

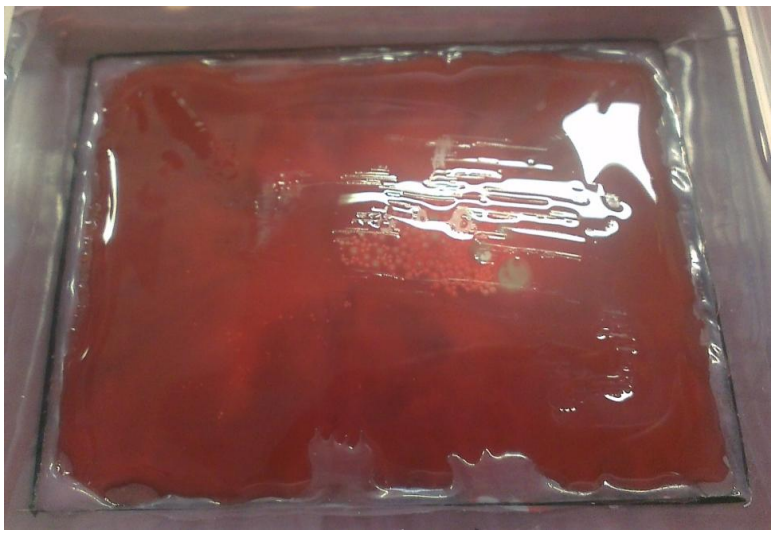


Figure 51: 'sitting' plasma on air side of control membrane at 3000secs

Despite the high TMP (approximately 40mmHg), the rate of plasma transfer was very slow and only amounted to a few millilitres over the 50 min (3000secs) observation period show in the TMP profile.

In order to make a basic initial appraisal as to what impact an even higher TMP would have on the rate of plasma transfer and on the membrane itself the same experiment was repeated but with 100ml instead of 80ml which was used previously.

Figure 52 shows the TMP profile across the control membrane for this larger volume. Again it can be seen that there is a 'spike' in pressure as the internal volume capacity of the small test rig (~76ml) is exceeded and further spikes in TMP as further blood is added to the rig. A maximum TMP of 83mmHg is reached when the full 100ml of blood had been added, and, as before, this is followed by a slow and steady decline in TMP as plasma slowly crossed the control membrane as observed previously.

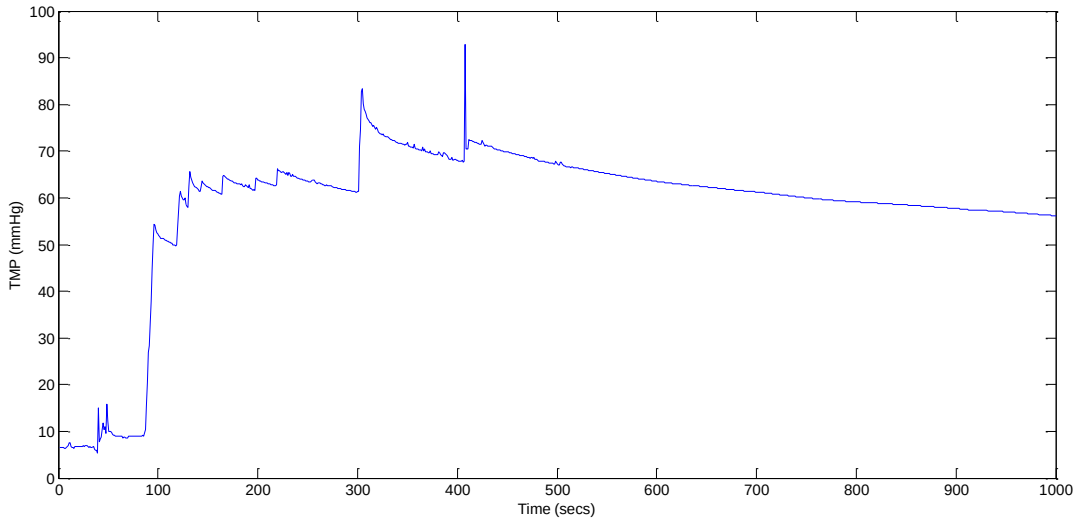


Figure 52: TMP profile as blood volume in fixed volume test rig is gradually increased far past its theoretical capacity

The rate of plasma transfer was still very slow and the volume leaving the rig via the membrane appeared too small to explain the drop in TMP seen, indeed upon inspection of the control membrane after the test rig had been cleared and dismantled it became clear another factor was contributing. Figure 53 shows that as the volume in the rig increased, the control membrane could be seen to bulge whilst the volume of plasma crossing the membrane remained low.

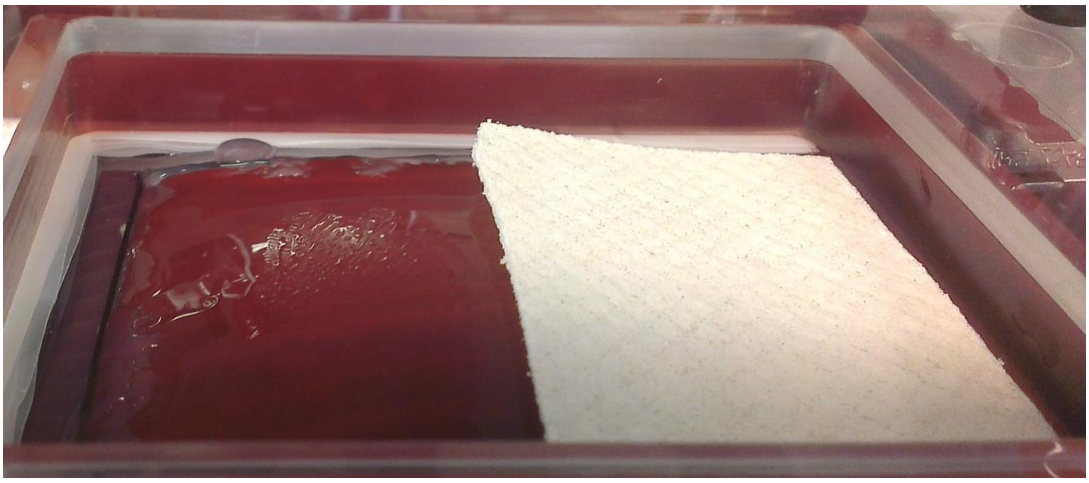


Figure 53: Bulging of control membrane whilst the volume of plasma crossing the membrane remains low.

After the experiment it could be seen that the bulging of the membrane was resulting in permanent stretching and bowing of the polycarbonate material, shown in Figure 54.

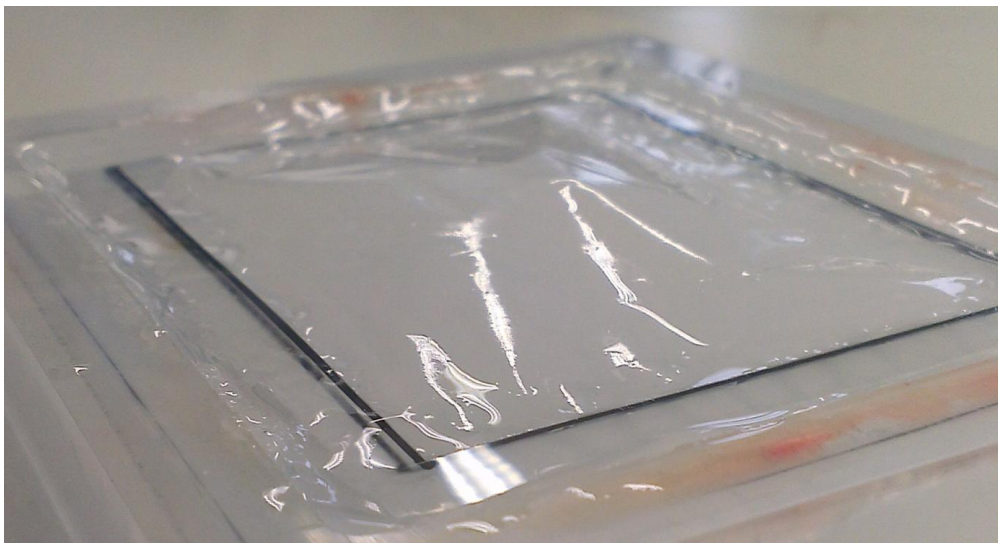


Figure 54: Permanent stretching and bowing of the polycarbonate membrane material

A section of the now stretched membrane was examined under the SEM using the same procedure described earlier (*3.2.1. Membrane Characteristics and Options*). The SEM scans of this piece of membrane are shown in Figure 55 and Figure 56.

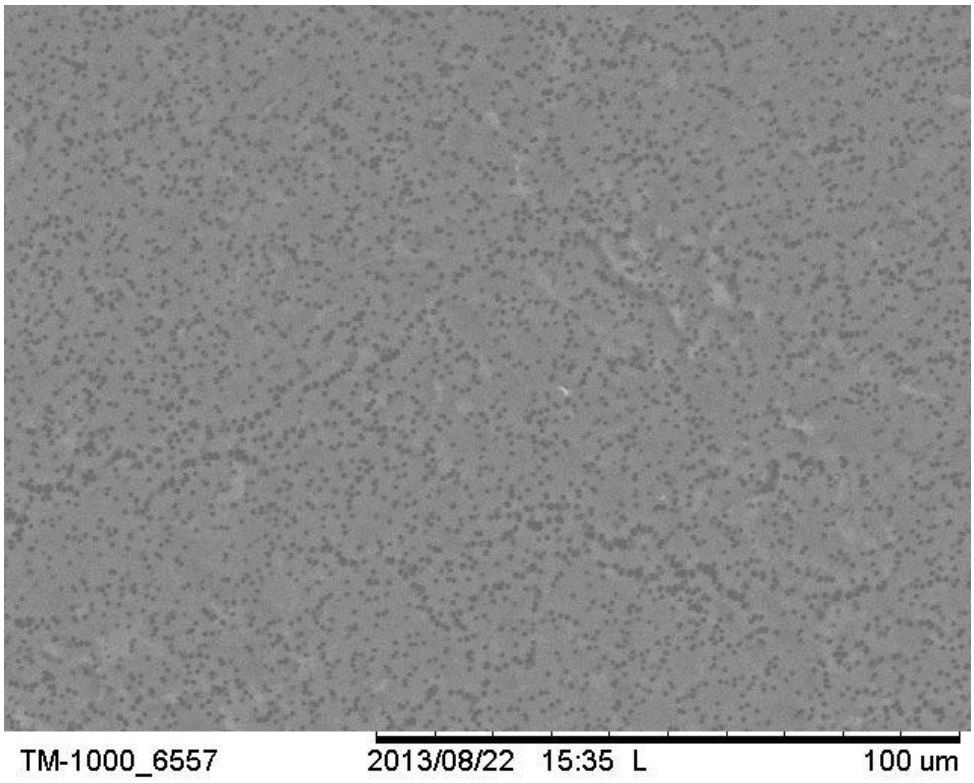


Figure 55: SEM image at x1000 magnification of permanently stretched membrane

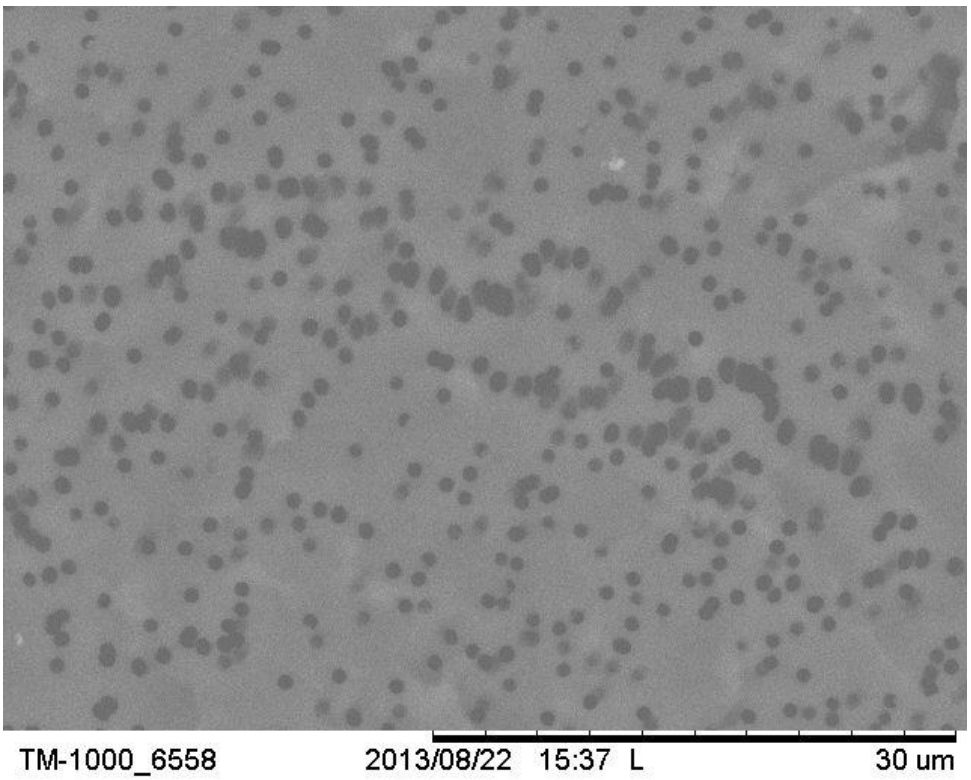


Figure 56: SEM image at x3000 magnification of permanently stretched membrane

It can be seen that there is an apparent change in the membrane pore structure as a result of the polycarbonate material stretching as it forms a convex bulge caused by filling the small test rig beyond capacity to induce high TMPs. The impact of the high TMP on the control membrane becomes even more apparent when SEM images of the stretched and unused membrane are shown side by side as in Figure 57.

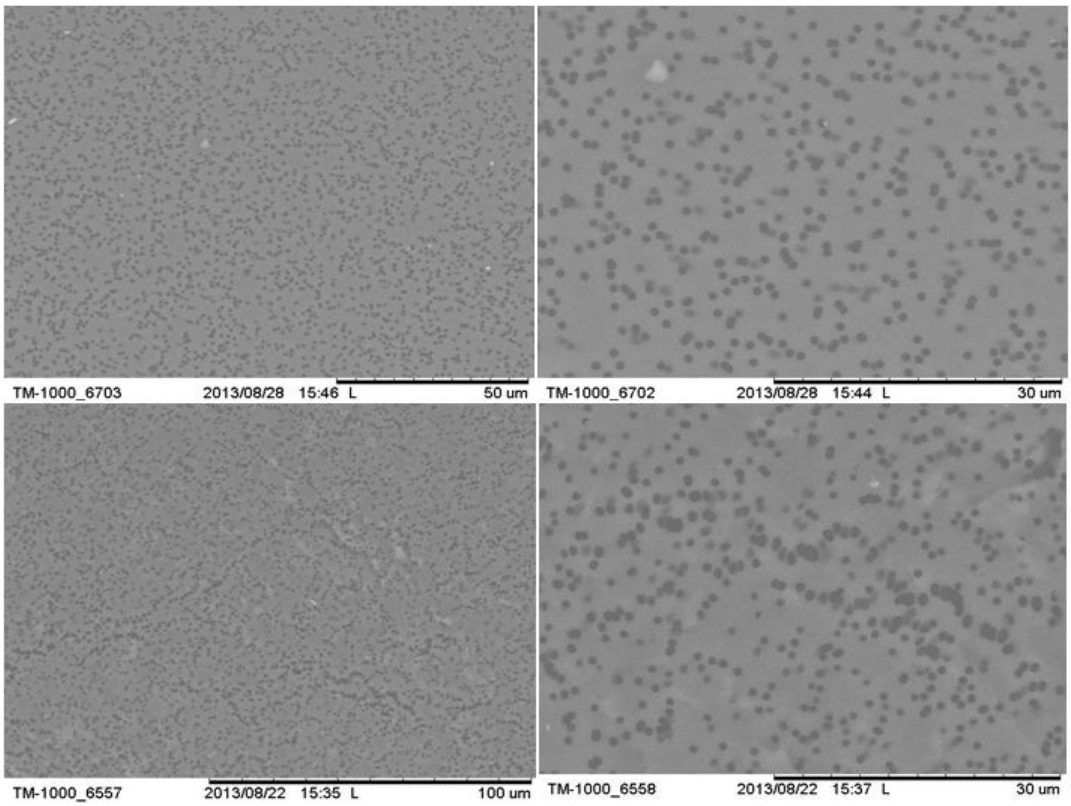


Figure 57: Comparison of SEM images at x1000 (left) and x3000 (right) magnifications of unused (top) and permanently stretched (bottom) membranes

There is some impression of tortuous lines over the membrane surface, possibly as a result of two or more merging to form a single large orifice, although this needs to be properly investigated. If the stretching of the membrane is resulting in the presence of larger pores, it would have a profound impact on the filtration capabilities of a membrane. As the pore diameter increases, the minimum size of particle which the membrane is able to retain also increases. In the case of MUF and the proposed device, if there was indeed an increase in mean pore diameter, this would likely mean the membrane would no longer reliably retain platelets and the device could have a detrimental effect on coagulation. This observation on the impact of high TMPs on the polycarbonate membrane to be used in the proposed device in a single sheet flat bed arrangement, coupled with the fact that the plasma transfer rate at these TMPs was very low, suggests that the TMP should be kept as low as the functioning of the device will allow and not intentionally raised in an attempt to assist the concentration-gradient-based uptake of plasma by the superabsorber material.

3.2.3. Membrane fouling

During early testing of the first prototypes of the proposed device there was little to no haemoconcentration measured. Theoretically the system should have functioned like the

Hemosep and thus emulated the repeatable performance of Hemosep. When trying to assess possible causes for the system's failure to haemoconcentrate it was noted that after the blood was drained from the test rig, the membrane had become stained from its contact with the blood as is shown in Figure 58.

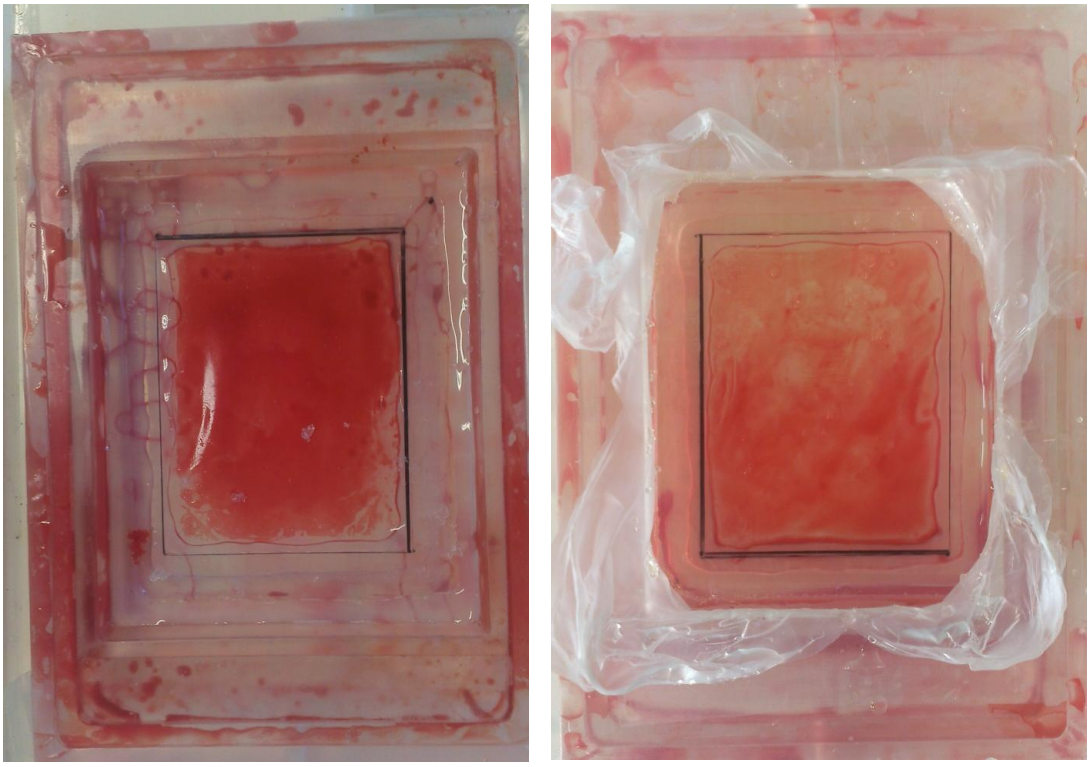


Figure 58: Staining of control membrane after contact with blood

It can be seen that the membrane is stained to a far greater degree than the surrounding cellulose acetate or acrylic glass. It was suggested that this staining may be indicative of blood constituents blocking the membrane pores and could possibly be an explanation for the lack of haemoconcentration achieved by the system. During one observation experiment it was seen that the membrane would allow both water and saline to permeate across its surface before contact with blood but not after, with no other parameters being changed. Interestingly, the staining seen was not immovable and could be washed away using water. Subsequently, SEM scans were taken of the membrane after it had been used to haemoconcentrate. One such scan is shown in Figure 59.

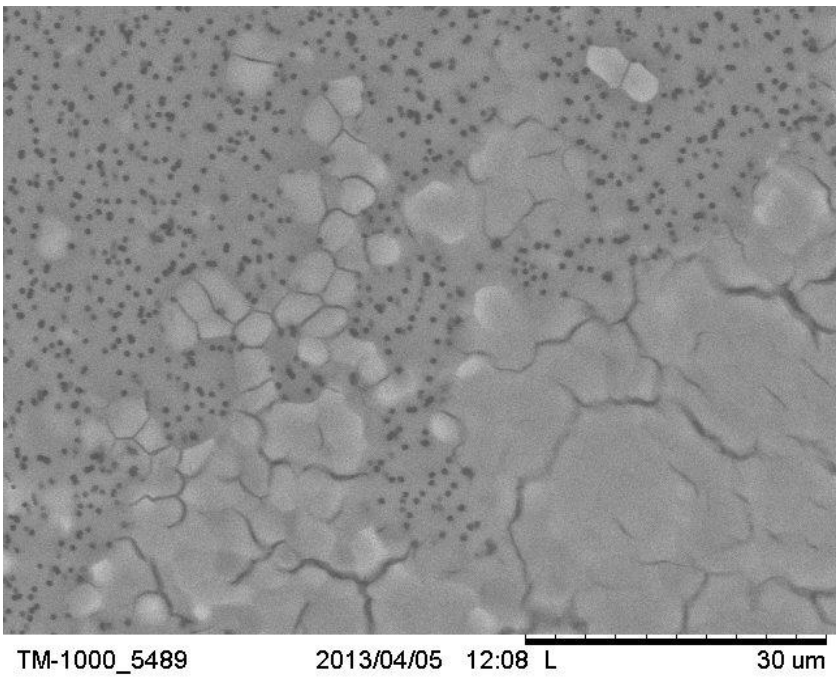


Figure 59: SEM image at x3000 magnification of used and stained control membrane showing clear signs of a thick deposit on the membrane surface in the bottom right corner of this image, whilst the upper left corner remains clear

There is clear evidence from the SEM scans that at least some areas of the membrane surface are obscured by some sort of deposit. As well as the more pronounced deposit seen in Figure 59, used membrane also showed the presence of either the same deposit in smaller amounts or another form of deposit which, when viewed under the SEM, manifested itself as areas of blurry light-in-colour 'deposit' as seen in Figure 60 and Figure 61.

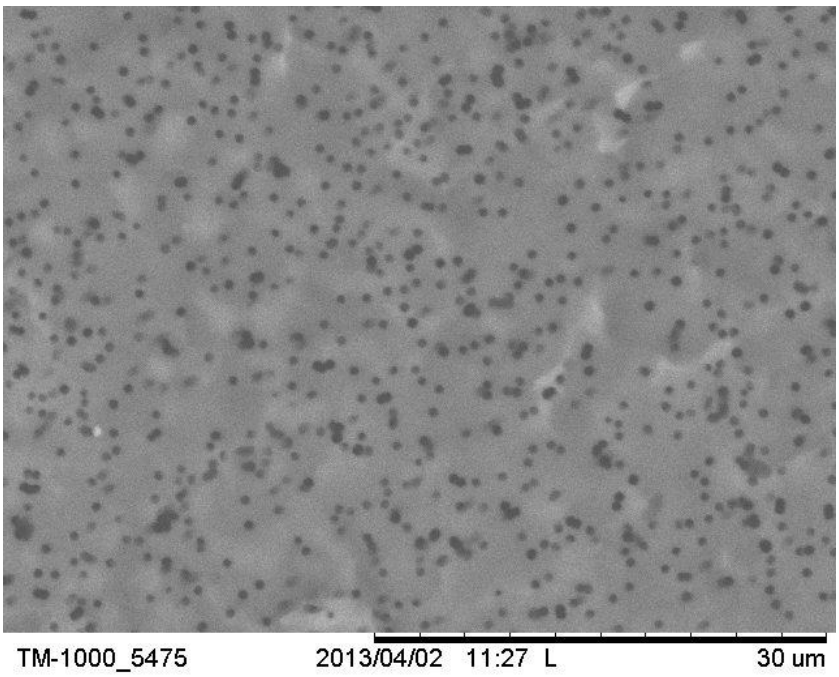


Figure 60: SEM image at x3000 magnification of less pronounced blurry light-in-colour 'deposit'

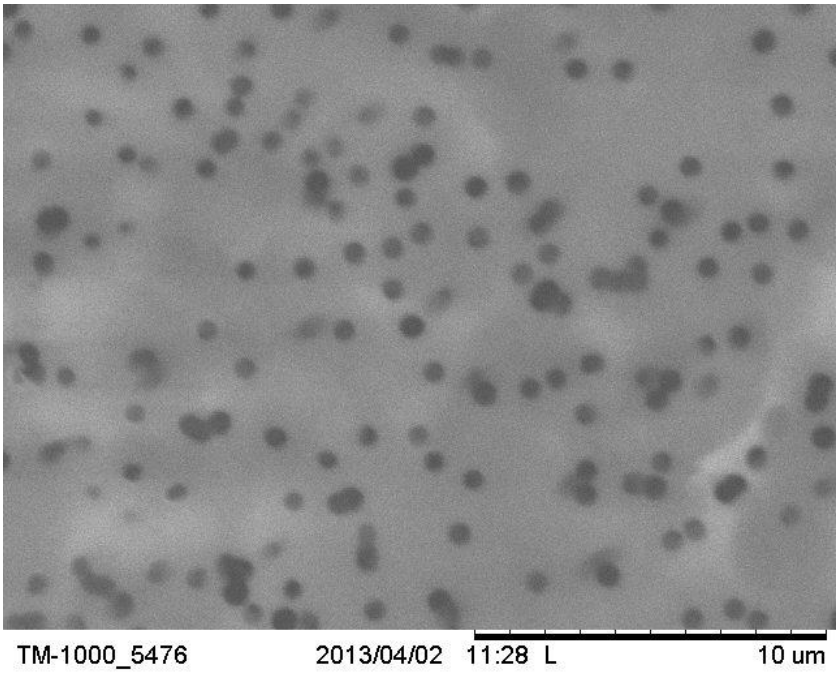


Figure 61: SEM image at x10000 magnification of less pronounced blurry light-in-colour 'deposit'

As mentioned earlier in this section, the membrane discolouration or staining which occurred after contact with blood during testing could be largely removed by washing the membrane with water, however, this form of 'deposit' was also seen to some extent in membranes after washing as is shown in Figure 62.

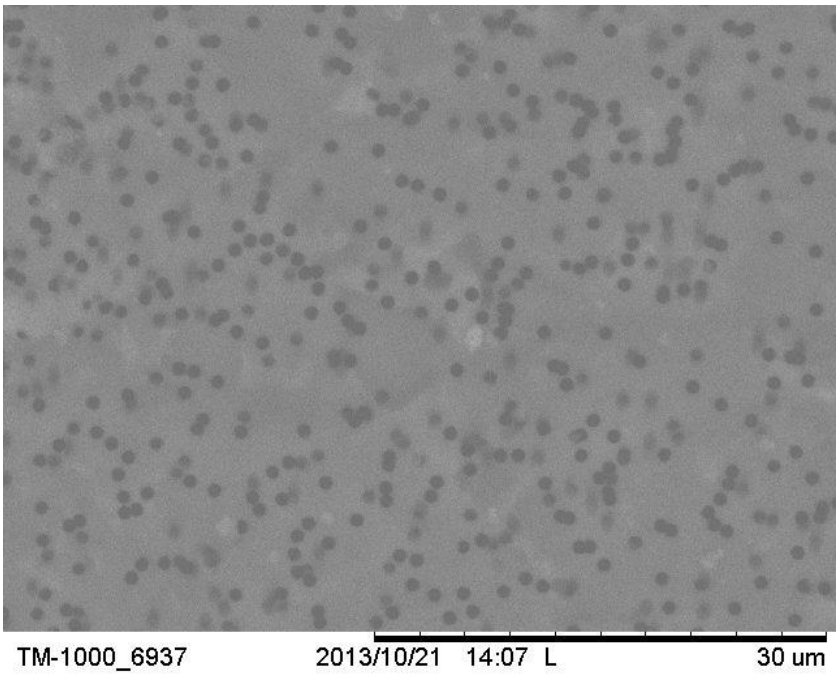


Figure 62: SEM image at x3000 magnification of less pronounced blurry light-in-colour 'deposit' after washing with water

The deposit seen in these SEM images could be an example of the protein which has adsorbed to the membrane surface, a phenomenon seen when protein rich solutions such as blood come in contact with foreign surfaces as explained below.

When blood comes in contact with a foreign material there are two important aspects of their interaction; platelet adhesion and blood coagulation (Beugeling 1979). The manner in which the initial contact is brought about, combined with the material surface properties and flow conditions will impact both platelet adhesion and blood coagulation (Beugeling 1979). The complexity of the blood response to a foreign surface makes it difficult to obtain meaningful information about the interaction itself (Courtney et al. 2003). Figure 63 from Beugeling et al shows the general phenomena which occur when blood interacts with a foreign material.

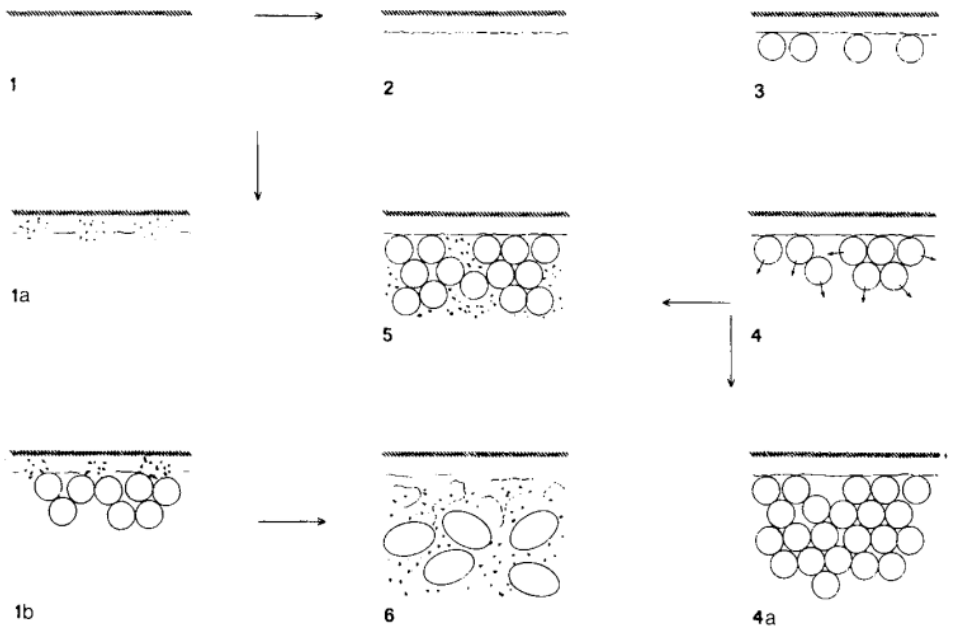


Figure 63: Interaction between blood and a foreign surface (Beugeling 1979) 1: Blood is exposed to a foreign surface. 2: proteins from the blood adsorb to the foreign surface. 3: Platelets adhere to the adsorbed protein layer. 4: ADP and other platelet constituents are released forming a platelet aggregate. 5: The coagulation process begins on the surface of the platelet aggregate forming an insoluble fibrin network. 6: Individual platelets lose integrity and fuse together while fibrin strands and entrapped blood cells form a red thrombus. Alternatively, 4a shows the formation of a white thrombus by aggregating platelets, whilst 1a shows the coagulation process being initiated by factor XII activation and 1b shows thrombin, formed during the coagulation process, causing platelet aggregation.

Additionally, Courtney et al list the phenomena involved in blood response to a biomaterial as: *"protein adsorption, platelet adhesion and aggregation, activation of intrinsic coagulation, participation of the fibrinolytic complement and kallikein-kinin systems, and the interaction of cellular elements"* (Courtney et al. 2003).

3.2.3.1 Protein Adsorption

The initial event which occurs when blood comes in contact with a foreign material (including blood contact with membranes in blood purification procedures) is the adsorption of proteins onto the material's surface, which it modifies, determining the nature of subsequent, sometimes undesirable, events (Beugeling 1979; Absolom et al. 1987; Su et al. 2011). This initial protein layer is often termed the *conditioning layer (Hastings 2012)*.

Protein adsorption is a spontaneous and often irreversible process that occurs when any protein solution such as blood is exposed to a foreign surface and is a critical feature of blood-biomaterial interactions (Lameire et al. 2000; Courtney et al. 2003; Somasundaran 2006). Protein adsorption is a common event which occurs within seconds of initial contact with the foreign surface and is the first step in many biological processes including the blood coagulation

cascade (Leber 2004; Rabe et al. 2011; Sultana 2012). As Rabe et al state: *"Whenever proteins come in contact with a solid surface they will most likely adsorb to it"* (Rabe et al. 2011).

This acquisition of a layer of host proteins by biomaterials and the foreign surfaces of medical devices is an immediate and spontaneous precursor to interactions with host cells (Anderson et al. 2008). Consequently, cells interact with the adsorbed protein layer rather than the material itself and thus the nature and conformation of the proteins which comprise this layer play an important role in determining the tissue reaction and overall response to contact with a foreign surface (Bohnert et al. 1986; Tang et al. 1995; Balasubramanian et al. 1999; Tang et al. 1999a; Tang et al. 1999b; Wilson et al. 2005; Anderson et al. 2008). The composition of the protein layer generally reflects the composition found of the plasma (Lameire et al. 2000). Protein adsorption can trigger the adhesion of cells, particles and bacteria, and additionally may promote fouling processes and inflammation cascades (Mueller et al. 1996; Elofsson et al. 1997; Wahlgren et al. 1997; Ghosh 2002; Ye et al. 2003; Yamamoto et al. 2005; Kalasin et al. 2009; Xu et al. 2009; Rabe et al. 2011; Su et al. 2011).

Host inflammatory cell interactions and adhesion are modulated by the presence of adsorbed proteins such as albumin, fibrinogen, complement, fibronectin, vitronectin, γ globulin (Jenney et al. 2000; Hu et al. 2001; Brodbeck et al. 2003; Anderson et al. 2008).

The three major plasma proteins are albumin, immunoglobulins and fibrinogen, whilst other factors such as factor VIII/vWF, Hageman factor and high molecular weight kininogen (HMWK) are also present to a lesser, or possibly similar, degree and will therefore also compose the adsorbed protein layer resulting from blood contact with a foreign surface (Anderson et al. 1990).

Albumin (molecular weight 69kDa (Tripathi 2011)) makes up 60-70% of plasma (Tang et al. 1993a; Tripathi 2011) but is not considered a mediator of inflammation (Tang et al. 1993a). While some evidence suggests that monocytes will adhere to albumin-coated surfaces, albumin is generally considered to greatly reduce the acute inflammatory response to a given material (Absolom et al. 1987; Tang et al. 1993a; Tang et al. 1995; Tang et al. 1996; Tang et al. 1999b), and it must be other plasma constituents which induce phagocyte adherence in inflammation (Tang et al. 1993a; Leber 2004).

Immunoglobulins are the second most abundant plasma protein class, comprising approximately 20% of the plasma (Tang et al. 1993a; Tripathi 2011). Despite some evidence suggesting immunoglobulin G adsorption onto biomaterial surfaces (Pankowsky et al. 1990; Tang et al. 1993a) it does not appear necessary for induction of inflammatory response to

biomaterial implants, based on the findings of Tang et al that the number of adherent phagocytes on PET disks was the same in mice with low lmmunoglobulin G as in normal mice (Tang et al. 1993a; Tang et al. 1995; Tang et al. 1999b).

Fibrinogen (molecular weight 34kDa (Tripathi 2011)) is the third major plasma component (Tripathi 2011) but unlike albumin and immunoglobulin G it appears to have a key role in the inflammatory response (Tang et al. 1993b). Fibrinogen immediately adsorbs onto the foreign surface and experiments suggest it subsequently undergoes denaturation leading to strong adhesion (Tang et al. 1993b; Tang et al. 1998; Balasubramanian et al. 1999). There is a strong correlation between the degree of fibrinogen denaturation and the degree of acute inflammatory response (Absolom et al. 1987; Tang et al. 1998; Tang et al. 1999a). Moreover, there is a lack of inflammatory response in mice which receive an implant but lack circulating fibrinogen except in the case when the implant itself is pre-coated with fibrinogen or plasma (Tang et al. 1993b; Tang et al. 1995; Szaba et al. 2002).

There is evidence to suggest that adsorption of fibrinogen and/or γ-globulin from the blood onto a foreign surface promotes subsequent platelet adhesion as there is a complex interaction between the adsorbed proteins and the platelets (Beugeling 1979).

Beugeling et al suggest that the surface activation of intrinsic coagulation by adsorbed proteins is dependent on the type of pre-adsorbed protein and surface properties of the material (Beugeling 1979). In their results, albumin coated surfaces exhibited longer plasma coagulation times than fibrinogen or γ-globulin coated surfaces, indicating that a polymer which preferentially adsorbs albumin will not only reduce platelet adhesion but also delay contact activation of intrinsic coagulation (Beugeling 1979).

Nonspecific protein adsorption has a wide range of negative implications in the medical device development field including; contact lens fouling, fibrous encapsulation, small-diameter artificial blood vessel occlusion ,acute and chronic inflammation, complement activation, and bioincompatibility of haemodialysis membranes (Somasundaran 2006). Additionally, non-specific protein adsorption is known to be a central factor for membrane fouling (discussed in more detail in *3.2.3.1 Protein Adsorption*) as plasma proteins such as albumin, fibrinogen and immunoglobulins form a layer which increases resistance to mass transfer (Lameire et al. 2000; Su et al. 2011). Evidence of this can be found when comparing solute sieving coefficients before and after exposing a membrane to plasma or another protein rich solution (Lameire et al. 2000). Outside of the medical device field, but related to MUF, nonspecific protein adsorption can also limit the effectiveness of water filtration membranes (Somasundaran 2006). However, protein adsorption can be exploited to improve performance or enact specific functions of certain

materials and systems by controlling which proteins adsorb as well as their orientation or conformational state e.g. in bioreactors, cell colonisation, biosensors, immunoassays and encouraging vascularisation in tissue scaffolds (Somasundaran 2006; Rabe et al. 2011).

Preventing nonspecific protein adsorption and subsequent denaturing of proteins is a primary concern for implanted biomaterials in particular, but also for devices which come in contact with protein rich bodily fluids such as blood (Courtney et al. 1999; Courtney et al. 2003; Binazadeh et al. 2013a). However, it is complicated to avoid protein adsorption and even the principles and long term stability of recent advances in protein rejection (self-assembled monolayers and polymer grafted surfaces e.g. PEG and PAA) remain to be fully understood (Huang et al. 2001; Heuberger et al. 2005; Zurcher et al. 2006; Rabe et al. 2011).

The unique overall structure of a given protein is principally determined by its amino acid sequence, however it has been noted that the conformation of a protein at its free energy minimum when near a surface is different to that seen in the same protein at its free energy minimum state in a solution, which suggests conformational changes occur in proteins near a foreign surface (Rabe et al. 2011; Binazadeh et al. 2013a). These conformational changes help the proteins to establish better contact with the foreign surface ultimately lead to non-specific protein adsorption, but can in some cases irreversibly alter the structure of a protein and cause protein denaturing thereby impacting upon its biological function and may instigate adverse reactions in the host system e.g. coagulation and inflammatory cell accumulation (Absolom et al. 1987; Lu et al. 1991; Ugarova et al. 1993; Hu et al. 2001; Collier et al. 2002; Rabe et al. 2011). Henry et al observed that on unmodified polycarbonate membranes (like the membrane to be used in the proposed device) albumin rapidly forms an adsorbed monolayer by changing its conformation which results in it losing its biologically active structure thereby affecting its functionality (Henry et al. 2003).

The extent of the conformational changes varies from protein to protein and are driven by protein-surface interactions (van der Waals, electrostatic, hydration, hydrophobic interactions), however entropy gain from structural rearrangements inside the protein and the release of surface adsorbed water molecules and salt ions is perhaps the major driving force of protein adsorption (Norde 1986; Haynes et al. 1994; Ramsden 1995; Norde 1996; Malmsten 1998; Rabe et al. 2011; Binazadeh et al. 2013a).

There are a variety of surface-induced forces between the foreign surface and protein solutions which are implicated in the non-specific adsorption of proteins (Andrade et al. 1987; Pankowsky et al. 1990; Nath et al. 2004; Shen et al. 2004; Vladkova 2010). It is thought that the low activation energy required to change conformations of proteins makes them vulnerable to

changes induced by surface interaction forces such as van der Waals, electrostatic, hydration and hydrophobic forces (Leckband et al. 2001; Binazadeh et al. 2013a).

London dispersion (induced dipole-induce dipole) forces between a protein and a surface may be most important since they represent ~95% of the van der Waals force (Van Oss 2006; Binazadeh et al. 2013a). The electrostatic force between a surface and a protein can either be attractive or repulsive depending on the charges of both the protein and the surface (Binazadeh et al. 2013a). In a physiological solution, such as blood, the various proteins and their varying conformations, charge densities and isoelectric points mean there will be a wide variety of electrostatic interactions across the general protein population, perhaps explaining the preferential adsorption of specific proteins seen (Binazadeh et al. 2013a). Hydrogen bonding also plays an important role in protein adsorption with hydration pressure and hydrophobic interactions possibly producing a driving force 100 greater than the electrostatic and dispersion forces combined, (Van Oss 2006; Binazadeh et al. 2013a). This highlights the importance of the hydrophobic/hydrophilic nature of membranes in the filtration of protein rich solutions such as blood.

The magnitude and range of all the aforementioned interactions depend upon the properties of the protein (conformation, isoelectric point, sequence), the surface (surface chemistry including charged character, surface free energy and topological structure) as well as external parameters e.g. those of the solution (temperature, pH and ionic strength) (Su et al. 2011; Binazadeh et al. 2013a).

3.2.3.2. External parameters influence on protein adsorption

The complex folded secondary and tertiary structure of proteins means that each protein has a specific distribution of hydrophobic and hydrophilic and positively and negatively charged side chains which will impact their adsorption characteristics but these characteristics and the behaviour of proteins are subject to change with changes in environmental conditions such as temperature, pH and ionic strength (Rabe et al. 2011). Higher temperatures can increase diffusivity of proteins towards the foreign surface, and thus generally result in increased protein adsorption (Koutsoukos et al. 1983; Rabe et al. 2011). pH influences the electrostatic state of proteins and therefore when pH is equal to the isoelectric point of a protein electrostatic repulsions between proteins are minimised and proteins can pack more tightly at the surface resulting in more protein adsorption (Jones et al. 2000; Rabe et al. 2011). Higher ionic strength will shorten electrostatic interactions between proteins resulting in higher packing densities and can also increase the tendency for protein aggregation (Jones et al. 2000; Vendruscolo et al. 2007).

3.2.3.3. Protein properties influence on protein adsorption

Certain proteins which are small in size and structurally rigid can be classified as 'hard' proteins and have a low tendency to change structure upon adsorption, while some higher molecular weight 'soft' proteins can undergo significant changes to their conformation e.g. lipoproteins which show a strong affinity for hydrophobic surfaces (to some extent explaining the high fouling tendency of hydrophobic membranes mentioned earlier in *3.2.1. Membrane Characteristics and Options*) (Rabe et al. 2011). By this measure, the majority of plasma proteins (Albumin, Transferrin, Immunglobulins) would be considered 'intermediate' and would generally undergo changes in their conformation upon contact with a surface (Rabe et al. 2011). Proteins of this type also have the ability to exist at two or more adsorbed states with differing adhesion energies (Rabe et al. 2011). Absolom et al found that the hydrophobic/hydrophilic nature of proteins themselves also impact upon their tendency to adsorb to a surface as in their study the most hydrophobic protein, fibrinogen, adsorbed to the greatest to all surfaces whilst the least hydrophobic protein, bovine serum albumin, adsorbed to the least extent to all surfaces studied (Absolom et al. 1987).

3.2.3.4. Surface properties influence on protein adsorption

Surface properties can dramatically impact the rate and magnitude of protein adsorption as well as the nature, concentrations and conformations of the adsorbed proteins (Grinnell et al. 1981; Anderson et al. 2008; Binazadeh et al. 2013b). Several studies have investigated the impact of different surface chemistries on protein adsorption but a detailed understanding of the mechanisms of the complex process of protein adsorption has not yet been achieved (Binazadeh et al. 2013a). Important surface parameters which influence protein adsorption are surface energy, polarity, charge, surface tension, wettability and morphology (Hlady et al. 1999; Rabe et al. 2011). Generally speaking, proteins will bind most strongly to non-polar (hydrophobic), high surface tension and charged substrates (Absolom et al. 1987; Rabe et al. 2011). The hydrophilic/hydrophobic nature of a surface is relatively important in determining its interaction with proteins (Su et al. 2011). A hydrophilic surface will preferentially adsorb water over solutes and may reduce protein adsorption and subsequent blood interactions, consequently many researchers have made efforts to use hydrophilic materials in membrane applications or modify surfaces to increase hydrophilicity in order to reduce protein adsorption and/or fouling (Courtney et al. 1999; Mockel et al. 1999; Xu et al. 2009; Hollinger 2011; Su et al. 2011). It has been suggested that non-polar surfaces will destabilise proteins allowing then to undergo conformational changes and form strong protein-surface and protein-protein interactions (Anand et al. 2010). Again, this goes some way to explaining the higher affinity for

proteins to hydrophobic surfaces and the higher degree of fouling in hydrophobic membranes (Andrade et al. 1986). Henry et al found that when albumin adsorbed to a polycarbonate membrane (similar to that to be used in the proposed device), it adopted a different conformation depending on if the surface was hydrophobic or hydrophilic (Henry et al. 2003).

There is a body of evidence to suggest that changes in nanoscale topography of artificial substrates significantly influences protein adsorption, and it even appears that different nanostructures influence different proteins (Lord et al. 2010). However, this area of research is still in its infancy both in terms of nanostructure synthesis and understanding of the mechanisms through which the nanoscale topographies influence protein adsorption (Lord et al. 2010).

3.2.3.5. Protein behaviour at a surface

When a protein adsorbs to a surface it does so in a certain favoured orientation determined by its free energy minimum state resulting from van der Waals interactions, hydrogen bonds and entropy gain from solvent molecule or counter ion release (Rabe et al. 2011). The complex structure of a protein means that different regions of its surface will have different affinities for adsorption to a given surface (Rabe et al. 2011).

As more and more proteins become adsorbed to a surface, the protein-protein interactions become more important in determining the free energy minimum state, and the initial orientation which was based on protein-surface interactions alone may no longer be the most favourable one (Rabe et al. 2011). For example, an initial adsorption orientation based on the electrostatic attraction between the protein and the surface may result in high electrostatic repulsion forces between neighbouring proteins. In order for further proteins to adsorb to the surface, the surface bound proteins may need to change their orientation to one which results in diminished protein-protein electrostatic repulsion as well as diminished protein-surface electrostatic attraction (Rabe et al. 2011). This is shown in Figure 64.

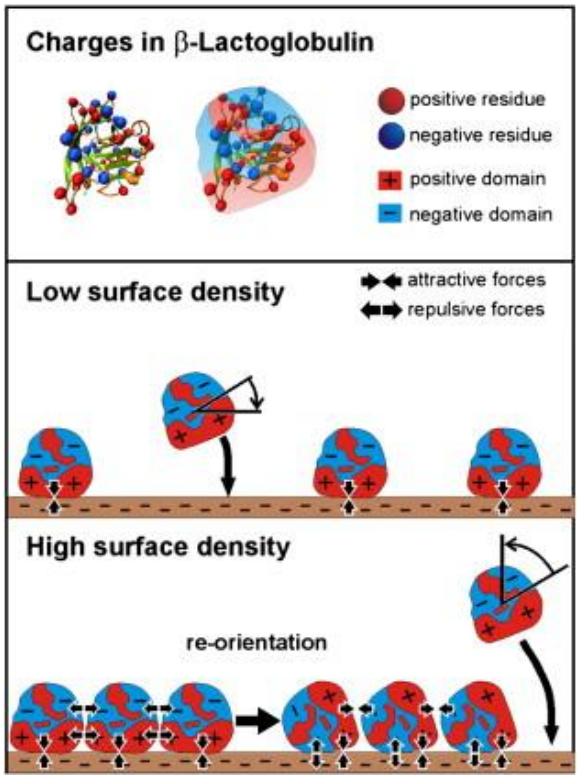


Figure 64: Orientation change of surface bound proteins in order for further proteins to adsorb to the surface which results in diminished protein-protein electrostatic repulsion as well as diminished protein-surface electrostatic attraction (Rabe et al. 2011)

Clearly protein adsorption is a highly complex topic and the complexity only rises further when we consider the collective behaviour of the proteins of the adsorbed layer, the details of which go beyond the scope of this thesis. At this point, especially when one considers the extreme difficulty in avoiding protein adsorption as well as the severe complexity of the protein adsorption process between plasma and polymer surfaces (as in the proposed device) as highlighted by Andrade et al (Andrade et al. 1987), it is sufficient to have an awareness of the phenomenon and the impact it may have on the functioning of the device and the membrane in particular. However, one element of the collective behaviour of proteins which is of particular interest with regards to haemofiltration is the Vroman effect.

In mixtures of different proteins such as blood, generally speaking it is the smaller proteins which will adsorb to the surface first since they can diffuse fastest and will make up the majority of the adsorbed layer in the earlier phases, however larger proteins will bond more strongly to the surface due to their larger contact area are even able to repel previously adsorbed proteins (Rabe et al. 2011). Consequently the mass of adsorbed proteins may peak during the adsorption process then decline rather than continuing to grow over time to a saturation point, this phenomenon is known as the Vroman effect (Rabe et al. 2011).

Vroman et al investigated the adsorption of blood plasma proteins to a surface and found that Fibrinogen would rapidly adsorb to the surface but that after a short time a fraction of it would be replaced by proteins with a higher affinity for the surface such as high molecular weight kininogen (Vroman et al. 1969; Vroman et al. 1971; Vroman et al. 1980). The Vroman effect has subsequently been confirmed by other groups (Brash et al. 1984; Elwing et al. 1987; Slack et al. 1995; Fang et al. 2003; Jung et al. 2003) and has been observed in both whole blood and plasma (Patrick et al. 1998). Steneker et al report the sequence of protein deposit in blood filtration applications as: albumin, immunoglobulin G, fibrinogen, fibronectin, high molecular weight kininogen, and factor XII (Steneker et al. 1995). Adsorbed proteins may desorb rapidly according to the Vroman Effect and thus in an in vivo environment the type of proteins cells will encounter can vary with time (Xu et al. 2007).

Another factor which must be mentioned is the aggregation of proteins. Minton suggests that protein monomers in the bulk solution exhibit a preference to aggregate with surface protein clusters rather than to adsorb directly to the surface material and additionally that the larger the cluster size the stronger the affinity (Minton 2000; Minton 2001). In this fashion protein can rapidly aggregate on a solid surface (Rabe et al. 2011).

As Rabe et al state; *"the often proposed cluster growth mechanism through a surface diffusion or a direct attachment of protein monomers cannot be observed directly but can only indirectly concluded from the increase of the cluster size"* (Rabe et al. 2011). This highlights the difficulty in determining the mechanism for deposition of the deposits seen in the SEM images shown previously (Figure 59) and, subsequently, the nature and consequences of such deposits. Rabe et al encourage caution in interpreting results from SEM scans since the microscope tip can cause protein displacement with repeat scans and also that some evidence shows protein clusters may grow normal to the surface making it difficult for SEM investigations to accurately assess the degree of protein aggregation. Rabe et al also point to an alternative mechanism, by which large protein clusters can manifest on a surface in which protein aggregates form in the solution and subsequently deposit onto and spread across the surface (Rabe et al. 2009).

Some proteins such as BSA (bovine serum albumin) can cluster in the solution and Rabe et al have shown that these clusters can deposit onto a surface and begin to spread (Rabe et al. 2009). Moreover, Rabe et al found that the rate at which the protein cluster spreads is highly influenced by surface chemistry with clusters spreading quickly on hydrophobic surfaces and slowly on hydrophilic surfaces based upon the notion that proteins on a hydrophobic surface have higher mobility and thus an existing protein layer can be disrupted to accommodate the anchoring of a protein cluster, a phenomenon which will not occur on hydrophilic surfaces

(Rabe et al. 2009). Again, this highlights the benefits of selecting the hydrophilic membrane for use in the proposed device.

The binding strength between proteins and a surface may be low at first but can increase over time with the conformational changes which can alter the secondary structure, consequently an adsorbed protein layer may be initially easy to remove by washing but after a certain time period (ranging from a few minutes to several hours) will exhibit and strong or insurmountable resistance to elution (Hlady et al. 1996; Malmsten 1998; Gray 2004; Lu et al. 2007; Rabe et al. 2011).

The deposit seen in the SEM images shown previously (Figure 59) resulted from contact time with blood of over an hour. Due to the strong binding nature of protein adsorption after long time periods, one would expect that a protein deposit to resist removal by gentle washing although it may be susceptible to the shear forces generated through more vigorous washing. In the study into protein adsorption on polycarbonate track etched membranes conducted by Tracey et al, and in other studies, membrane samples were rinsed and allowed to dry before being viewed under SEM, further suggesting that gentle washing should not remove such deposits (Tracey et al. 1994; Gaell et al. 1996).

In order to better ascertain the impact of washing in the deposit seen with a view to better understanding the nature of the deposit, the impact of two different washing techniques was observed.

The 'blood side' of a heavily stained used membrane is shown in Figure 65.

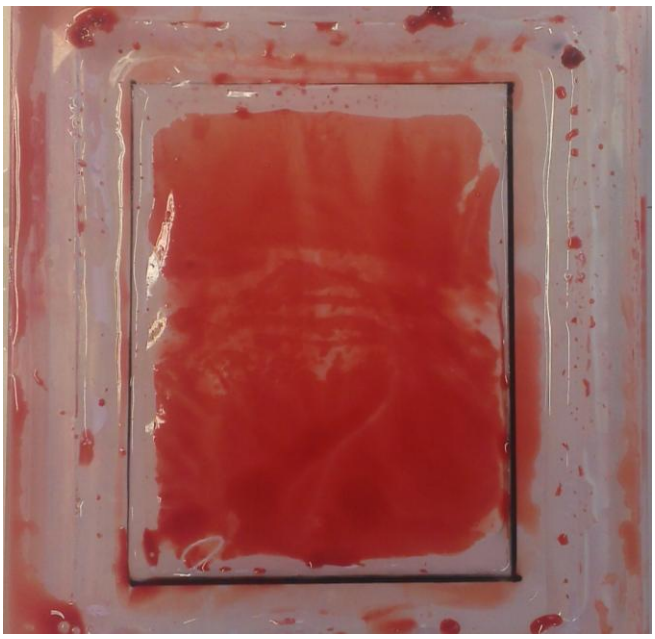


Figure 65: Control membrane at end of experiment heavily stained with blood

The upper half of this membrane was washed by gently pouring saline onto the blood side of the membrane surface whilst the lower half of this membrane was washed more vigorously under a stream of water. Figure 66 shows the visual result of these two techniques.

Figure 66: Membrane after washing, upper half washed by pouring saline onto it, lower half washed more vigorously with water from tubing attached to tap

The more vigorous technique appears to have removed much more of the deposit although some discoloration was visible. Figure 67 and Figure 68 show the SEM scans of the upper half of the membrane which was gently washed with saline.

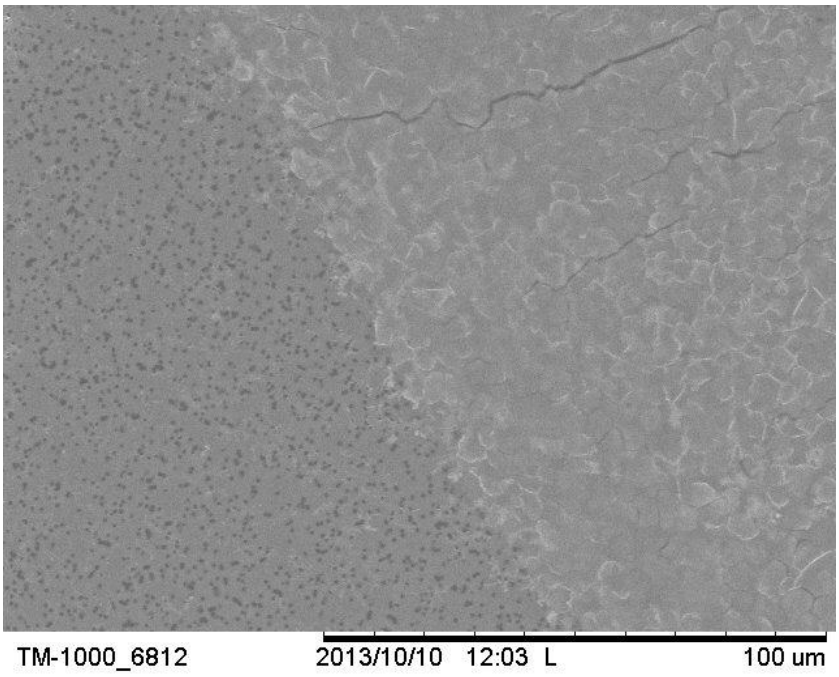


Figure 67: SEM image at x1000 magnification of the upper half of the membrane which was gently washed with saline showing a deposit frontier

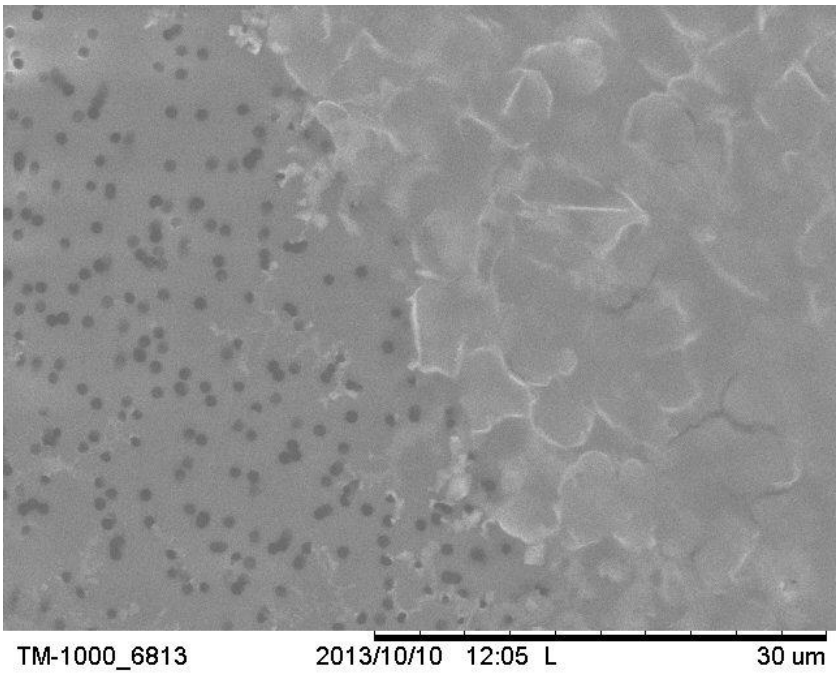


Figure 68: SEM image at x3000 magnification of the upper half of the membrane which was gently washed with saline showing a deposit frontier

Although there were some areas clear of deposit it is clear that despite gentle washing with saline, some areas still show the same more pronounced deposit seen earlier (Figure 59). This suggests that the deposit exhibits at least some resistance to its removal. In addition to this form of deposit, SEM scans showed examples of 'plugged' pores as seen in Figure 69 and Figure 70.

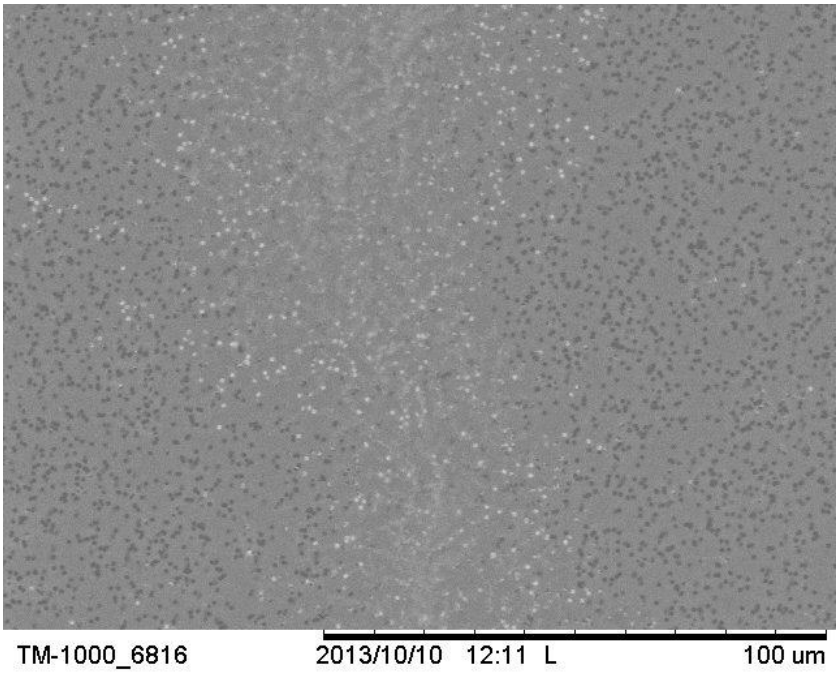


Figure 69: SEM image at x1000 magnification of the upper half of the membrane which was gently washed with saline showing areas of 'plugged' pores

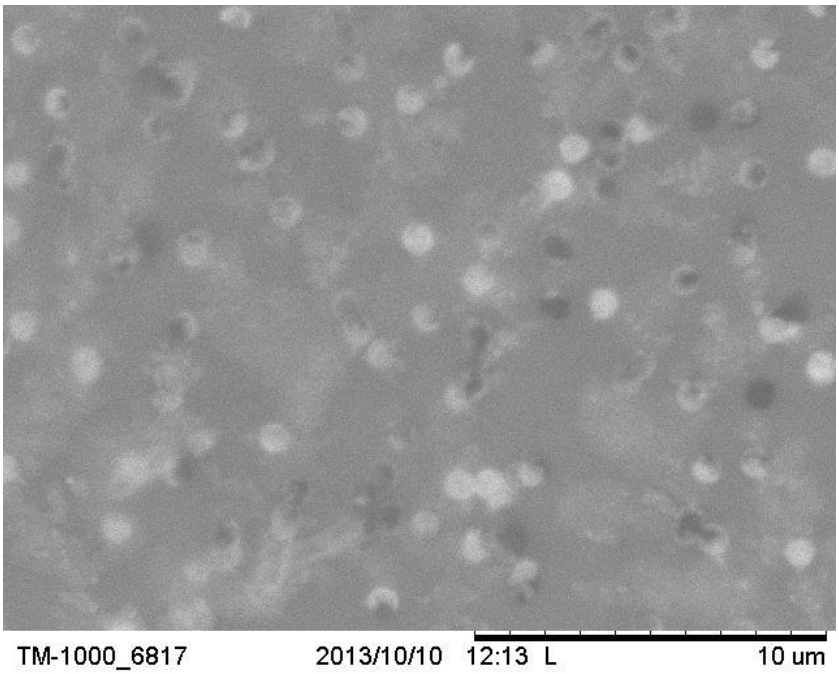


Figure 70: SEM image at x10000 magnification of the upper half of the membrane which was gently washed with saline showing 'plugged' pores

The particles of this 'pore plugging' material must be smaller than 1μm in diameter in order to pass into the pores and block them internally. Upon further inspection with the SEM it appeared that the source of this 'pore plugging' may in fact be the washing saline itself. In some instances, as shown in Figure 71 and Figure 72, the 'plugged' pores appear immediately adjacent to areas of 'dendritic' deposit.

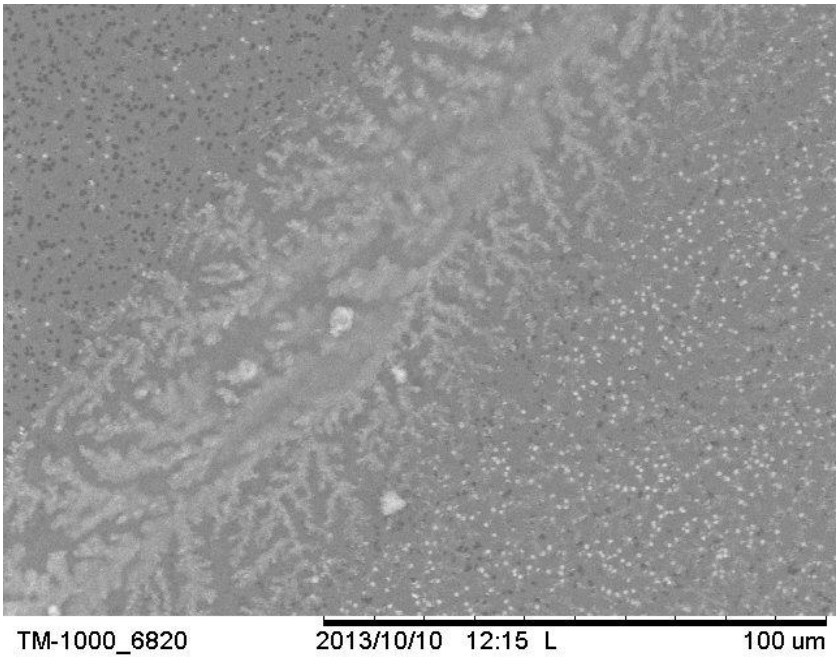


Figure 71: SEM image at x1000 magnification of 'plugged' pores appearing immediately adjacent to areas of 'dendritic' deposit

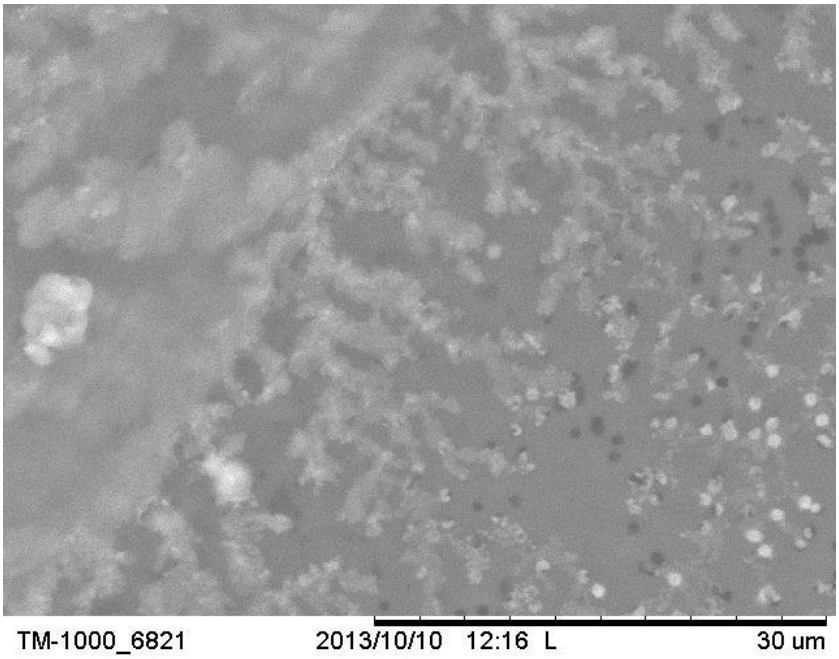


Figure 72: SEM image at x3000 magnification of 'plugged' pores appearing immediately adjacent to areas of 'dendritic' deposit

This ‘dendritic’ deposit had not been seen previously on membranes which had been in contact with blood and bears a resemblance to SEM images of salts from other research groups shown in Figure 73.

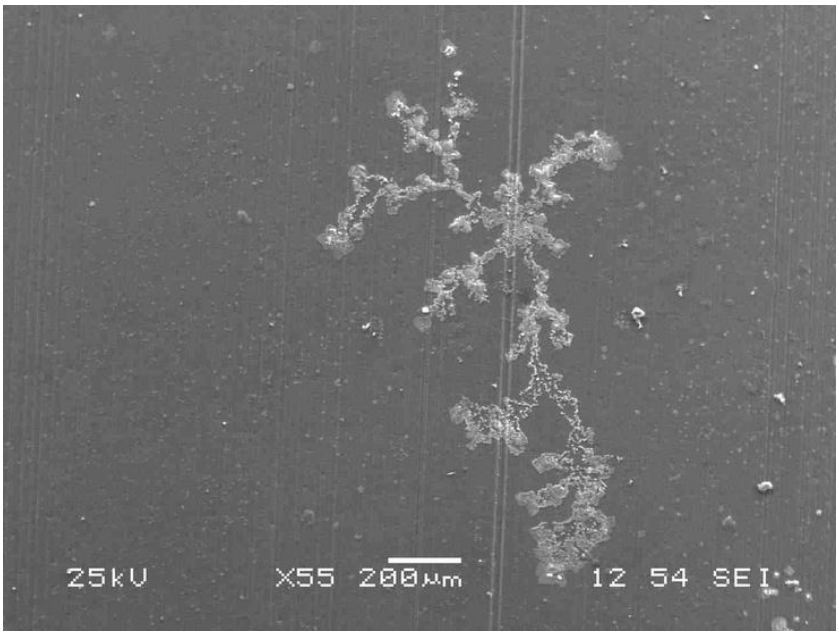


Figure 73: SEM image of a dendritic salt crystal residue on aluminium (Jerner 2015)

As the initial photograph in Figure 66 suggests the lower half of the membrane which underwent more vigorous washing with water was largely free of the more pronounced deposit although, as Figure 74 and Figure 75 show, there was some evidence of ‘pore plugging’.

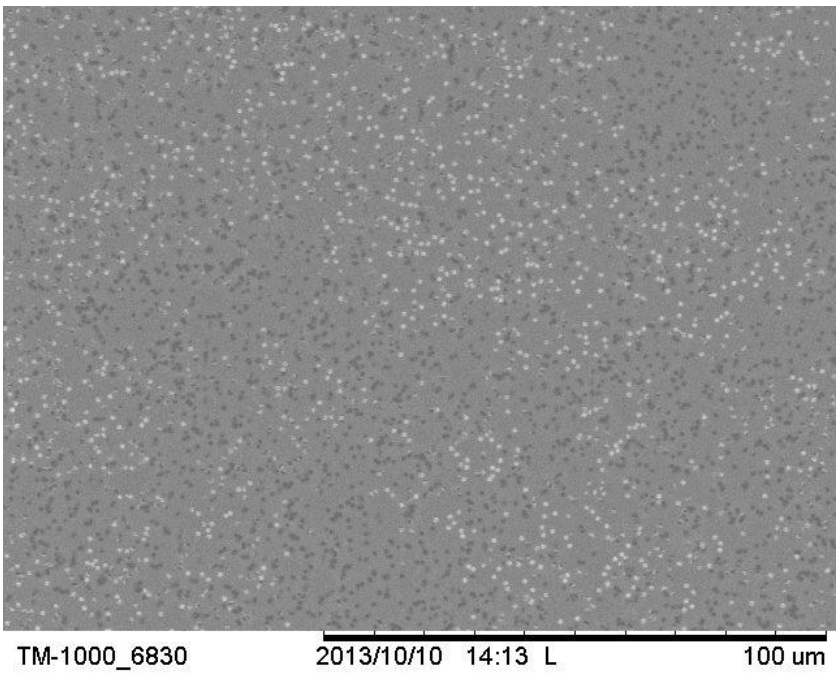


Figure 74: SEM image at x1000 of the lower half of the membrane which was more vigorously washed with water magnification showing areas of 'plugged' pores

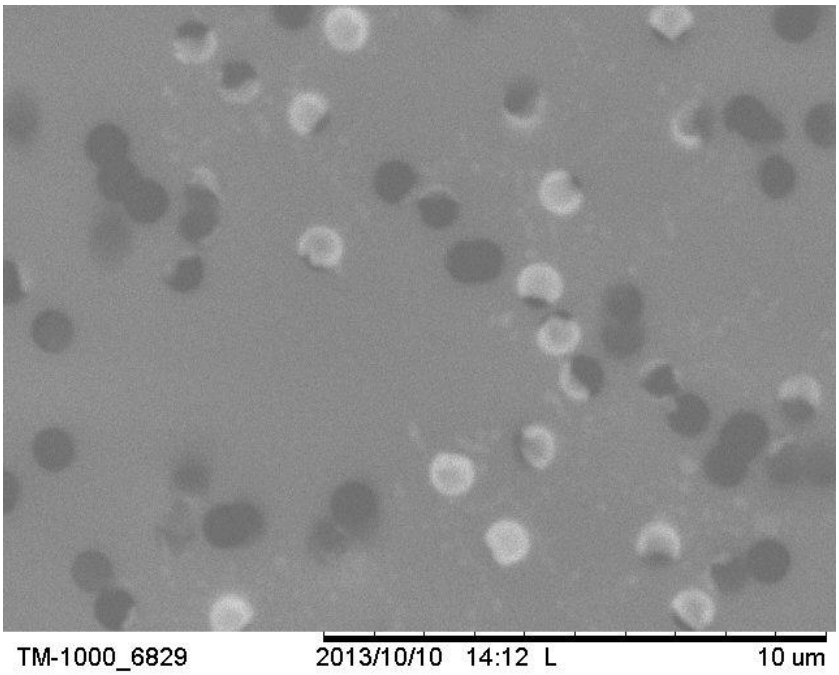


Figure 75: SEM image at x1000 of the lower half of the membrane which was more vigorously washed with water magnification showing areas of 'plugged' pores

Although this lower half of the membrane was washed with water and not saline, there may have been some 'spill over' from the upper half of the membrane which was washed with saline resulting in the replicated 'pore plugging' which as we have seen is most likely from saline salt.

The deposit appears to have some resistance to rinsing with saline but can be largely remove with more vigorous washing with water. It is certain that some degree of protein adsorption would occur when blood comes in contact with the polycarbonate membrane, but whether this is the 'thick' layer of deposit seen which completely obscures the membrane pores is still not clear and is difficult to ascertain. It is possible that the 'thick' layer of deposit is comprised of adhered platelets.

As mentioned earlier (*3.2.3. Membrane fouling*), when blood comes in contact with a foreign surface, protein adsorption is a precursor for platelet adhesion, which is a feature of blood coagulation along with activation of clotting mechanism and ultimately thrombus formation, and there is evidence to suggest that adsorption of fibrinogen and/or γ-globulin from the blood onto a foreign surface promotes subsequent platelet adhesion (Beugeling 1979; Chandy et al. 1985; Courtney et al. 2003).

Platelet adhesion to the surfaces of medical devices which come in contact with blood is a fundamental event in the formation of thromboses on such surfaces (Courtney et al. 2003; Su et al. 2011). Adhesion and activation of platelets can induce thrombin production leading to further coagulation (Keuren et al. 2002; Monroe et al. 2002; Su et al. 2011). For clinical applications such as CPB, interest in understanding and controlling the blood response has shifted from the erythrocytes to the platelets, leukocytes, complement activation and the inflammatory response (Courtney et al. 1996; Courtney et al. 1999). SEM is the method of choice for determining the magnitude of platelet adhesion and the morphology of the adhered platelets (Su et al. 2011).

Figure 76 shows examples of the typical morphology of platelets adhered to polyethersulfone (PES) membranes (Su et al. 2011).

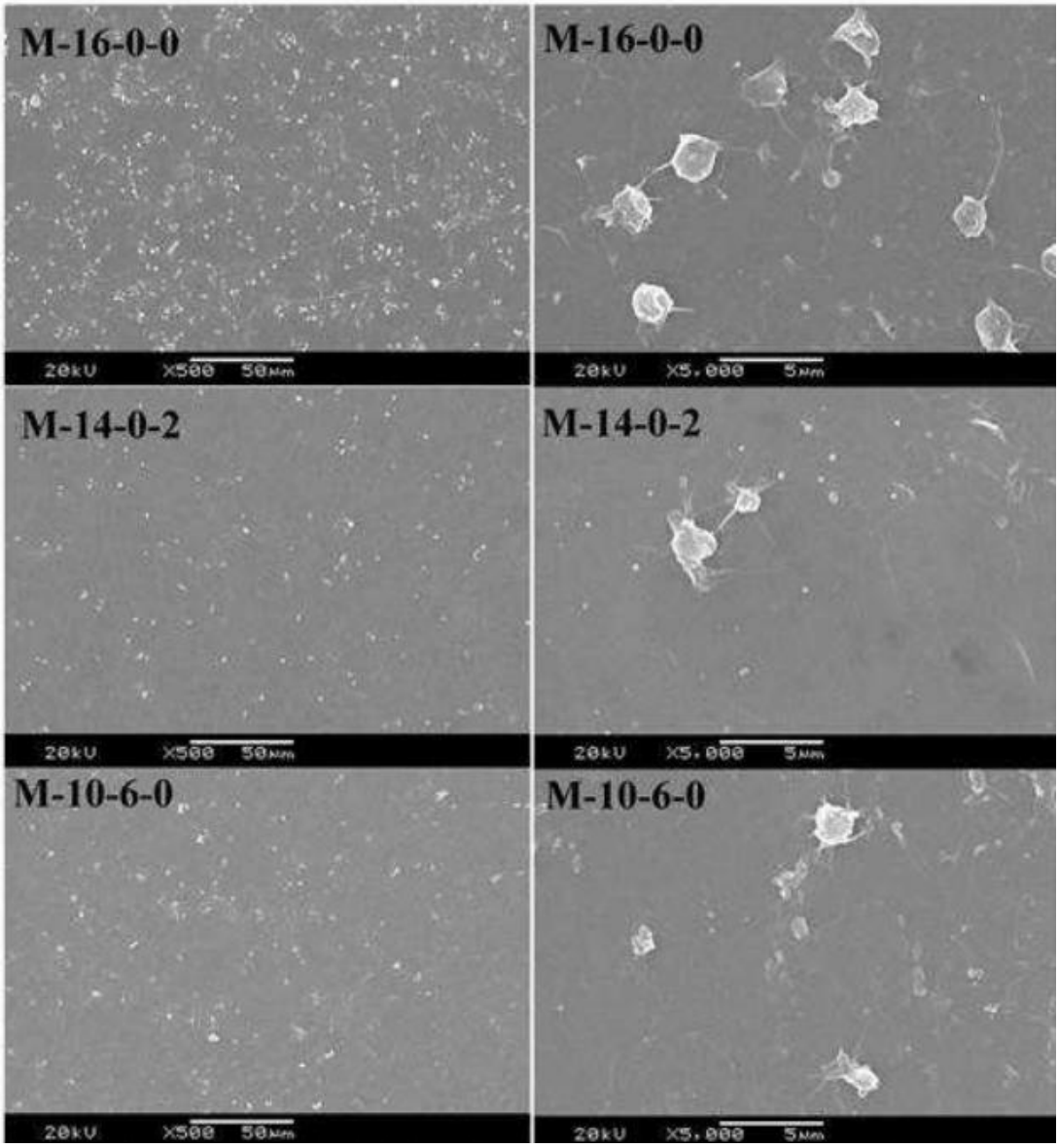


Figure 76: Typical morphology of platelets adhered to polyethersulfone (PES) membranes (Su et al. 2011)

Figure 76, depicting differing degree of platelet adhesion on differently modified PES membranes, shows large amounts of adhered and aggregated platelets to the membrane surface with a 'pancake' shape (suggesting spreading as the pseudopodium retract) and also more rounded platelets with nearly no pseudopodium and deformation (Su et al. 2011). The different stages of adhesion and the associated shape changes platelets undergo is shown in Figure 77.

Figure 77: SEM images showing different stages of platelet adhesion: left: resting platelet x10,000, middle: attached platelet showing shape change and pseudopodia x3000, right: spread platelet x3000 (Ginés Escolar 2015)

Options for surface modification to reduce the platelet response to foreign surfaces exist but the complex nature of the overall blood response to artificial surfaces means there is often a subsidiary negative effect (Courtney et al. 2003). For example, increased negative charge could reduce platelet response but increase the contact activation phase of intrinsic pathway, whilst increased hydrophilicity by introducing hydroxyl groups could also reduce platelet response yet increase complement activation (Courtney et al. 2003).

The platelets seen in the SEM images from Su et al are not found in the SEM images taken during preliminary testing of the proposed device and do not bear much resemblance to the deposit seen. It is likely therefore that the 'thick' obscuring layer of deposit seen is not the result of mass platelet adhesion, although platelet adhesion may be occurring to some degree.

Based on the SEM images of the stained membrane and of washed membrane from early testing shown previously in this section, it is still difficult to ascertain the nature and severity of the deposit seen. Clearly the more pronounced 'thick' deposit seen has the potential to obstruct the membranes and could hinder the functioning of the proposed device if it is bound with sufficient vigour during the haemoconcentration procedure. The washing regimen comparison indicates that the deposit exhibits at least some resistance to it removal but that more vigorous washing it able to largely eradicate it. What is difficult to ascertain is whether this deposit is bound to the membrane in some fashion during the haemoconcentration procedure or merely the result of fluid 'settling' on the membrane surface by surface tension as blood is drained from the rig after the experiment. One factor determining this, related to the response of the deposit to membrane washing, is whether the flow through the device acts to 'wash' the membrane itself. Again, this is very difficult to determine at this stage but this topic will be discussed in detail later in this chapter.

The presence of the deposit seen could well be indicative of two phenomena which affect almost all filtration processes; concentration polarisation and membrane fouling.

3.2.3.6. Concentration Polarisation and Membrane Fouling

In filtration processes, the retained or rejected elements which are unable to pass through the membrane naturally accumulate at the membrane surface, resulting in concentration polarisation and membrane fouling, both of which reduce filtration performance (Sablani et al. 2006). The theory of concentration polarisation was first conceived by Blatt et al in 1970 (Blatt et al. 1970; Jaffrin et al. 1992).

Concentration polarisation is a natural consequence of filtration in which the accumulation of particles unable to pass through the membrane pores results in a concentration gradient leading to back-transport of the rejected elements to the bulk flow (Sablani et al. 2006). The accumulated particles create significant osmotic pressure at the membrane wall, reducing the effective TMP and resulting in reduced filtrate flux (Sablani et al. 2006). The relationship between concentration polarisation and filtration flux (J) can be expressed by the equation: (Sablani et al. 2006)

$$J = k\ ln\left(\frac{C_w - C_P}{C_B - C_P}\right)$$

Where k is the mass-transfer coefficient and C_w, C_B and C_P are the species concentrations at the membrane, in the bulk and in the filtrate respectively (Sablani et al. 2006). It can be seen that filtration flux can be increased by either increasing the mass-transfer coefficient or reducing the bulk concentration of the species.

In other haemofiltration techniques such as renal replacement therapy, concentration polarisation is primarily related to plasma proteins which accumulate at the membrane surface leading to a concentration gradient back into bulk plasma along which 'back-diffusion' can occur (Lameire et al. 2000). In this way, concentration polarisation can negatively impact upon clinical procedures such as continuous veno-venous haemofiltration (Lameire et al. 2000). However, Lameire et al state that in renal replacement therapy applications, concentration polarisation actually enhances the removal of proteins between 30kDa and 70kDa in mass which, under normal conditions, would undergo minimal removal by convection (Lameire et al. 2000).

Concentration polarisation is a reversible phenomenon which affects the performance of the filtration system without altering the intrinsic properties of the membrane itself (Sablani et al. 2006). However, concentration polarisation is the primary cause of membrane fouling which is a more complex, severe and sometimes irreversible phenomenon which can reduce flux by up to 95% by causing changes to the membrane or the formation of a fouling layer (Sablani et al. 2006; De et al. 2013).

Membrane fouling refers to the undesired accumulation and deposition of particulate matter, solutes, colloids and microorganisms over the membrane surface and the associated decrease in permeate flux which this results in (Sablani et al. 2006; Mohanty et al. 2011; De et al. 2013). There are three types of fouling; cake layer formation (which has been described earlier in *2.2.2.1. Filter Cake*), pore blockage and internal pore fouling (Sablani et al. 2006). The relative preponderance and importance of each of these types of fouling depends on the properties of both the membrane and the fluid to be filtered, for example, if the size of the foulant particles is comparative to or smaller than the pore diameter then fouling by pore blockage and internal pore fouling is more likely to occur than if the foulant particles are larger than the pore diameter, in which case fouling by cake layer formation is more probable (Sablani et al. 2006; Mohanty et al. 2011). In pore blocking and internal pore fouling scenarios, adsorption of solutes onto and/or within the membrane reduce the open area through which filtrate can pass thus reducing the effective pore diameter and filtration flux (Song 1998; Sablani et al. 2006; Mohanty et al. 2011). These forms of membrane fouling are particularly common when filtering macromolecules such as proteins and are irreversible, with chemical cleaning required to restore the functionality of membrane (Sablani et al. 2006). Cake layer formation on the membrane surface has been described earlier in this thesis (*2.2.2.1. Filter Cake*) and is the result of the retained or rejected elements which are unable to pass through the membrane accumulating at the membrane surface and increasing resistance to filtrate flow through the membrane pores (Song 1998; Sablani et al. 2006; Mohanty et al. 2011). Figure 78 illustrates the difference between cake layer formation and pore blocking and internal pore fouling

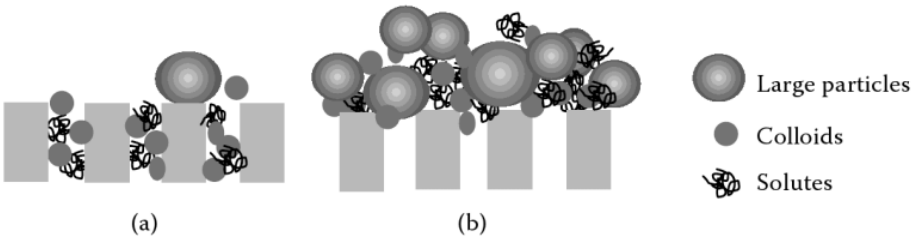


Figure 78: a) pore blockage and internal pore fouling, b) cake layer formation (Meng et al. 2009)

Membrane fouling can be subcategorised according to the nature of the foulant as being either; biofouling, organic fouling or inorganic fouling (Mohanty et al. 2011). Biofouling refers to the deposition, growth and metabolism of bacterial cells on the membrane (Pang et al. 2005; Mohanty et al. 2011). Biofouling can be important in filtering applications such as water treatment but for MUF applications this form of fouling would only likely be important in the case of a patient with sepsis. Inorganic fouling refers to deposits of inorganic scales e.g. silica forming on the membrane resulting from concentrations of such substances beyond their

saturation point (Mohanty et al. 2011), again this form of fouling is less relevant in MUF applications. Organic fouling refers to the deposition of organic matter such as proteins and polysaccharides onto the membrane (Mohanty et al. 2011) and is the most relevant on the three forms of fouling to a MUF device. The relatively small size of organic matter means that they are more readily deposited on the membrane by the filtrate flow since the lift forces acting on the particles from the feed flow are smaller thus reducing the prevalence of back transport into the bulk phase (Mohanty et al. 2011).

In 1995, Field et al introduced the concept of critical flux, and suggested that operation of a filtration system below a certain critical flux would avoid irreversible fouling (Field et al. 1995; Mohanty et al. 2011). For short term applications, if permeate flux is kept below this critical flux, TMP remains stable and any fouling which occurs will be removable (Mohanty et al. 2011). In long term applications, irreversible fouling can occur even below this critical flux (Ognier et al. 2004; Guglielmi et al. 2007). The value of critical flux is dependent on operating conditions and the characteristics of the fluid and the membrane (Mohanty et al. 2011). The principle of critical flux has been used to control membrane fouling in reverse osmosis and nanofiltration membranes, though some have questioned its suitability (Lisitsin et al. 2005; Vrouwenvelder et al. 2009; Mohanty et al. 2011).

In 1991 Meireles et al assessed the impact of membrane fouling on ultrafiltration performance (Meireles et al. 1991). The group compared membrane permeability before and after either adsorption or ultrafiltration with bovine serum albumin. In the absence of protein denaturation, the membrane permeability was reduced by the same fraction by both fouling and adsorption regardless of ultrafiltration conditions (Meireles et al. 1991). When proteins were denatured, fouling was more severe. The results of this comparison suggested that membrane fouling is primarily due to adsorption of proteins and also deposition of denatured proteins on the membrane surface (Meireles et al. 1991). Meireles et al also measured retention coefficients and ultrafiltration fluxes of various membrane sizes (10kDa, 40kDa and 100kDa) and found that fouling resulted in increased retention coefficients and reduced ultrafiltration fluxes, the magnitude of which were larger for the higher MWCO membranes (Meireles et al. 1991).

It is clear that membrane fouling can have a profound impact upon ultrafiltration rates in any filtration technique, it is important to be aware of this phenomenon and, in particular, the specific and special fouling challenges raised by to blood filtration. Since blood is a protein rich solution, the fouling response of the filtration membrane to blood is likely to bear some resemblance to the response to other protein solutions which have been studied and modelled by several groups (Tracey et al. 1994; Kelly et al. 1995; Guell et al. 1996; Song 1998).

Models of membrane fouling in protein rich solutions generally involve two consecutive phases: first pore blocking followed by cake layer formation (Tracey et al. 1994; Kelly et al. 1995; Song 1998). Tracey et al suggest that the pore blocking phase is the result of 'internal fouling' where proteins are adsorbed or deposited at the internal wall or mouth of pores (Tracey et al. 1994). Tracey et al model internal fouling according to two principles; the first being that pore blocking is caused by protein adsorption to the internal walls of the pores thus reducing their radius while the pore density remains constant, giving rise to the 'standard blocking model', whilst the second principle is that pore blocking is the result of some pores being completely obscured whilst others are left unaffected, thereby reducing the pore density while the pore diameters remain constant, giving rise to the 'pore blocking model' (Tracey et al. 1994). Usually, only one of these models is used when interpreting results but it is likely that both are happening simultaneously (Guell et al. 1996). Tracey et al propose that the cake layer formation phase is the result of 'external fouling' where proteins and protein aggregates accumulate, deposit and grow at the membrane surface (Tracey et al. 1994). The 'cake filtration model' assumes cake resistance increases with time as rejected proteins accumulate at the membrane surface. Kelly and Zydney investigated mechanisms for membrane fouling with BSA and proposed that the pore blocking phase is the result of protein aggregates being deposited on the membrane surface whilst the cake layer formation phase is the result of chemical attachment of additional proteins to the initial protein deposits (Kelly et al. 1995).

Tracey et al studied the protein fouling specific to track etched polycarbonate microfiltration membranes with pore diameters of 0.05μm and 0.2μm (Tracey et al. 1994). Since, the membrane to be used in the proposed device is a track etched polycarbonate membrane with a pore diameter of 1μm; the protein fouling behaviour observed by Tracey et al could be a good indicator of the protein fouling phenomena possible in the proposed device.

Figure 79 shows the rapid decline in flux observed with a polycarbonate membrane in three protein solutions (Guell et al. 1996).

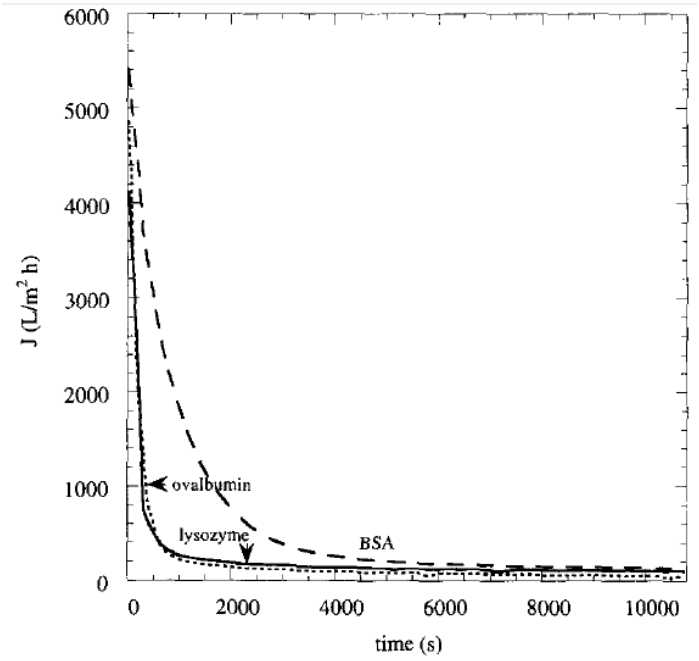


Figure 79: Rapid decline in flux observed with a 0.2µm pore diameter polycarbonate membrane in three protein solutions (Guell et al. 1996)

Guell et al also suggest that the narrow pore size distribution along with the low surface porosity of the polycarbonate membrane made it easier for the lysozyme protein aggregates to foul the membrane and reduce the flux (Guell et al. 1996).

Tracey et al point to the work of other groups which found that protein aggregates are a major fouling mechanism (Chandavarkar 1990; Hlavacek et al. 1993; Kelly et al. 1993). Despite the fact that protein and polysaccharide molecules are considerably smaller than the pore diameter of most microfiltration membranes, they still contribute to fouling by forming aggregates in the bulk phase which are subsequently deposited onto the membrane surface, leading to severe flux resistance in cross-flow filtration (Chandavarkar 1990; Belfort et al. 1994; Guell et al. 1996). Guell et al state that protein fouling during microfiltration is not related to protein size but rather the size of the aggregate a protein forms (Guell et al. 1996). Tracey et al observed the formation of a cake layer on the feed side of the membrane over the duration of their experiment as is shown in the SEM from their study shown in Figure 80 (Tracey et al. 1994).

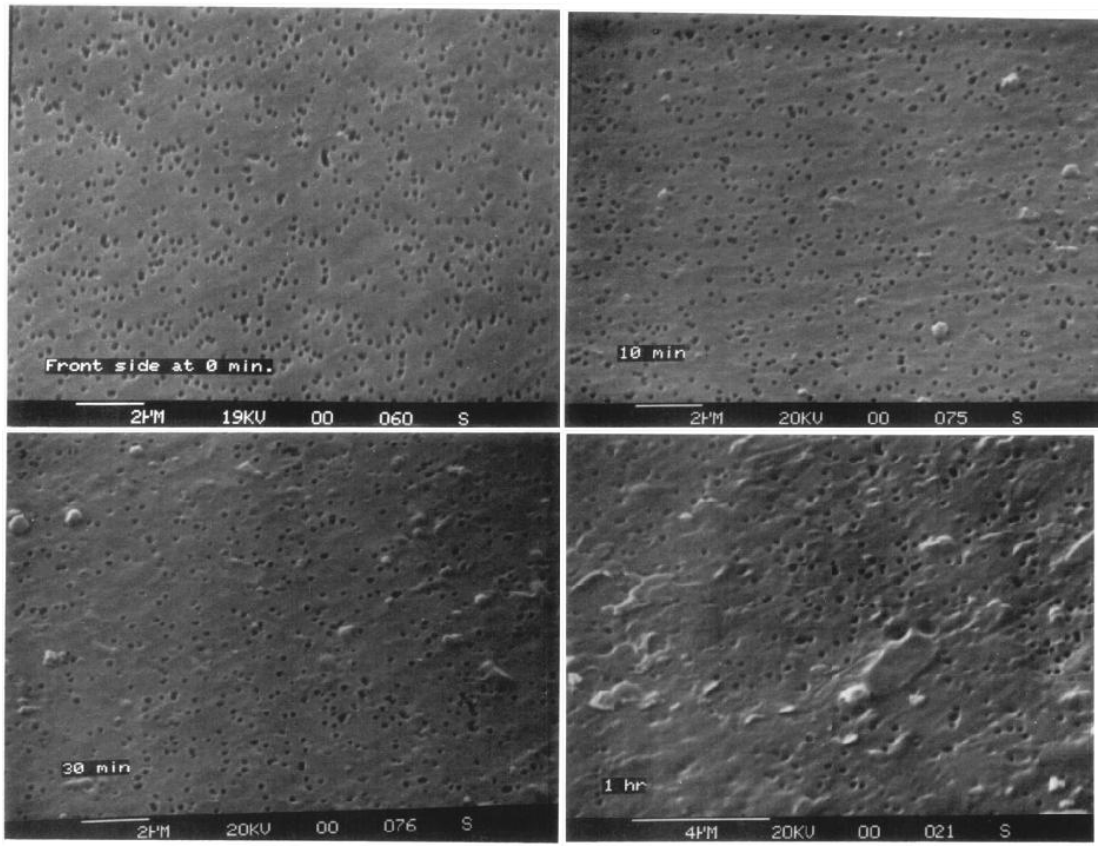


Figure 80: Formation of a cake layer on the feed side of the membrane over the duration of an experiment by Tracey el al, 0-60mins (Tracey et al. 1994)

The accumulation of the cake layer and the associated reduction in pore density and pore diameter resulted in a decrease in permeate flux (Tracey et al. 1994). Tracey et al state that for the 0.2µm pore diameter membrane both standard and pore blocking models which results in decreased pore density and decreased pore diameter respectively (defined earlier in *2.1. Principles of Ultrafiltration*) fit the data recorded and suggest, based on the SEM images, that these internal fouling mechanisms lead to a build-up of rejected protein and subsequent external fouling (Tracey et al. 1994). For the 0.05µm pore diameter membrane, Tracey et al observed that fouling occurred immediately most likely due to cake layer formation from the rejection of protein aggregates (Tracey et al. 1994).

The cake layer deposit seen in the SEM images from Tracey et al bear some resemblance to the more pronounced deposit seen in the SEM images from early testing of the proposed device, lending weight to the notion that the deposit seen is also the result of cake layer build-up from protein adsorption driven membrane fouling, yet the evidence is far from conclusive.

There are some aspects of membrane fouling which are of specific interest when investigating haemofiltration. As stated earlier (*3.2.3. Membrane fouling*), in the filtration of macromolecular

solutions such as blood, concentration polarisation and consequent protein adhesion and pore plugging are the initial steps in the triggering of several pathways (complement, coagulation, cell adhesion etc), and lead to decreases in permeate flux and thus are the major cause of membrane fouling, a phenomenon which is particularly severe in pressure driven systems (Mueller et al. 1996; Elofsson et al. 1997; Wahlgren et al. 1997; Ghosh 2002; Ye et al. 2003; Yamamoto et al. 2005; Kalasin et al. 2009; Xu et al. 2009; Rabe et al. 2011; Su et al. 2011). Protein adsorption significantly decreases sieving coefficient and diffusive permeability of high molecular weight solutes but only slightly decreases these parameters for low molecular weight solutes (Rockel et al. 1986; Yamamoto et al. 2005).

In microfiltration of plasma from whole bovine blood the concentration polarisation layer develops within 3-4 seconds and is reversible in this period (Jaffrin et al. 1992). Once the concentration polarisation layer is established the permeate flux is no longer affected by changes in TMP (Jaffrin et al. 1992). Jaffirn et al suggest an explanation for this could be that the concentration polarisation layer becomes a tightly packed, stationary layer of erythrocytes which shields the membrane from changes in TMP (Jaffrin et al. 1992). RBCs have the ability to pack to very high concentration, approximately 98% volume fraction for compressive pressures above 13kPa (100mmHg) (Zydney et al. 1989). Stepner et al studied the hydraulic resistance of a RBC layer on a membrane and found the layers of approximately 20μm thick could account for the flux reduction seen in plasmapheresis (Stepner et al. 1985). Later, Zydney et al conducted a similar study and estimated the thickness of this concentration polarisation induced erythrocyte layer to be as low as between 4μm and 9μm for filtration velocities between $4x10^{-4}$ cm/sec and 10^{-3} cm/sec in plasmapheresis (Zydney et al. 1989). Jaffrin et al themselves estimated the layer to be <13μm (Jaffrin et al. 1992).

In plasma separation applications which utilise synthetic membranes, the plugging of pores by erythrocytes is a common challenge (Lin et al. 2000). The deformability of erythrocytes from their usual circular shape measuring 7μm in diameter to a cylindrical shape 2μm in diameter through shear forces (Peterson 1992; Lin et al. 2000). Consequently, most membranes for plasma separation have pore diameters below 0.65μm to efficiently separate the cells but this results in the need for a higher TMP to drive the plasma through the membrane (Lin et al. 2000).

Aside from the concentration polarisation and membrane plugging, one limitation particular to blood related filtration techniques is the need to avoid haemolysis, which is discussed in more detail later (*3.3.3. Effect of flow on blood*) (Jaffrin 1989).

It has been made quite clear the extent to which protein adsorption, polarisation concentration and membrane fouling impact the effective functioning of haemofiltration technologies such as

the proposed device. Much research has been conducted regarding the prevention and/or minimisation of these phenomena and examples of these, along with the efforts made to that end regarding the proposed device are describe later in this thesis (*3.4.2. Fouling Control Strategies*).

The fact that it utilised the same 1μm pore diameter polycarbonate track etched 'Cyclopore' membrane from Whatman mean that the Hemosep device could be a useful point of comparison for potential fouling mechanisms and thus, prior to exploring mechanisms for fouling prevention, it was decided that SEM images of the Hemosep membrane surface after use should be obtained and inspected.

A Hemosep bag was used to processes 340ml of bovine blood (diluted to 16% Hct with saline) using the standard Hemosep apparatus and procedure, that is, the bag was primed with saline and subsequently filled with the diluted blood and agitated for 40 minutes on Hemosep agitator. Unlike the proposed device testing procedures, this involved no blood flow. After the 40 minutes a final volume of 150ml of concentrated blood was extracted from the Hemosep bag and the Hct was measured to be 39%. SEM samples were taken from the topside and underside of the Hemosep bag membrane as shown in Figure 81. The membrane from the Hemosep bag showed the same staining as was seen previously (*3.2.3.5. Protein behaviour at a surface*) with the membrane from early testing of the proposed device.

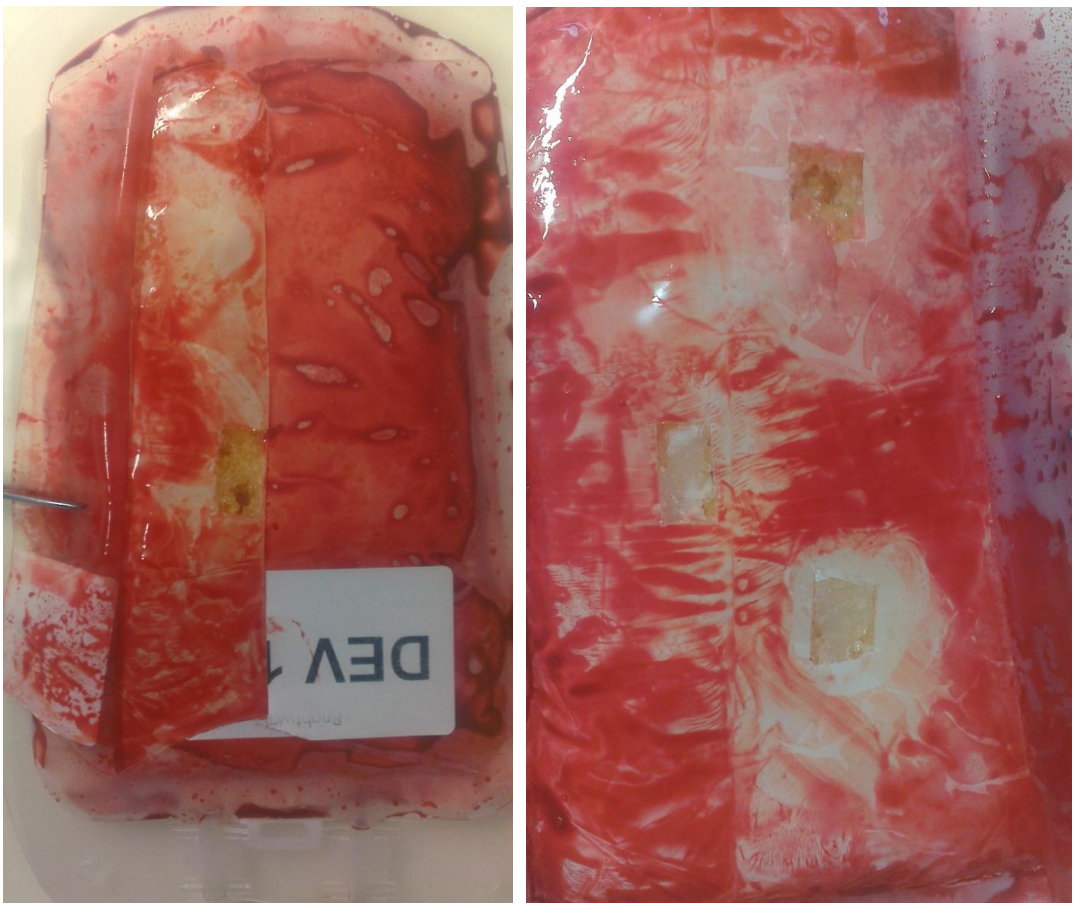


Figure 81: Images showing the locations from which the SEM samples were taken from the topside (left) and underside (right) of the Hemosep bag membrane.

In total four samples were taken, a sample from the topside which was left unwashed, a sample from the underside which was left unwashed, a sample from the underside which was washed with saline, and a sample from the underside which was washed with water. Each sample is shown in Figure 82.

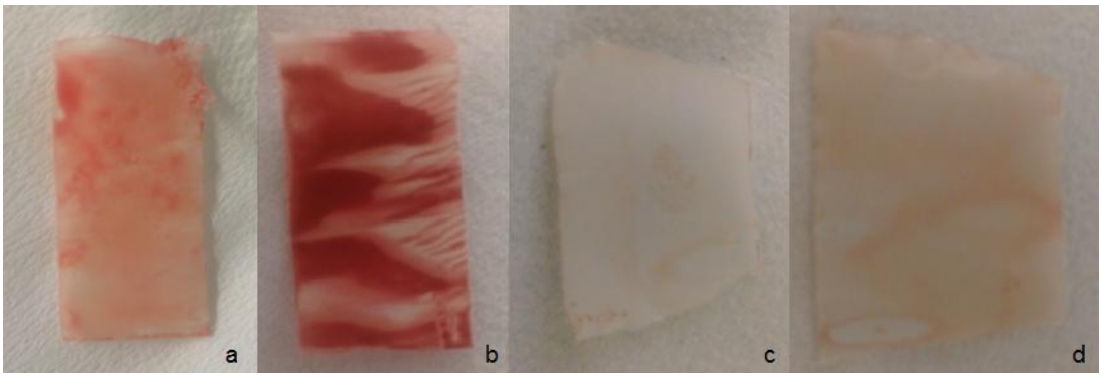


Figure 82: Photograph of each sample taken from Hemosep with the blood contacting side facing upwards ready to be imaged using the SEM. a) Topside unwashed b) underside unwashed c) underside saline washed and wiped d) underside water washed and wiped

First, the sample taken from the topside and left unwashed was scanned using the SEM. It was observed that there were large areas showing what appeared to be the same deposit seen from the membrane used in early testing of the proposed device. The coverage was not as extensive as the degree of staining initially suggested. Figure 83 and Figure 84 show the deposit seen.

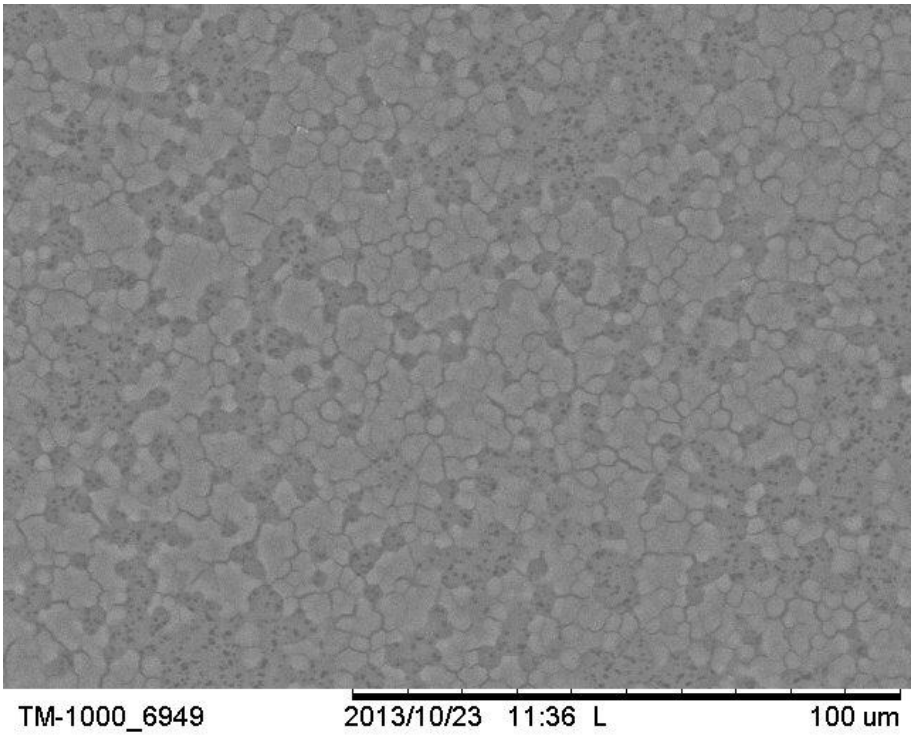


Figure 83: SEM image at x1000 of large areas of thick deposit on surface of unwashed topside of membrane from Hemosep after successful haemoconcentration

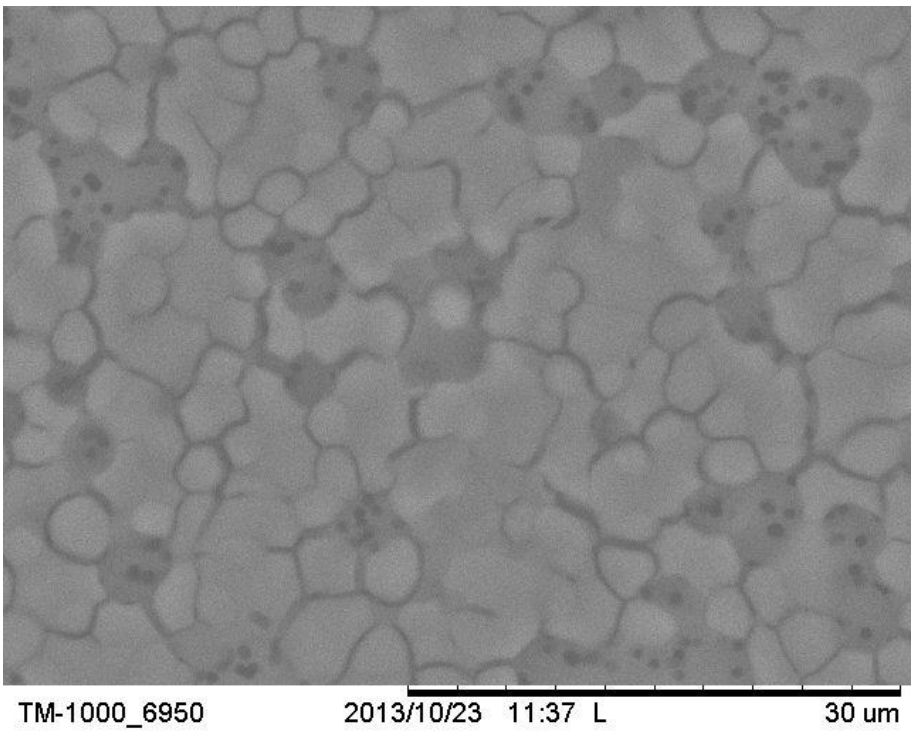


Figure 84: SEM image at x3000 of thick deposit on surface of unwashed topside of membrane from Hemosep after successful haemoconcentration

Additionally, there were signs of salt crystals on the membrane surface, again as observed previously (*3.2.3.5. Protein behaviour at a surface*) with the membrane used in early testing of the proposed device, which, due to the fact that this sample was not washed with saline, suggests that this was most likely residual salt from the initial priming phase. The fact that the Hemosep bag was only filled with 340ml of blood, which is far from the capacity of 1L, meant that a large portion of the blood may have pooled in the low half of the bag, leaving the topside membrane less influenced by the movement of blood under agitation and allowing salt crystals to remain settled on the membrane surface. The 'salt residue' is shown in Figure 85 and Figure 86.

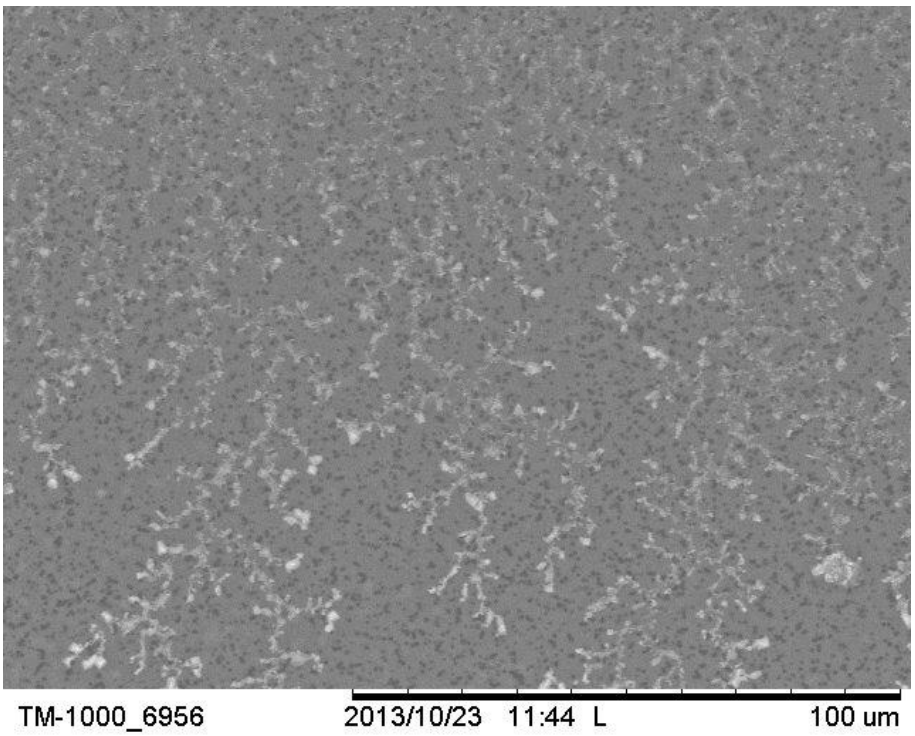


Figure 85: SEM image at x1000 of dendritic salt residue on unwashed topside of Hemosep membrane surface

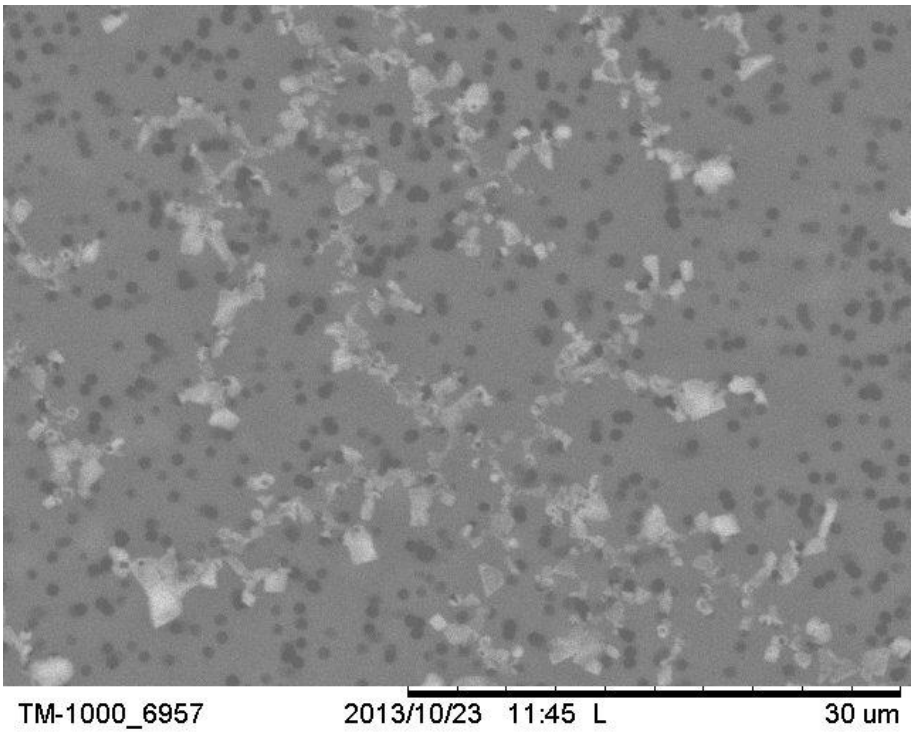


Figure 86: SEM image at x3000 of dendritic salt residue on unwashed topside of Hemosep membrane surface

Despite the fact that the entire sample was stained to some degree, there were areas of the membrane which were clear of any deposit.

Next, the membrane sample from the underside and left unwashed was scanned using the SEM. It was observed that there were large areas in which the membrane was densely covered with a 'thick' deposit layer. The majority of the right hand side of the sample, which exhibited the darkest staining, showed this form of deposit. The SEM scans displaying this are shown Figure 87 Figure 88.

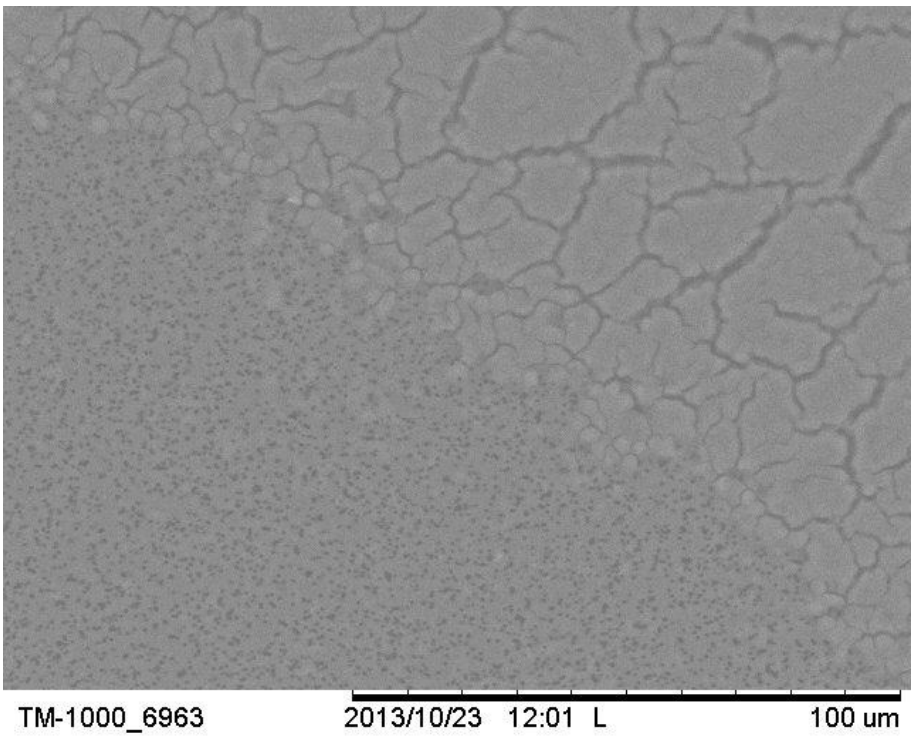


Figure 87: SEM image at 1000x magnification of thick deposit frontier on unwashed underside membrane from Hemosep after successful haemoconcentration

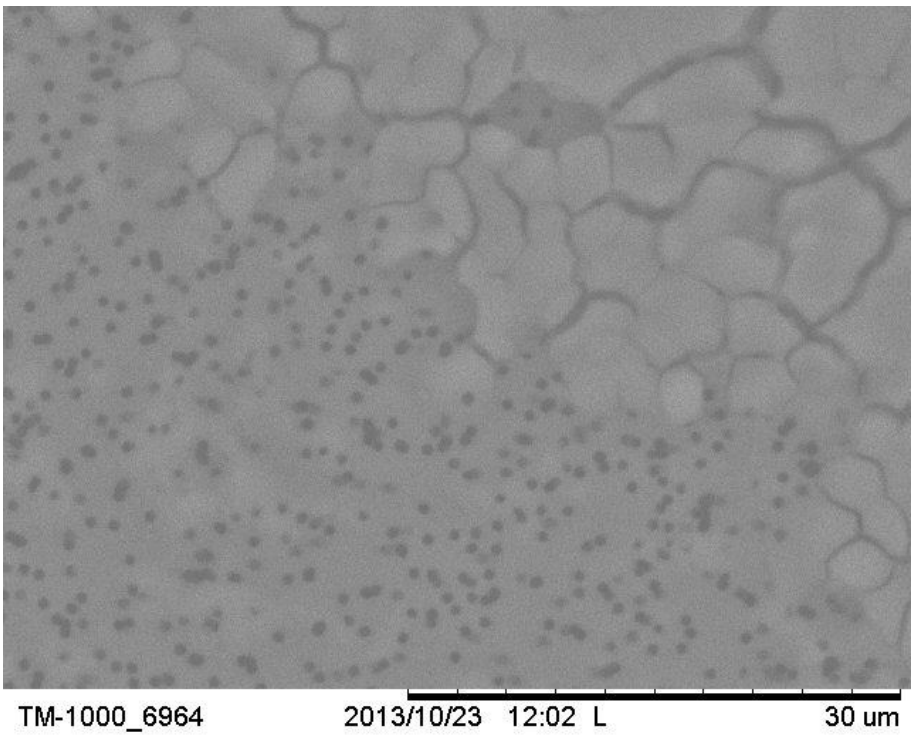


Figure 88: SEM image at 3000x magnification of thick deposit frontier on unwashed underside membrane from Hemosep after successful haemoconcentration

Again, despite the fact that the entire sample was stained to at least some degree there was not complete coverage of the membrane across the sample and some areas were left clear of any deposit.

Next, the membrane sample from the underside and washed with saline was scanned using the SEM. Scans of the saline washed membrane sample from the underside of the Hemosep bag showed that the majority of the membrane was clear of the previously seen deposit, with a very small amount of what may have been salt residue present as shown in Figure 89 and Figure 90.

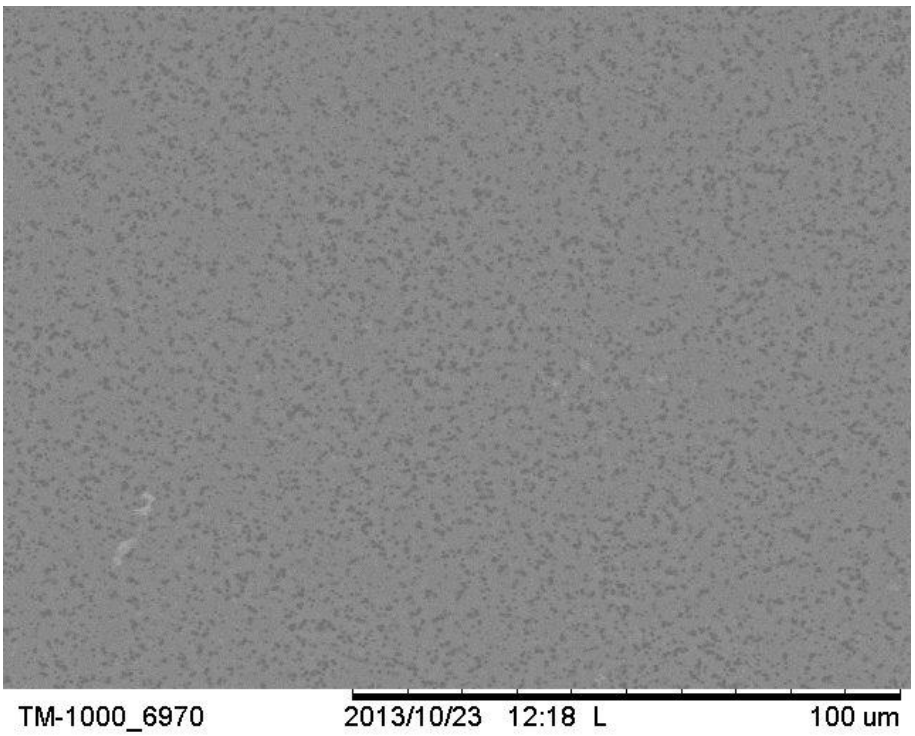


Figure 89: SEM image at 1000xmagnification of saline washed underside of Hemosep after successful haemoconcentration showing no deposit and a very small amount of what may have been salt residue

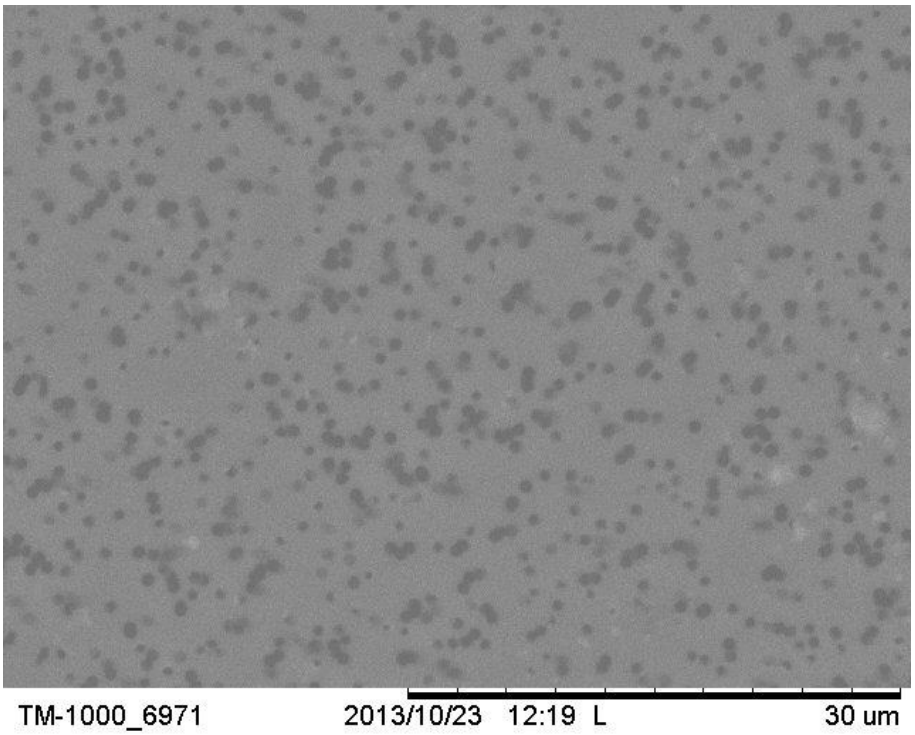


Figure 90: SEM image at 3000xmagnification of saline washed underside of Hemosep after successful haemoconcentration showing no deposit and very small amount of what may have been salt residue

However, in some areas of the membrane there appeared to be a marked decrease in the pore density. This is shown in Figure 91 and Figure 92.

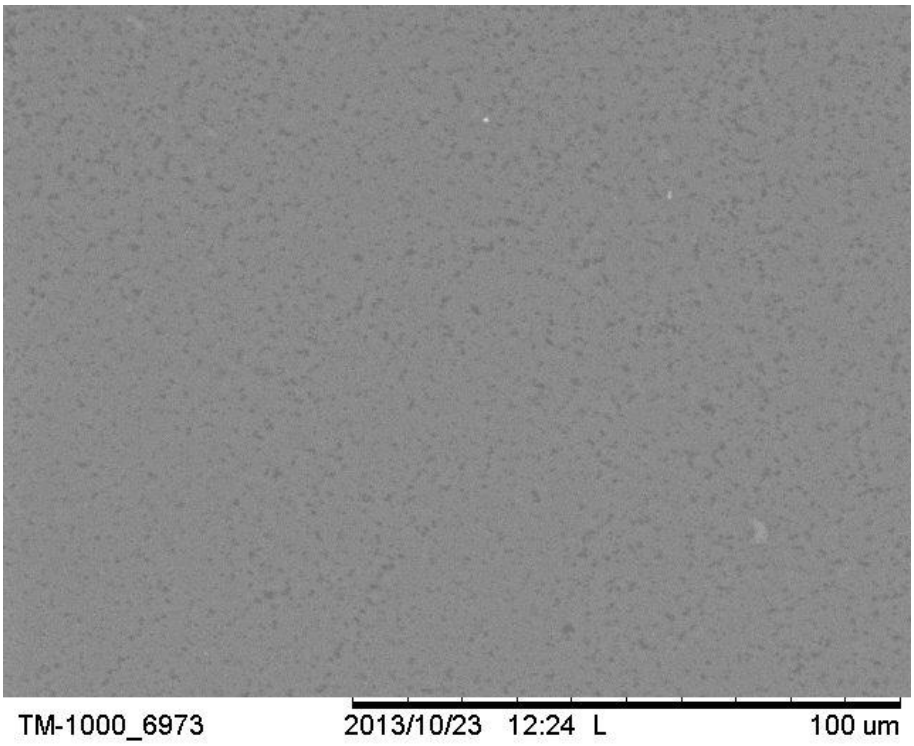


Figure 91: SEM image at 10000xmagnification of saline washed underside of Hemosep after successful haemoconcentration appearing to shown marked decrease in pore density

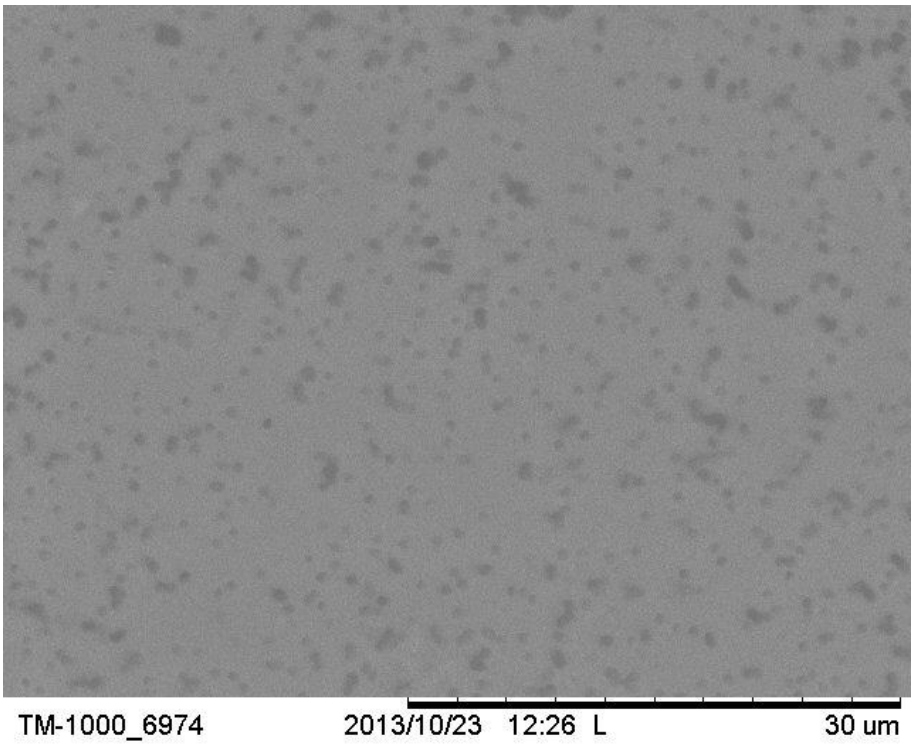


Figure 92: SEM image at 30000xmagnification of saline washed underside of Hemosep after successful haemoconcentration appearing to shown marked decrease in pore density

Whether this was the result of blood contact and indicative of protein deposit or merely an aberration in the membrane itself was difficult to ascertain.

Finally, the membrane sample from the underside and washed with water was scanned using the SEM. Scans of the water washed membrane sample from the underside of the Hemosep bag showed that the vast majority of the membrane was clear of the previously seen deposit as is shown in Figure 93 and Figure 94.

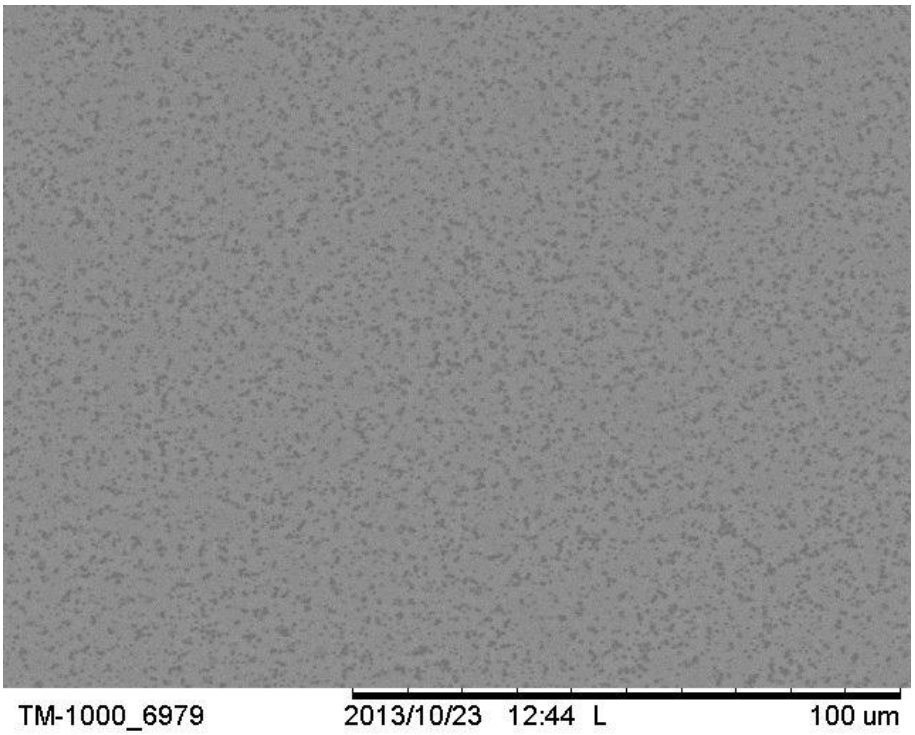


Figure 93: SEM image at 1000xmagnification of water washed underside of Hemosep after successful haemoconcentration showing no deposit

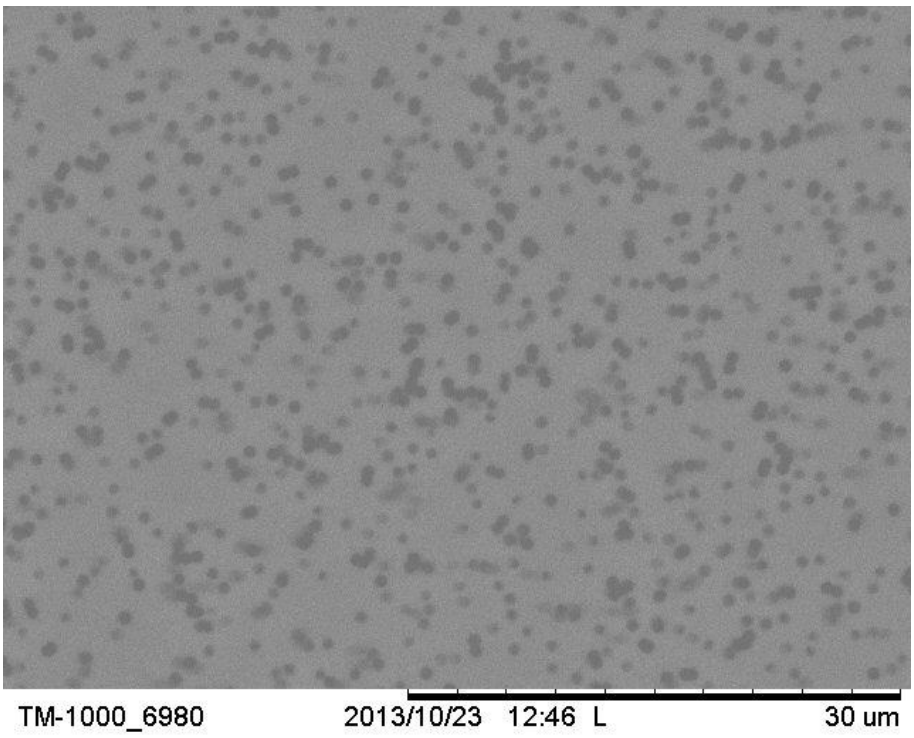


Figure 94: SEM image at 3000xmagnification of water washed underside of Hemosep after successful haemoconcentration showing no deposit

The deposit seen on the Hemosep bag closely resembled the previously seen deposit on the membrane used during early testing of the proposed device. The deposit on the Hemosep membrane seemed to exhibit less of a resistance to removal by washing (indicated by the fact that it was more effectively removed) but this could be explained by the shorter processing time in the Hemosep system (40mins) compared to the early testing of the proposed device (~2hrs) and thus shorter time for the deposit layer to be firmly established. Based on the appearance of the deposit in the SEM images it is likely the deposit seen in early testing of the proposed device and Hemosep are similar in nature. Thus, the fact that Hemosep was able to effectively haemoconcentrate the blood (raising the Hct from 16% to 39% in a volume of 340ml in 40mins) whereas the early prototype of the proposed device was unable to elicit any change in Hct, suggests that the presence of the deposit is not sufficient explanation for the failure of early prototypes to achieve haemoconcentration. The problem of ascertaining whether the deposit seen was the result of membrane fouling, or merely the settling of blood by surface tension during draining of the test rig, remains. The observations made up until this were aimed at being the first step in finding an explanation for the lack of haemoconcentration seen and although it cannot be said that the deposit is the explanation, it is still wise when developing a haemofiltration technology to make attempts to prevent/minimise protein adsorption, concentration polarisation and/or membrane fouling where possible. At this point, further investigation into the reason for the lack of haemoconcentration was required.

3.2.4. Impact on design

The polycarbonate track etched 'Cyclopore' membrane from Whatman appears to be the best choice of membrane for the single sheet flat bed membrane system to be used in the proposed device due to its suitable properties; hydrophilic whilst exhibiting very low levels of water adsorption and non-specific protein binding, biologically inert, applicability to blood filtration procedures, and regular topography arising from it uniform flat surface and straight through pores (Zydney et al. 1989; Fisher Scientific 2014).

Although it is still unclear as to what impact the observed deposit on the membrane has on the performance of the system, steps must be taken to avoid fouling of the membrane, and the strategy employed to control fouling will need to be incorporated into the design of the device. The membrane is susceptible to stretching under high TMP which appears to result in permanent deformation and enlargement of the pores which would greatly impact upon the functionality of the proposed device and thus the proposed device must be designed to avoid reliance on high TMP for successful haemoconcentration.

3.3. Blood flow

3.3.1. Blood flow in the proposed device

Despite the fact that Hemosep could function well whilst exhibiting what appeared to be the same 'deposit' as was observed in early testing of the proposed device, the system was still failing to successfully haemoconcentrate the diluted blood. Aside from the notion that the deposit seen was obscuring the membrane pores and preventing plasma removal, one possible explanation posited for the lack of haemoconcentration was that there was insufficient flow coverage across the exchange surface.

The flat-bed cross flow design of the proposed device works on the principle that blood flowing along shallow yet wide channel allows for a large surface area across which the plasma is subject to the forces driving absorption by the superabsorber via the membrane pores. This large surface area should allow for simultaneous plasma extraction from a relatively large volume of blood and thus a rapid rate of haemoconcentration. However, the presence of a large exchange surface area is not as important as a large area of blood flow 'coverage'. Despite the fact the blood may 'cover' the entire exchange surface, blood may only be flowing across a small fraction of this area, that is, rather than blood flow being evenly distributed and largely uniform

across the channel, the majority of blood flow is along narrow pathlines which leaves the remaining areas of the channel in stasis. This phenomenon stems from the tendency of a fluid to follow the path of least resistance (Hillegass et al. 2010). The result is the restriction of the effective exchange area to that immediately adjacent to these pathlines which, if the pathlines are such that they exist outside the extremities of the exchange surface, could almost entirely prevent the occurrence of plasma extraction. In oxygenators the portion of blood which flows through the device without being oxygenated is referred to as the 'shunt fraction', but this principle of 'shunting' applies equally to a MUF device (Segers et al. 2001).

If fluid shunting was occurring during early testing the proposed device, this could be an explanation for the lack of haemoconcentration seen. In addition, undesirable blood flow can impact the functioning of the proposed device by affecting the blood itself. The impact of flow on the blood along with the modelling methods utilised are described later in this section (*3.3.3. Effect of flow on blood*), after a discussion on the effect of flow on filtration.

3.3.2. Effect of flow on transfer rate

It has been seen previously (*2.1. Principles of Ultrafiltration*) how blood flow rate is related to pressure through Pousille's equation, and how this in turn can have an impact on TMP and ultrafiltration rate. Additionally, it has been seen how above a given TMP threshold, ultrafiltration rate becomes 'boundary limited' after which blood flow rate becomes a dominant factor (Figure 9) (Jacobs 1996). Moreover, the importance of controlling phenomena such as concentration polarisation and membrane fouling in achieving and maintaining good filtration rates have been discussed in detail (*3.2.3.6. Concentration Polarisation and Membrane Fouling*). The various methods employed to reduce these phenomena will be discussed in more detail later (*3.4.2. Fouling Control Strategies*) but at this point it would be prudent to briefly mention the impact that flow rate can have on fluid transfer rates and how it can be manipulated to act as a control method in itself.

Flow manipulation is the main focus of operating parameter alterations aimed at controlling concentration polarisation and membrane fouling and increasing filtration rate (Hilal et al. 2005). Flow manipulation encompasses increased flow rates, backflushing, pulsatile and reversed flow (described earlier in *2.2.3. Cross flow (tangential flow) filtration*), but many flow manipulation methods aimed at improving filtration rate rely on increased shear at the membrane surface often by inducing turbulence (Hilal et al. 2005).

3.3.2.1. Increased Shear

Shear rate at the membrane surface is known to be an important factor in controlling membrane fouling (Jaffrin 1989; Si-Hassen et al. 1996; Song 1998; Valette et al. 1999). Vassiliev et al postulated that a shear induced erosion mechanism controls cake layer formation at the membrane surface (Jaffrin et al. 1992), and, as Si-Hassen et al state, it has since become a well known observation that "cake thickness decreases with increasing fluid velocity to reach the critical erosion stress" (Si-Hassen et al. 1996).

In cross-flow filtration in particular, shear flow has a profound impact upon membrane fouling (Song 1998). In cross-flow filtration, unlike in dead-end filtration processes, the ultimate permeate flux (i.e. the steady state filtration flux that the system tends towards over time) is non-zero (Song 1998). Figure 95 from Song et al shows that increased shear rate both increases the equilibrium flux and reduces the time to that equilibrium (Song 1998).

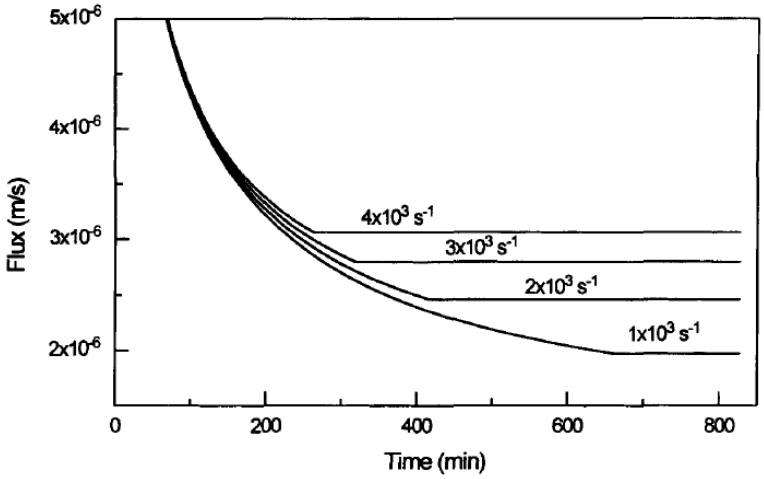


Figure 95: Increased shear rate both increases the equilibrium flux and reduces the time to that equilibrium (Song 1998)

A detailed description of the physical mechanisms by which shear affects filtration flux will be given in the next section.

3.3.2.2. Turbulence and Vortices

One of the most common methods of inducing shear stresses to thin and mix the fouling boundary layer with the bulk flow is to increase fluid velocity until the flow becomes turbulent (Costigan et al. 2002). Turbulence can also be achieved by placing flow-disrupting inserts into the flow channel, varying the geometry of the membrane surface, utilising pulsatile flow, and for

flat plate systems the introduction of a rotating blade (Costigan et al. 2002). In order to improve performance of filtration systems, some groups have sought to exploit secondary flow, flow instabilities or vortices to reduce the concentration polarisation layer and increase mixing with the bulk solution (Kroner et al. 1988; Moulin et al. 1996; Mallubhotla et al. 1997). An example of this is depicted in Figure 96.

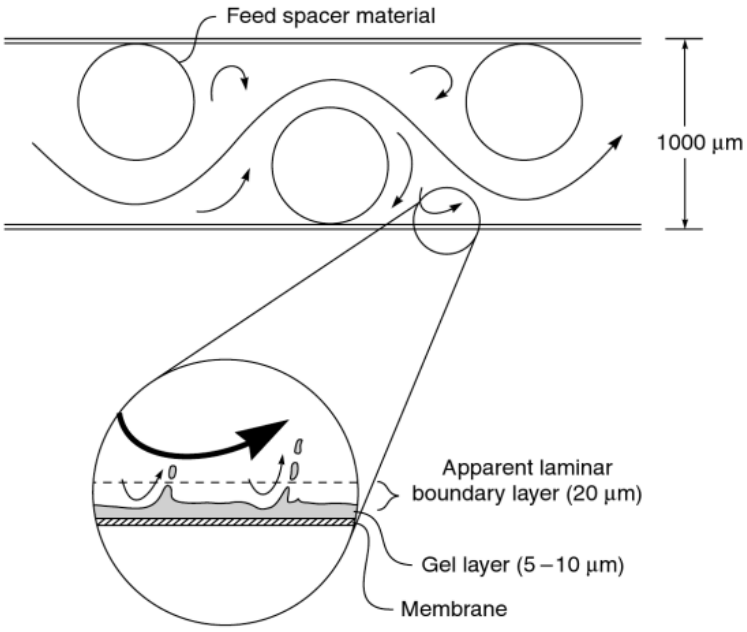


Figure 96: Inducing turbulence to thin and mix the boundary layer (Baker 2004)

In the early 1970s and through to the 1980s, Brian Bellhouse of the University of Oxford worked on improving the efficiency of blood oxygenators (Bellhouse 1976a; Bellhouse 1976b; Bellhouse 1992). Previous oxygenators involved direct injection of oxygen bubbles into a blood reservoir which was found to be damaging to the blood, whilst membrane oxygenators at the time, although less damaging to the blood, were inefficient and highly susceptible to fouling (Bellhouse 1992). Bellhouse developed several methods of reducing membrane fouling in the oxygenators including the use of pulsatile flow, and an important innovation of flowing blood across a furrowed surface to induce turbulence and vortices at the exchange surface and increase blood gas mixing (Bellhouse 1992). Bellhouse later employed the same technique to the development of a membrane based blood plasma separator for use in blood salvage from the bypass circuit (Bellhouse et al. 1988).

Several groups have continued the use of vortices and turbulence at the exchange surface to develop efficient membrane filtration systems (Kroner et al. 1988; Moulin et al. 1996; Mallubhotla et al. 1997). Millward et al utilised a combination of slow oscillatory flow with flow

deflectors to generate a vortex wave in a flat bed membrane channel to assist separation of plasma from whole bovine blood and found a significant improvement in plasma flux with minimal blood damage (Millward et al. 1995a; Millward et al. 1996a). Millward et al also utilised screw thread flow promoters in tubular membrane systems and found it to be an effective anti-fouling technique in the ultrafiltration of BSA but could not significantly improve plasma separation from whole bovine blood (Millward et al. 1995b). Using similar methods, Najarian et al utilised pulsatile flow in combination with ladder-like flow disruptors within a flat plate membrane system to aid separation of T-globulins from albumin in bovine plasma and in combination with a helical insert to aid separation of plasma from whole bovine blood in a tubular membrane system, however, unlike Millward et al, Najarian et al found that plasma separation was enhanced with the helical insert (Najarian et al. 1996a; Najarian et al. 1996b). Hwu et al utilised oscillatory flow through a channel with flow deflectors to generate vortex waves and mathematically modelled the concentration dispersion these vortex waves caused (Hwu et al. 1996; Hwu et al. 1997). Gomaa et al have recently studied the combination of turbulence promoters and a laterally oscillating membrane and found a significant improvement in mass transfer at the solid-liquid interface (Gomaa et al. 2007; Gomaa 2012).

Knowledge of the impact which shear and turbulence have upon filtration flux has led to several innovative processes. A selection of the processes include: utilisation of a rotating disk or rotating or vibrating membrane to induce shear forces (Jaffrin 2008), introducing granular medium in suspension to the flow to mechanically scour the membrane surface (Pradhan et al. 2012), blowing air across the membrane known as air scouring (Hong et al. 2002; Pradhan et al. 2012), application of an external DC electric field across the membrane to fractionate proteins (Sarkar et al. 2009), introduction of intermittent phases of high-velocity low-pressure flow (Si-Hassen et al. 1996), and the injection of gas into the feed stream to create a two phase gas-liquid crossflow system known as gas sparging which is widely used (Bellara et al. 1996; Cui et al. 1996; Ghosh et al. 1998; Li et al. 1998; Sur et al. 2001; Gupta et al. 2005; Wang et al. 2010). Several of these methods will be discussed in detail later (*3.4.2. Fouling Control Strategies*).

3.3.3. Effect of flow on blood

The blood response to contact with a foreign material has been discussed previously (*3.2.3. Membrane fouling*), however for medical devices involving flowing blood, the blood response to mechanical forces of flow are highly important too. As mentioned previously (*3.2.3.6. Concentration Polarisation and Membrane Fouling*), one limitation to haemofiltration techniques is the need to avoid haemolysis (Jaffrin 1989).

Haemolysis is extreme erythrocyte trauma which results in the rupturing of erythrocyte membranes and the release of intracellular haemoglobin into the plasma (Alkhamis et al. 1990; Sowemimo-Coker 2002; Sakota et al. 2008). There are a number of clinical complications associated with haemolysis including: hypercoagulation, bleeding, thromboembolism, renal and neurological damage, anaemia, fatigue, jaundice, haematuria and kidney failure (Kameneva et al. 2004).

Erythrocyte damage is a major complication associated with blood flow through extracorporeal circulation and some degree of haemolysis (and the associated haemoglobin release into the plasma) almost always occurs (Lampert et al. 1972; Kameneva et al. 2004). Haemolysis in these cases is not osmosis-induced but rather is the result of a complex combination of blood contact with foreign surfaces for prolonged periods in areas of flow stasis (Bacher et al. 1970; Kameneva et al. 2004), cavitation (Kameneva et al. 2004), the presence of other plasma components (Bernstein et al. 1967; Kameneva et al. 1997), high shear forces (Blackshear et al. 1966; Lampert et al. 1972; Leverett et al. 1972; Yeleswarapu et al. 1995; Sowemimo-Coker 2002; Grigioni et al. 2005) and elevated Reynolds shear stresses (turbulence) (Sowemimo-Coker 2002; Kameneva et al. 2004). However, the mechanisms of haemolysis through these phenomena are not fully identified (Kameneva et al. 2004). The adsorbed layer of proteins on foreign surfaces is highly influential in haemolysis just as it influences biocompatibility by obscuring the bulk blood from the native material surface (Lampert et al. 1972).

Even prior to extracorporeal circulation there are several factors (aside from other sources of mechanical induced haemolysis) which can induce erythrocyte damage. Sowemimo-Coker et al list a vast array of causal factors: preparation procedures including delays between collection and processing, variations in storage container material and rapid anticoagulation (including mixing anticoagulant with blood) have all been associated with haemolysis (Sowemimo-Coker 2002). Bacterial contamination, osmotic and pH changes, activation of leukocytes, platelets and complement, administration of drugs including penicillin and vitamin C and subjection to temperatures above 40°C or below 1°C are all factors which may also induce haemolysis (Utoh et al. 1992; Kameneva et al. 1999; Sowemimo-Coker 2002). Additionally, certain erythrocytes may have a higher propensity for haemolysis due to natural defects, age or other biological variations (Sowemimo-Coker 2002).

High shearing stress is one of the more important causal factors in haemolysis (Nevaril et al. 1969). In the vasculature, shear stress in blood is caused by the force associated with its flow; the velocity difference between layers at different distances from the vessel wall determines shear rate, which is directly proportional to shear force and inversely proportional to blood viscosity (Ikeda et al. 1991). In the normal vasculature, the highest physiological shear stress

which erythrocytes are subjected to is approximately 10 N/m^2 (100$dynes/cm^2$) (Waters et al. 2007). In artificial organs for extracorporeal blood filtration, blood comes in contact with non-biological surfaces and can be subjected to higher mechanical forces for longer durations compared to physiological arteriovenous circulation (Vitale et al. 2011). Subjecting erythrocytes to these elevated forces and shear stresses can result in erythrocyte deformation and haemolysis indicated by the release of haemoglobin into the plasma through a partially damaged (sub-lytic) or destroyed (haemolysis) membrane (Yeleswarapu et al. 1995; Vitale et al. 2011). Erythrocytes can become damaged in narrow or partially occluded or obstructed openings and in kinked or twisted intravenous tubing (Sowemimo-Coker 2002). The blood path within the haemofilter is also important as narrow channels may lead to increased shear stress and thus increased haemolysis (Segers et al. 2001).

Particularly relevant to the proposed device is the impact of Hct. Mechanical trauma of erythrocytes is more likely to occur in undiluted blood with high Hcts than in low Hct, low viscosity diluted blood (American Association of Blood Banks Standards Committee 1989; Sowemimo-Coker 2002). This suggests that if haemolysis is to occur in the proposed device it is more likely to occur later in the procedural timeframe as the blood is haemoconcentrated and Hct increases.

Several studies have attempted to determine the critical shear stress and exposure time which induce haemolysis, but variation among haemolysis studies has resulted in a wide range of quoted haemolysis thresholds (Bernstein et al. 1967; Nevaril et al. 1969; Leverett et al. 1972; Sutera 1977; Kameneva et al. 2004). The threshold of shear stress which erythrocytes can withstand is highly dependent upon exposure time to the shear stress (Yeleswarapu et al. 1995) but is normally taken as 1500$dynes/cm^2$ for 'moderate' exposure times although erythrocytes can withstand shear stresses up to $10^5$$dynes/cm^2$ for durations of a few microseconds (Leverett et al. 1972). 1500 $dynes/cm^2$ is the threshold for widespread shear induced haemolysis and is sometimes also used as the dividing line between the two regimes of shear induced haemolysis: 'high stress' and 'low stress' (Lampert et al. 1972; Leverett et al. 1972).

At shear stresses in excess of 1500-3000$dynes/cm^2$ (150-200N/m^2), erythrocyte damage is due to shear stresses acting directly on the cells themselves and other sources of damage such as interacting with other cells or solid surface are unimportant (Nevaril et al. 1969; Lampert et al. 1972; Leverett et al. 1972; Waters et al. 2007). At this level, shear stresses can cause permanent cell damage, cell rupture, and very high levels of haemolysis in erythrocytes (Nevaril et al. 1969). However, in this 'high stress regime', stress is not the only factor with cells being able to withstand shear stresses greater than 3000$dynes/cm^2$ for short periods of time, indicating that haemolysis is related to both stress and time (Lampert et al. 1972). This reflects the rupture

behaviour of other viscoelastic materials (Lampert et al. 1972). In the high stress regime, surface material does not appear to have an impact (Lampert et al. 1972).

In the low stress regime (<1500dynes/cm^2), stress in itself is not a factor and is generally insufficient to cause haemolysis (Lampert et al. 1972; Alkhamis et al. 1990; Sowemimo-Coker 2002). However, shearing forces promote cell rotation and migration and subsequent surface interactions (Leverett et al. 1972) and as such, interactions with solid surfaces do cause erythrocyte damage (Bernstein et al. 1967; Shapiro et al. 1970; Leverett et al. 1972). Consequently, in the low stress regime where there is minimal cell damage directly due to shear stress, solid surface interactions are highly important and are related to the relative velocities of cells and solid surfaces along with the surface material, cell-cell interactions, and interaction with any air interfaces (Shapiro et al. 1970; Lampert et al. 1972; Leverett et al. 1972). As a result, in the low stress regime, haemolysis per unit time is very low; however, if the processing time is sufficient, noticeable haemolysis will occur making exposure time an important parameter (Leverett et al. 1972). Exposing erythrocytes to low stresses for short periods of time results in reversible elastic elongation, but if the same stress is applied for a longer period it can result in an irreversible shape change (Grigioni et al. 2005). When subjected to high stresses the erythrocyte membrane will yield resulting in a more severe permanent deformation (Kuypers 1998; Grigioni et al. 2005). It can be seen therefore that even below the threshold of haemolysis, erythrocyte damage can occur, a fact which some modelling systems (described later in this section) ignore (Grigioni et al. 2005).

Unlike in the vascular system where blood flow is mostly laminar, cardiovascular devices often introduce turbulent flows (Kameneva et al. 2004). Some sources of turbulent stress in blood processing systems include: the edges of kinked tubing, partially opened tube closures, or partially obscured inlets/outlets (Sowemimo-Coker 2002). Agitation of blood bags during mixing before filtration can lead to haemolysis of older and more fragile erythrocytes (Sowemimo-Coker 2002).

In 1972 Sutera et al found that turbulent flow in itself does not affect the onset of haemolysis for stresses as high as 2500dynes/cm^2 for a 1 hour period (Sutera et al. 1972). It was suggested that an explanation for this could be that the smallest eddies generated by their apparatus would be 29μm in diameter and thus on the scale of erythrocytes the flow would appear laminar (Sutera et al. 1972). Sutera et al suggested that if turbulence is indeed non-haemolytic then mass transfer can be improved without the need for small channel dimensions with laminar flows by inducing turbulent flow at the exchange surface, thereby increasing convective diffusion (Sutera et al. 1972). Moreover the average time cells spend near the exchange surface could be reduced (Sutera et al. 1972). Similarly Najarian et al reported negligible haemolysis after microfiltration

of bovine blood using oscillatory flow through a tubular membrane system with a screw-threaded insert (Najarian et al. 1996b).

However, subsequently, it has been more widely accepted that haemolysis can be caused by turbulent shear stress (Reynolds stress) (Monroe et al. 1981; Nakahara et al. 1986; Sowemimo-Coker 2002; Kameneva et al. 2004). Kameneva et al demonstrated in their study that turbulent stresses generate considerable increases in haemolysis at high Reynolds number in turbulent flows (Kameneva et al. 2004). The mechanisms of turbulence induced haemolysis are not well understood but Kameneva et al suggested that small turbulent eddies may lead to erythrocyte stretching and subsequent fatigue fracture of the cell membrane (Kameneva et al. 2004).

Free haemoglobin in the plasma is a result of haemolysis and is indicated by a pink discolouration (Sowemimo-Coker 2002). Plasma free haemoglobin is toxic to the kidney and thus excessive haemolysis can result in organ failure in addition to anaemia (Olsen 2000; Sakota et al. 2008). Consequently, upon seeing pink discolouration after a form of blood processing, one should immediately seek to identify any possible sources of haemolysis (Sowemimo-Coker 2002). Free haemoglobin levels as low as 25mg/dL (0.09% Haemolysis at 45% Hct and 16g/dL of total haemoglobin in erythrocytes) will result in clearly visible pink discolouration of plasma (Sowemimo-Coker 2002). The FDA recommends no more than 1% haemolysis for storage of erythrocytes, while the EU set a maximum haemolysis level of 0.8% for transfusions (Sowemimo-Coker 2002). Figure 97 from Sowemimo-Coker et al shows the discolouration of plasma with free haemoglobin.

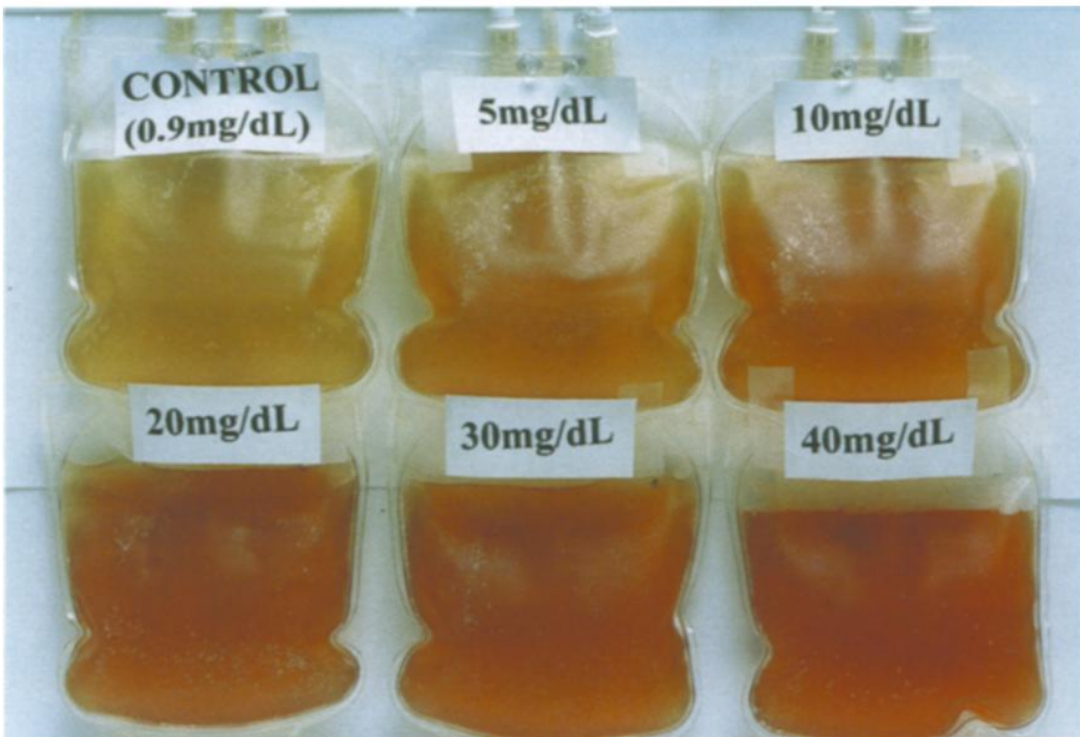


Figure 97: Discolouration of plasma with different levels of free haemoglobin (N.B. 40mg/dL is <1% haemoglobin) (Sowemimo-Coker 2002)

Holt et al previously observed that in haemofiltration *"increasing positive pressure increases the shear force which is more hemolytic" (Holt et al. 1982)*. Blood salvaged from the bypass circuit commonly contains elevated concentrations of free haemoglobin, thought to be a result of haemolysis induced by the suction process used to retrieve it (Gregoretti 1996). Suction induced haemolysis is particularly pertinent to MUF devices and the proposed device as suction, or a pressure differential, is the main driving force for plasma extraction in several ultrafilters, but not the proposed device which relies on a concentration gradient. Gregoretti et al investigated the contribution negative pressure made to haemolysis and found that increased negative pressure was associated with increased haemolysis but that the trauma was only moderate; at 300mmHg, the highest suction in the study, haemolysis rate was only 0.3% (Gregoretti 1996). However, when air was allowed to be drawn into the suction system whilst blood was salvaged, haemolysis was significantly increased at across a range of negative pressures (150mmHg, 200mmHg, 250mmHg and 300mmHg) and was much more severe, ranging from 1.45% on average at 150mmHg to 2.85% on average at 300mmHg (Gregoretti 1996). The aspirated air forms fast-moving bubbles which in the negative pressure environment expand and collide resulting in the erythrocytes being subjected damaging to mechanical stresses (Waters et al. 2007). The results suggest that negative pressure in itself is not severely haemolytic but that under certain conditions it can be a driving factor for significant haemolysis.

In 1981, Wright investigated the thresholds of various levels of blood damaged induced by cardiotomy suction during CPB as is shown Figure 98.

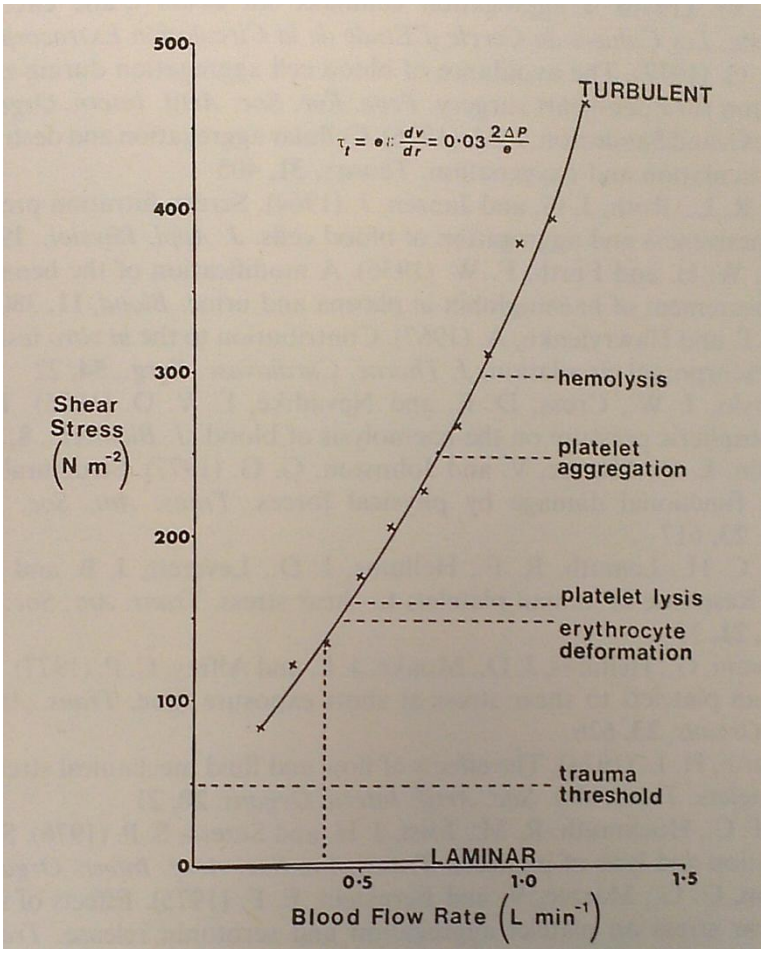


Figure 98: Laminar and turbulent shear stresses for blood and air flowing through a cannulae of 4mm internal diameter (Longmore et al. 1981)

More recently, Waters et al also studied suction induced haemolysis during blood salvage (Waters et al. 2007). Waters et al also found that increased negative pressure was associated with increased haemolysis but that other factors were more important (Waters et al. 2007). Again, the introduction of air into the suction system (in this case whilst aspirating blood from a flat surface) led to increased haemolysis, but this could be counteracted by diluting the blood with saline to reduce viscosity, resulting in approximately 60% reduction in haemolysis (Waters et al. 2007). The nature of the proposed device means that in the early stages the system will be processing diluted blood and thus should suffer less from haemolysis. However, as the blood becomes more concentrated and the viscosity increases, the blood may be more prone to shear induced haemolysis, highlighting the importance of studying the entire procedure period when analysing haemolysis.

Tamari et al investigated the impact of a specific haemofilter on the blood and found that the leukocytes and platelets were significantly decreased after haemofiltration (2.5% and 15.3% decrease respectively), but that there was no significant loss of albumin and fibrinogen, and no significant change in plasma free haemoglobin suggesting minimal haemolysis (Tamari et al. 1984). A previous study by Sanford et al found that the same haemofilter led to significant haemolysis but Tamari et al suggest this may have been due other factors such as the application of suction to times of low or no blood flow (Tamari et al. 1984).

Karliczek et al attempted to estimate how much additional blood trauma is caused by haemofiltration (Karliczek et al. 1986). The results of their study showed that during the operative period there was no significant difference in erythrocyte, leukocyte and platelet populations, platelet function, active clotting time or free plasma haemoglobin between the haemofiltration and control groups, indicating that there was little additional blood trauma attributable to the haemofilter (Karliczek et al. 1986). It was noted however that platelet levels were lower on the first two days postoperatively and thus some negative impacts of haemofiltration did occur (Karliczek et al. 1986).

In plasma filtration systems, haemolysis can occur when blood comes in contact with the microporous membrane (Jaffrin 1989). It has been suggested that haemolysis may occur when a red cell becomes trapped within one of the membrane pores and is subjected to tension (σ) induced by the TMP and proportional to the pore radius according to Laplace's Law:

$$\sigma = \frac{\Delta P_{TM} R_P}{2}$$

where: σ is tension, P_{TM} is the TMP and R_P is the pore radius (Zydney et al. 1987; Jaffrin 1989; Bronzino et al. 2006). If the time in the pore is sufficient for the tension exerted to induce a strain (degree of stress induced deformation) greater than the critical value of strain a red cell can withstand, then haemolysis will occur (Baskurt et al. 2003; Bronzino et al. 2006). The red cell can become dislodged from the pore by collisions from other cells or by the fluid shear stress and thus increased shear stress will decrease the time spent in the pore resulting in a decrease in haemolysis (Bronzino et al. 2006).

Solomon et al found when TMP was raised above a certain threshold, haemolysis would occur and increased with increasing pore size (Solomon et al. 1978). Consequently, Jaffrin et al suggested that in plasmapheresis systems, pore size should be limited to 0.65μm (Jaffrin 1989). A TMP of 70mmHg has been suggested as a safe limit to avoid haemolysis (Jaffrin 1989), however, as initially noted by Solomon el al (Solomon et al. 1978) and later confirmed by bovine

blood haemolysis analysis by Ding et al (Ding et al. 1986), the TMP threshold for haemolysis will increase with increasing shear rate as is shown in Figure 99 (Jaffrin 1989).

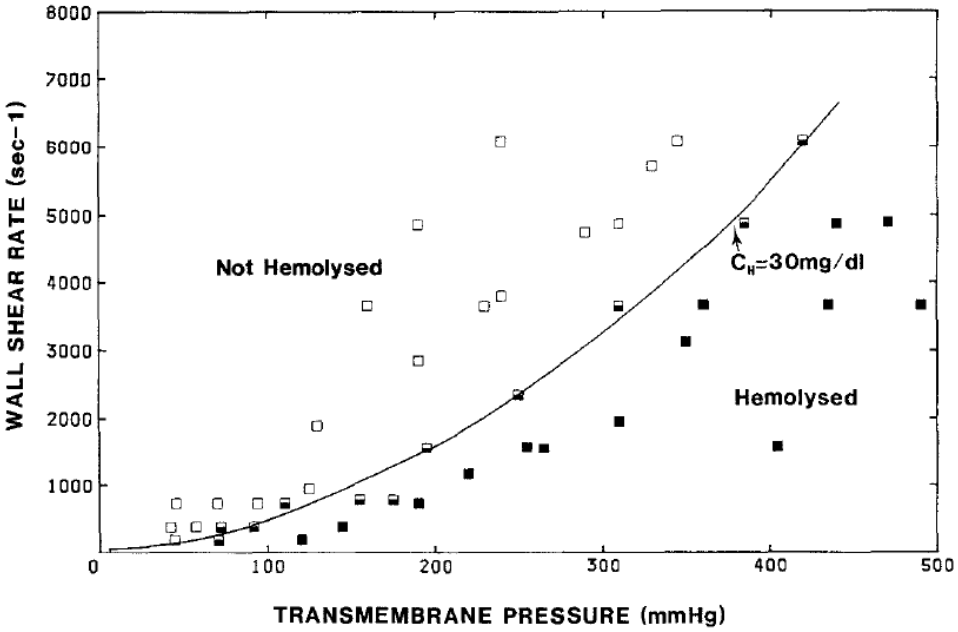


Fig. 3. Hemolysis boundary in wall shear rate–mean transmembrane pressure plane for bovine blood and polypropylene fibers (from ref., 20). (□) not hemolysed, $C_H < 25$ mg/dl; (⬓) hemolysis limit, $C_H = 30$ mg/dl; (■) hemolysed, $C_H > 35$ mg/dl.

Figure 99: TMP threshold for haemolysis increases with increasing shear rate (Jaffrin 1989)

There are two important implications of this relationship; first that a plasma filter can theoretically operate at high TMPs without inducing haemolysis, providing the wall shear rate is high enough, and secondly, given the fact that shear rate and TMP increase with increased blood flow, increasing flow rate in a non-haemolytic filter system should theoretically not lead to haemolysis provided the membrane length is not too high (Gupta et al. 1986; Jaffrin 1989).

However, as has been discussed in this section, shear is an important factor in causing haemolysis. The consequence of this is that there is an upper bound on which increasing shear rate can assist in reducing haemolysis, beyond which the shear stresses in the bulk fluid will instead contribute to haemolysis (Bronzino et al. 2006). This is illustrated in Figure 100 where haemolysis occurs at very low shear rates due to red cells spending longer in the membrane pores, and at much higher rates due to bulk shear stresses (Bronzino et al. 2006).

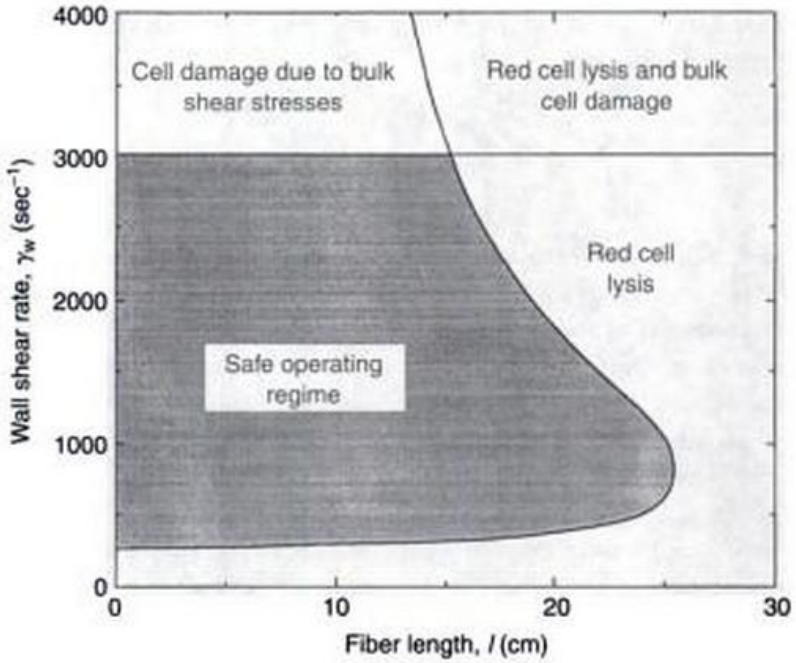


Figure 100: Example of an upper bound on which increasing shear rate can assist in reducing haemolysis, beyond which the shear stresses in the bulk fluid will instead contribute to haemolysis (Bronzino et al. 2006) Here haemolysis occurs at very low shear rates due to red cells spending longer in the membrane pores, and at much higher rates due to bulk shear stresses.

Early research into haemolysis modelling focussed on relating haemolysis to both magnitude and duration of mechanical loading (Leverett et al. 1972; Grigioni et al. 2005; Mohandas et al. 2008). Lampert and Williams found that haemolysis is in general a nonlinear function of time and that this is particularly pertinent to the validity of short duration testing of long term applications (Lampert et al. 1972). Leverett et al summarised the work of several predecessors and found a hyperbolic relationship between exposure time and the shear stress threshold of damage to erythrocytes as is shown in Figure 101 (Leverett et al. 1972).

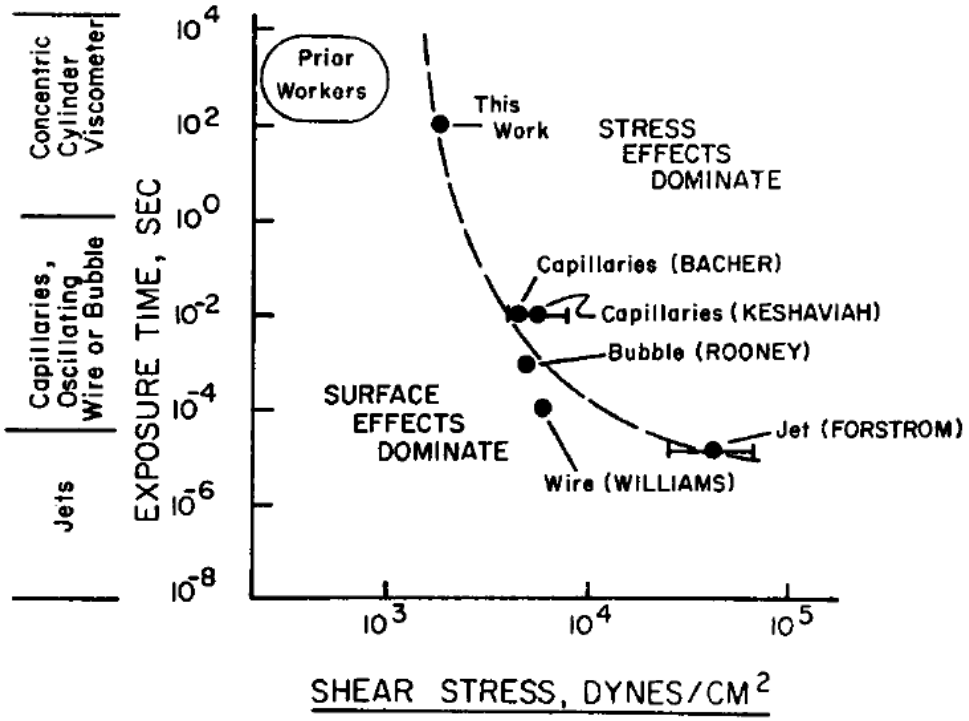


Figure 101: Hyperbolic relationship between exposure time and the shear stress threshold of damage to erythrocytes (Leverett et al. 1972)

With pulsatile flow and oscillatory flow induced by rotary pumps (as used in the proposed device), the shear stress profile will be highly non-uniform, involving cyclical long-term profiles and unsteady short term stresses (Yeleswarapu et al. 1995). Additionally, it is important to consider the impact of damage accumulation with regards to shear induced haemolysis (Yeleswarapu et al. 1995). As a result, using a constant shear trauma threshold is problematic as is highlighted in Figure 102 from Yeleswarapu et al.

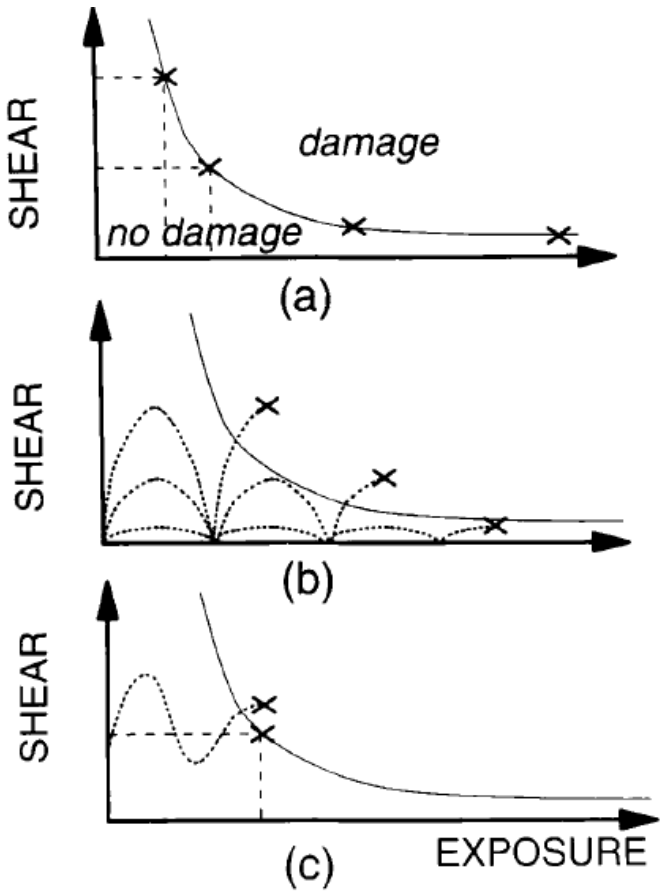


Figure 102: Unsuitability of a fixed trauma threshold with non-uniform shear stress profile which arises from pulsatile and oscillatory flow. a) Typical relationship between shear and exposure time when modelled from constant stress experiments. These models can be unsuitable for b) repeated cyclical exposure or c) unsteady transient exposure e.g. rotary pumps (Yeleswarapu et al. 1995)

Yeleswarapu produced a model for haemolysis which aimed to take into account accumulated damage to erythrocytes and was based on a power-law relationship between the critical time of exposure to haemolysis and the shear stress at constant stress exposure (Yeleswarapu et al. 1995). This model contrasted with the hyperbolic relation observed by Leverett et al and suggested that different ranges of stress levels required a different set of constants for the model (Yeleswarapu et al. 1995).

Subsequently, however, Grigioni et al demonstrated that the model put forward by Yeleswarapu et al did not accurately account for the impact historical shear on erythrocytes has (Grigioni et al. 2005). Progressing on from the power-law model of Yelewarapu and others (Giersiepen et al. 1990; Yeleswarapu et al. 1995), Grigioni et al produced a model which described haemolysis with respect to historical load magnitude and duration, as well as acting load magnitude and exposure time (Grigioni et al. 2005).

More recently however, Vitale et al criticised the previous models for being *"based on data obtained by exposing blood to a time-constant, monodimensional stress"* and thus not being representative of the actual physical phenomena involved in haemolysis (Vitale et al. 2011). Vitale et al called for new modelling approaches for haemolysis analysis based instead on the physical description of the haemolysis and haemoglobin release (Vitale et al. 2011).

Although the work covered in this thesis does not extend to mathematical modelling of haemolysis in the proposed device, the complexity of effectively modelling the phenomenon shown above highlights the difficulty in making informed design decisions to accurately limit haemolysis in the proposed device. Being aware of what general conditions induce haemolysis and the negative impact it can have, in combination with a mindful approach to minimise the shear stresses applied to the blood during the functioning of the proposed device, was sufficient during the initial development stage. Subsequent haemolysis analysis on haemoconcentration with bovine blood was conducted and is described in detail in *4.3 Complete Blood Counts*.

Aside from haemolysis, blood contact with foreign surfaces for prolonged periods in areas of flow stasis, the presence of other plasma components, high shear and elevated Reynolds shear stresses may activate platelets, leukocytes, complement, and increase concentrations of inflammatory mediators (Kameneva et al. 2004).

Aside from haemoglobin, damaged erythrocytes will release ADP into the surrounding plasma (Alkhamis et al. 1990). In fact, due to its smaller size, ADP would be expected to leak more readily from erythrocytes compared to haemoglobin (Alkhamis et al. 1990).

There is strong evidence to suggest that this shear-induced release of ADP will in turn chemically induce the adhesion of platelets to artificial surfaces and the aggregation of platelets in the bulk (Born 1962; Alkhamis et al. 1990; Puri et al. 1997). ADP is a major cause of primary platelet aggregation, and is also heavily involved in secondary aggregation (Puri et al. 1997). Aside from ADP release, erythrocyte motion can increase the effective diffusivity of platelets, thereby assisting their transport in the bulk blood phase which, coupled with ADP release, can cause the build up and adhesion of a protein rich layer at surfaces (Alkhamis et al. 1990). Alkhamis et al found that intact erythrocytes will release ADP and haemoglobin under low-stress shearing and those erythrocytes released twice as much ADP as platelets (Alkhamis et al. 1990). Moreover, erythrocyte contribution to platelet aggregation in the bulk phase was six times that of platelets, and twice the platelet contribution to platelet adhesion to artificial surfaces (Alkhamis et al. 1990). The threshold above which erythrocyte contribution to ADP release, platelet adhesion and aggregation was greater than that of the platelets themselves was found to be 35% Hct, suggesting that below this level, erythrocyte populations are too low to

allow for sufficient traumatic interactions which may result in ADP release (Alkhamis et al. 1990). Not only does this further highlight the importance of Hct with regards to the impact of shear forces on the blood, but it demonstrates how it is not only erythrocytes which are impacted by shear forces in the blood as a result of blood flow.

It is known that aside from haemolysis, shear stress can cause platelet activation (Konstantopoulos et al. 1995; Konstantopoulos et al. 1998; Hu et al. 2003; Nesbitt et al. 2009; Yong et al. 2011). Under low shear force conditions (~12 dynes/cm2), platelet aggregation is initiated by compounds such as thrombin and mediated by fibrinogen binding to the glycoprotein GPIIb-IIIa complex, cm2 (O'Brien 1990; Ikeda et al. 1991). The aggregates formed are unstable and reversible, becoming disaggregated at ~68 dynes/cm2 (O'Brien 1990; Ikeda et al. 1991). Anti-platelet drugs such as aspirin and GPIIb-IIIa inhibitors can be used to attenuate this form of platelet aggregation (O'Brien 1990; Rupprecht et al. 1998; Cadroy et al. 2000; Lev et al. 2001; Yong et al. 2011). Conversely, under higher shear force conditions (~80 dynes/cm2), the shearing forces alone initiate platelet aggregation, fibrinogen is not involved and instead the formation of stable aggregates is mediated by von Wildebrand factor binding to both the GPIIb-IIIa and GPIb-IX complexes (O'Brien 1990; Ikeda et al. 1991). This shear-induced process can be suppressed by GPIIb-IIa inhibitors but is insensitive to aspirin and similar drugs (O'Brien 1990; Konstantopoulos et al. 1995; Merten et al. 2000; Goto et al. 2003; Yong et al. 2011). Moreover, it was shear-induced platelet aggregation to occur independent of ADP (O'Brien 1990; Nesbitt et al. 2009; Yong et al. 2011). In this way, shear alone can induce platelet aggregation and adhesion, without the need for prior haemolysis.

Aside from haemolysis and platelet activation and aggregation, shear stress is also implicated in platelet-leukocyte aggregation and leukocyte activation (Konstantopoulos et al. 1998; Hu et al. 2003; Yong et al. 2011). In turn, leukocyte activation can promote platelet activation (Faraday et al. 2001). Moreover, platelet, leukocyte and complement activation may induce the release of chemicals which are damaging to erythrocytes and increase the susceptibility to haemolysis (Rosse et al. 1980; Himmelfarb et al. 1999; Sowemimo-Coker 2002). Thus it can be seen that mechanical shear in the blood as a result of flow through extra-corporeal devices can induce a series of interacting negative effects on the blood constituents. Analysis of flow through medical devices involving extracorporeal blood flow is therefore an important step in the development of such devices. In order to assist in the design of devices which have minimal haemolytic effect, computational fluid dynamics (CFD) analysis and subsequent haemolysis analysis with animal blood is the standard approach (Sakota et al. 2008).

3.3.4. Assessing blood flow

In accordance with the aforementioned 'standard approach', assessment of the blood flow within the proposed device and any potential impact it may have upon haemolysis and ultrafiltration rate was performed by utilising computational fluid dynamics (CFD) followed by haematology analysis with bovine blood.

3.3.4.1. Difficulties in modelling blood and haemofiltration

There are several difficulties in modelling the fluid flow within the proposed device, some arising from the nature of the device, and some arising from the nature of the blood, or its rheology.

Blood rheology is the field of study related to the physical and flow properties of blood (Connes 2011). The finer details of blood rheology are still under investigation, and are of particular clinical interest as it is known to be altered under the influence of several disease states (Baskurt et al. 2003). As discussed previously (*2.3.5. Retention of different blood components*), blood is a complex fluid comprising cells suspended in liquid plasma which is mainly water with proteins, hormones, glucose and mineral ions (Brust et al. 2013). The complexity of studying and modelling blood rheology becomes clear when first attempting to characterise blood since blood can be regarded as one of three possible combinations of physical states; a two-phase liquid (the most common model), a solid liquid suspension comprising solid cellular elements in the liquid plasma, or, based on the liquid behaviour of the red blood cells under shear, a liquid-liquid emulsion (Baskurt et al. 2003). Due to the high RBC content of blood (as mentioned earlier Hct is typically approximately 46% for men and 41% for women (Burton 2000)), the rheological behaviour of whole blood is largely determined by the behaviour of the RBCs (Connes 2011; Brust et al. 2013).

Several fluids, for example water, can be described as being Newtonian, meaning that at a fixed temperature and pressure its viscosity is independent of shear stress (Merrill 1969). Blood is a non-Newtonian suspension, that is, its viscosity depends on shear stress and can be calculated as the ratio of shear stress to shear rate as is shown in Figure 103 (Merrill 1969; Baskurt et al. 2003; Pedrizzetti et al. 2003; Connes 2011; Lowe et al. 2012).

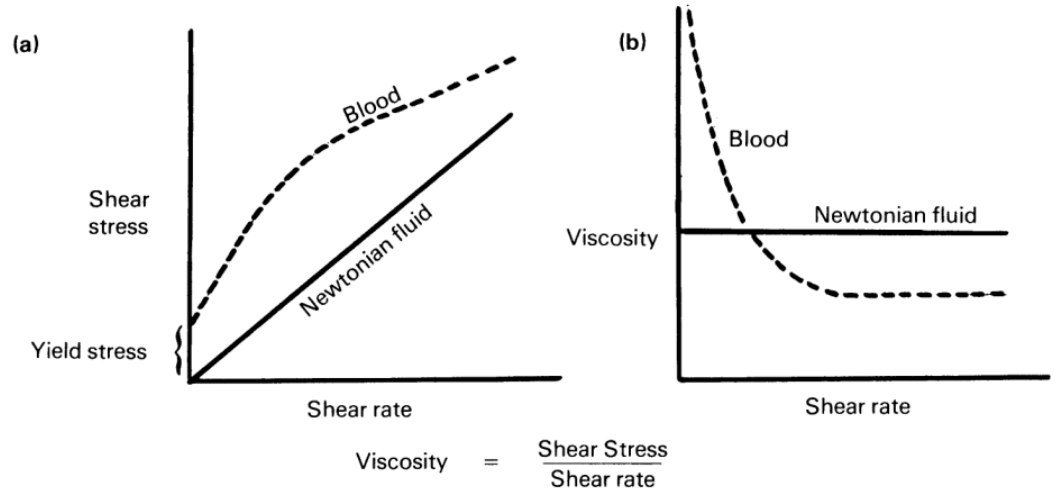


Figure 103: Relationships between Shear rate and a) shear stress / b) viscosity for Newtonian fluids and blood (a Non-Newtonian fluid) (Lowe et al. 2012)

Also shown in Figure 103 is a yield stress point which is present in some non-Newtonian fluids and represents a point below which shear rate is zero (no flow) despite the fact that stress may be finite and thus results in an infinitely high apparent viscosity (Lowe et al. 2012).

The apparent viscosity of normal human blood is high at low shear rates or shear stresses but will decrease with increasing shear forces (Rand et al. 1964; Merrill 1969; Baskurt et al. 2003). This behaviour is known as 'shear-thinning' and is shown in Figure 104 (Baskurt et al. 2003; Connes 2011; Chang et al. 2012; Lowe et al. 2012).

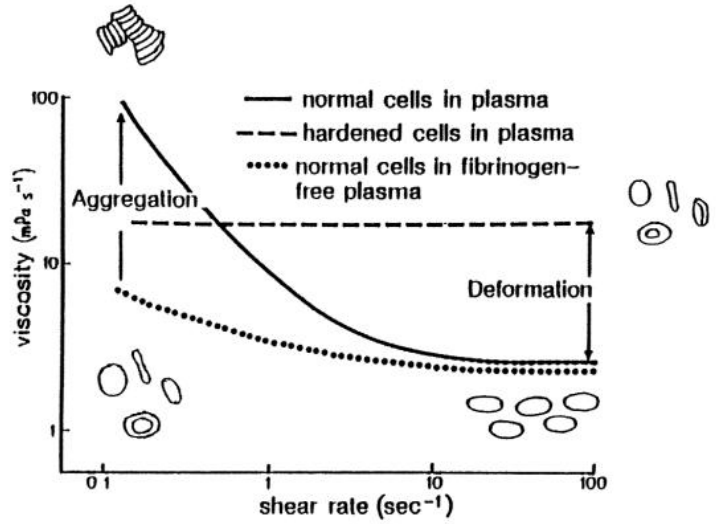


Figure 104: Shear-thinning behaviour of blood as apparent viscosity decreases with increased shear rate (Chang et al. 2012)

The viscosity of normal blood at 37°C is relatively insensitive to increases in shear beyond high shear rates of 100-200 s^{-1}, however below $100s^{-1}$ blood viscosity exponentially increases as shear rate decreases (Baskurt et al. 2003). The non-Newtonian and shear-thinning behaviour of blood greatly increases the complexity of modelling its flow in the proposed device.

As mentioned previously (*2.1. Principles of Ultrafiltration*), the specific flow behaviour of blood is largely determined by its structure (Connes 2011). Blood is a two-phase liquid, the two phases being the plasma and cellular components, and consequently the rheological properties of blood are determined by the rheological properties of these two phases as well as their relative contribution to the blood volume as a whole (Baskurt et al. 2003; Pedrizzetti et al. 2003; Connes 2011). As discussed previously (*1.2. Conventional Ultrafiltration*), Hct is the broad measure of fraction of the blood which is cells (and in turn the fraction which is plasma), and as such is a major determinant of blood viscosity (Baskurt et al. 2003; Pedrizzetti et al. 2003; Connes 2011; Brust et al. 2013). Given that RBCs constitute 99% of the cellular components, compared to RBC concentration the contribution of WBCs and platelets to blood rheology is largely insignificant (Pedrizzetti et al. 2003; Connes 2011). When blood is under conditions of laminar flow, the cellular elements will disturb the streamlines and consequently lead to blood viscosity being higher than plasma viscosity (Baskurt et al. 2003; Connes 2011). Higher Hct and thus more RBCs per unit volume leads to more disruption of the flow and higher viscosity as is illustrated in Figure 105 which shows the exponential relationship between Hct and blood viscosity (Levitzky 2008).

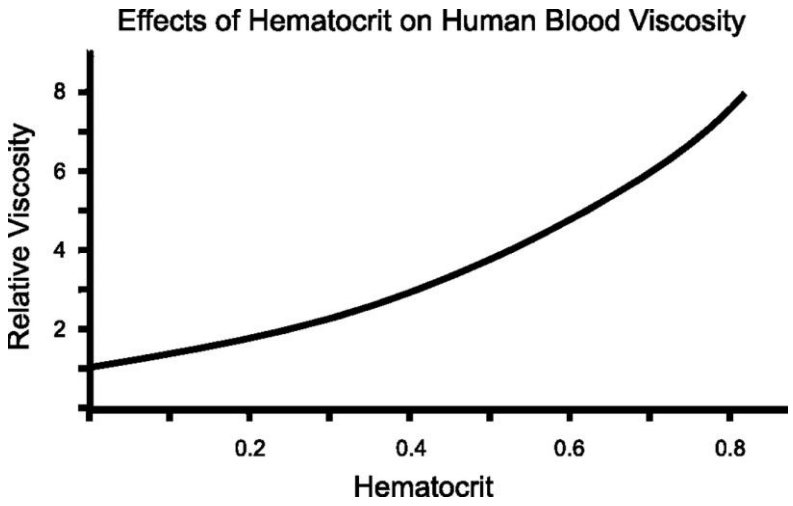


Figure 105: Exponential relationship between Hct and blood viscosity (Levitzky 2008)

The contribution of RBCs to the whole blood viscosity it not just related to their concentration, but also to their behaviour in the blood which, as it will transpire, also explains the shear-thinning behaviour of blood described previously in this section.

The disturbance of flow streamlines and the corresponding impact upon blood viscosity is not only affected by the concentration of the RBCs but also upon their behaviour in response to shear forces and their deformability to and aggregability under specific haemodynamic conditions (Wells et al. 1969; Schmid-Schonbein et al. 1971; Baskurt et al. 2003; Pedrizzetti et al. 2003; Rampling et al. 2004; Connes 2011).

At high shear rates (characterised by higher shear forces), RBCs have been observed to behave to like fluid drops by exhibiting a shape change and slightly deforming and orientating themselves with flow streamlines which reduces flow disruption and the internal resistance to flow thus reducing blood viscosity (Wells et al. 1969; Schmid-Schonbein et al. 1971; Baskurt et al. 2003; Connes 2011). The deformability of RBC, (determined by cell geometry, membrane properties and cytoplasm composition), is thus an important factor in determining the high shear rate viscosity of blood (McKay et al. 1993; Connes 2011). To further complicate the problem of modelling blood flow in the proposed device, the deformability of RBCs described is in itself highly complex with the extent of RBC geometry changes being functions of both the magnitude and orientation of the force as well as the aforementioned cellular properties (Baskurt et al. 2003; Connes 2011). The reversibility of the RBC deformation means RBCs behave as elastic bodies, but they also exhibit viscous behaviour and thus are said to respond as a viscoelastic body, giving rise to the notion of blood being, to a slight extent, a viscoelastic substance in itself i.e. one that has *"capacity to recover its shape after a briefly applied stress, but flows after long periods of stress"* (Merrill 1969; Evans et al. 1975; Baskurt et al. 2003; Pedrizzetti et al. 2003). Thurston first studied the viscoelasticity of human blood and found that for oscillatory flow blood does indeed possess elastic properties (Thurston 1972). However, there are few studies regarding the influence viscoelastic blood behaviour has on flow patterns in large vessels but it has been suggested that the affect is minimal compared to the shear-thinning effect (Pedrizzetti et al. 2003). To compound the modelling complexity further, under some pathological circumstances the RBC membrane can, under excessive shear forces, exhibit plastic changes and be permanently deformed, described previously in *3.3.3. Effect of flow on blood* as sub-lytic damage (Yeleswarapu et al. 1995; Baskurt et al. 2003).

At high flow rates, the influence which RBCs have upon blood viscosity is dependent upon their behaviour as individual particles which, although they may deform, do not change their surface area or volume (Pedrizzetti et al. 2003). However, at low flow rates ($\gamma<10s^{-1}$) RBCs are able aggregate and form linear arrays called rouleaux which are arranged like a stack of coins, thereby increasing the size of the flow obstructing particles and resulting in an increase in blood viscosity (Baskurt et al. 2003; Pedrizzetti et al. 2003; Rampling et al. 2004; Connes 2011; Brust et al. 2013; Steffen et al. 2013). If shear rate is decreased further these rouleaux will form three

dimensional structures thus exacerbating flow disruption and leading to an additional increase in blood viscosity (Baskurt et al. 2003; Pedrizzetti et al. 2003; Rampling et al. 2004). The impact of aggregation and deformation of RBCs is shown in Figure 106.

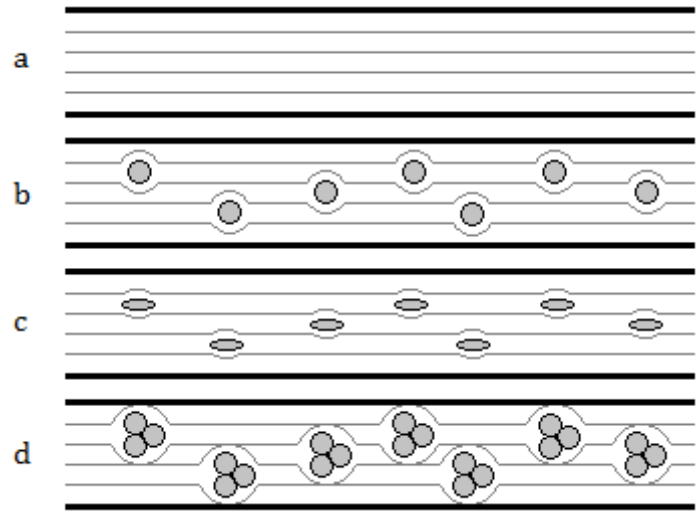


Figure 106: The impact of aggregation and deformation of RBCs on flow streamlines a) streamlines in plasma without RBCs b) distorted streamlines in presence of undeformed RBCs c) steamlines less distorted in presence of deformed RBCs d) streamlines more distorted in presence of aggregated RBCs

The aggregation of RBCs is determined by cellular properties and plasma composition and is promoted by fibrinogen and other large plasma proteins, most likely through a depletion effect in which the distance between RBCs is reduced sufficiently to prevent other macromolecules from occupying the space between them, thereby depleting their concentration in this region and leading to an effective osmotic pressure inducing attraction between the RBCs (Baskurt et al. 2003; Connes 2011; Brust et al. 2013; Steffen et al. 2013). Figure 107 shows a photograph of a RBC rouleaux in a dextran solution (Steffen et al. 2013).

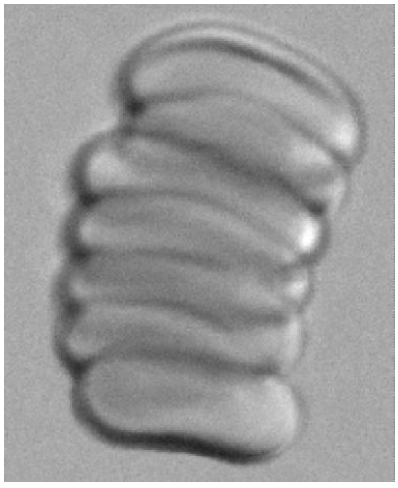


Figure 107: Photograph of a RBC rouleaux in a dextran solution (Steffen et al. 2013)

Fortunately, these aggregates are reversible and under high shear rates the rouleaux are disrupted and broken up or disaggregated back to individual RBCs and again align with flow resulting in a decrease in blood viscosity (Baskurt et al. 2003; Pedrizzetti et al. 2003; Connes 2011; Brust et al. 2013; Steffen et al. 2013). This process explains the shear thinning behaviour of blood (Brust et al. 2013). The formation of the rouleaux is dependent upon shear rate and Hct, but for normal human blood in a shear field of $10s^{-1}$, aggregation time is approximately 10secs, whilst the breakdown of rouleaux will occur almost instantly once shear rate passes a given threshold (Pedrizzetti et al. 2003). Consequently, due to the fact that pulsatile flow in the natural vasculature induces intermittent high shear rates over a period much shorter than the RBC aggregation time, rouleaux are unable to form in relatively undisturbed medium sized artery flow (Pedrizzetti et al. 2003). It can be seen therefore how areas of stasis in the flow channel of the proposed device can be problematic in inducing RBC aggregation which could potentially lead to an increase in blood viscosity and resistance to flow (Connes 2011).

As the suspending phase for the cellular elements in the blood, the viscosity of plasma has a direct impact upon whole blood viscosity regardless of the concentration and condition of the cellular elements and makes a particularly significant contribution to whole blood viscosity under high shear conditions (Baskurt et al. 2003; Connes 2011). Plasma viscosity is mainly dependent upon its protein content and temperature (Brujan 2010). Plasma in itself is a Newtonian fluid, yet some groups have reported non-Newtonian behaviour, although some groups suggest these behaviours are surface effects rather than bulk effects (Baskurt et al. 2003; Connes 2011; Jaishankar et al. 2011; Brust et al. 2013).

It was previously stated in this section that RBCs exhibited a viscoelastic behaviour which led to some viscoelastic behaviour of whole blood. Brust et al found that plasma too exhibits pronounced viscoelastic behaviour and were able to demonstrate that the elongational properties of blood are largely due to the elongational properties of plasma proteins (Brust et al. 2013). Brust et al suggest that this viscoelasticity may lead to viscoelastic flow instabilities, reduction of turbulent instabilities by plasma elongational properties, and a pronounced cell depleted layer near wall boundaries (Brust et al. 2013).

Related to this notion, it is known that when RBCs are flowing through a cylindrical pipe they are not evenly distributed across the cross-section of that pipe but rather will tend to accumulate along the central axis resulting in a cell-depleted layer close to the pipe wall (Baskurt et al. 2003). This is because central fluid layers have the lower shear forces and higher velocities than wall adjacent regions (Baskurt et al. 2003). This can be seen clearly in the simulation model produced by Tsubota et al shown in Figure 108 (Tsubota et al. 2006).

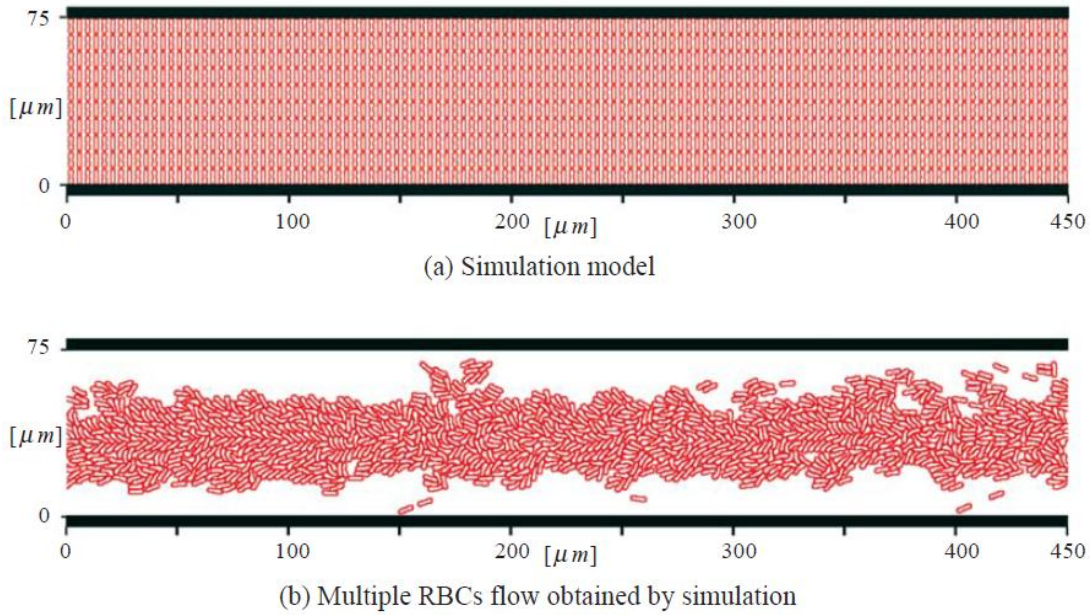


Figure 108: Model by Tsubota et al demonstrating the accumulation of RBCs at the central axis of a cylindrical pipe (Tsubota et al. 2006)

The consequence of this is clear, namely that there will be a Hct disparity between the central and peripheral areas of the flow channel, and an associated viscosity disparity, further complicating the modelling of blood flow (Goldsmith et al. 1989; Baskurt et al. 2003).

Clearly, Hct and plasma viscosity have a significant impact on the flow conditions of whole blood which has some implications particularly relevant to attempting to model the proposed device. Haemofiltration is known to result in increased blood viscosity and decreased flow velocity (Yamamoto et al. 2005).The nature of the device is such that both Hct and blood viscosity will not be constant but rather continually increasing as plasma is extracted from the whole blood. Importantly, the rate of plasma extraction is not yet known but will be dependent upon the operating conditions of the superabsorber material, the membrane and in all probability other parameters not yet known. It is unlikely, therefore, that the rate of Hct and blood viscosity increase will be linear, adding a further complex function to the model. Additionally, aside from the aforementioned radial gradient of Hct (and therefore viscosity) across the cross section of the flow channel, the fact that plasma is extracted along the length of the flow channel means there will also be a Hct and viscosity gradient between the inlet and outlet of the device with lower Hct and lower viscosity blood near the flow channel inlet and higher Hct and higher viscosity blood near the flow channel outlet. Furthermore, the way in which fluid enters the flow channel via a narrow inlet, coupled with the pulsatile flow induced by the roller pump will lead to inevitable mixing between the diluted and reconcentrated blood. The pulsatile flow itself is an

important haemodynamic aspect which would need to be considered if the model involved studying downstream vortex structures (Politis et al. 2008). It has been discussed previously (*3.2.3.6. Concentration Polarisation and Membrane Fouling*) how filtration leads to concentration polarisation and there will be a chemically induced suction force associated with the superabsorber and the concentration gradient across the membrane, both of which will impact flow. Not only that, but, as has been discussed (*3.1.8. Agitation rate*), the entire flow channel will be agitated which will massively impact the flow pattern of the blood. Even without these immense complications in modelling the fluid and flow within the device, the fact that the membrane exchange surface contributes a significant portion of the flow channel surface area, couple with the flexibility of the polycarbonate membrane when compared to the cellulose acetate used to construct the remainder of the flow channel, adds complexity to the modelling of the device itself.

Clearly, accurately modelling the blood flow through the proposed device during its operation would be an extremely difficult undertaking and beyond the scope of this thesis. Therefore, it was important to focus attention on which information, which could be realistically obtained from CFD modelling, would best contribute towards validating the design of the proposed device with regards to producing good quality, haemoconcentrated blood, in a timely manner.

It was decided that CFD modelling could be utilised to inspect bulk flow of a simplified 'blood' fluid in the proposed device and ensure that there was consistent flow across the entire membrane exchange surface and that there were no areas of stasis in the flow channel. Achieving this would ensure a good throughput of blood across the membrane exchange surface, allowing for better plasma extraction, and furthermore a reduction in haemolysis could be achieved by eliminating points of stasis which are known to contribute to haemolysis and RBC aggregation (Kameneva et al. 2004; Connes 2011). Additionally, CFD modelling can give an insight into potential sites of elevated shear forces which maybe induce haemolysis and reduce the quality of the blood leaving the proposed device.

3.3.4.2. Early flow analysis

Before CFD analysis began, water based dye tracing was used to assess flow in the proposed device. It should be noted that, as described earlier (*3.3.4.1. Difficulties in modelling blood and haemofiltration*), blood is a highly complex fluid and thus this water model has limited predictive capabilities for how blood will behave under similar conditions, yet it is still useful as an initial basic qualitative visualisation technique. Water was passed through the flow channel of a prototype device and a blue dye was injected at the inlet. The process was video recorded from above to capture the flow pattern adopted by the dye as it moved along the exchange

surface towards the outlet. Figure 109, Figure 110, Figure 111, and Figure 112 show the progression of the flow pattern as captured on the video.

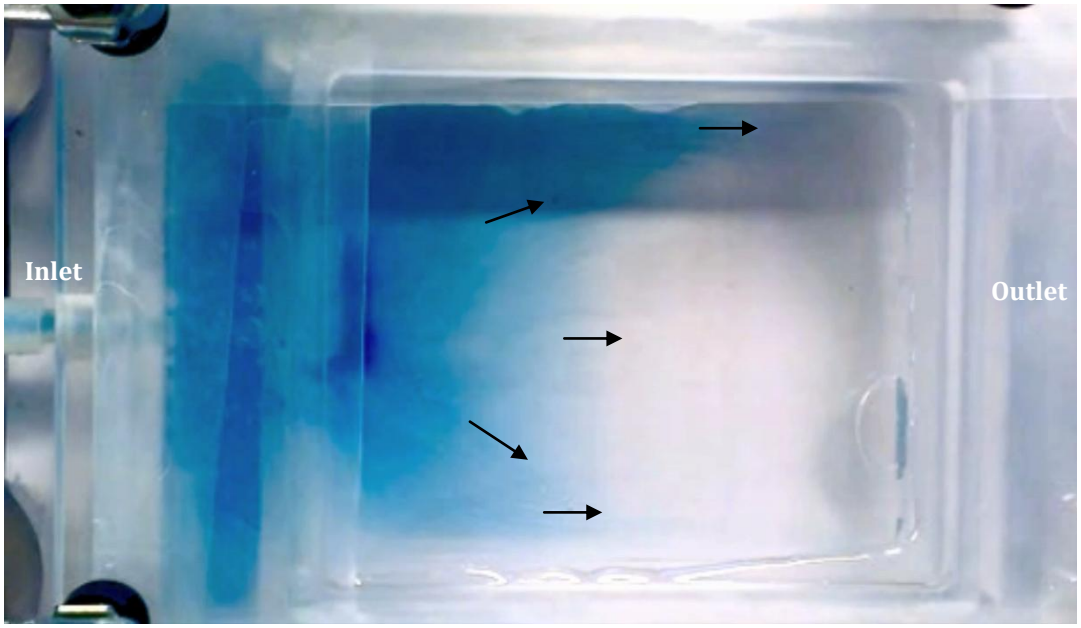


Figure 109: First image from dye tracing showing streamlines propagating from inlet to outlet (not visible in diagram). Darker horizontal band in upper section artefact created by a shadow cast on the test rig.

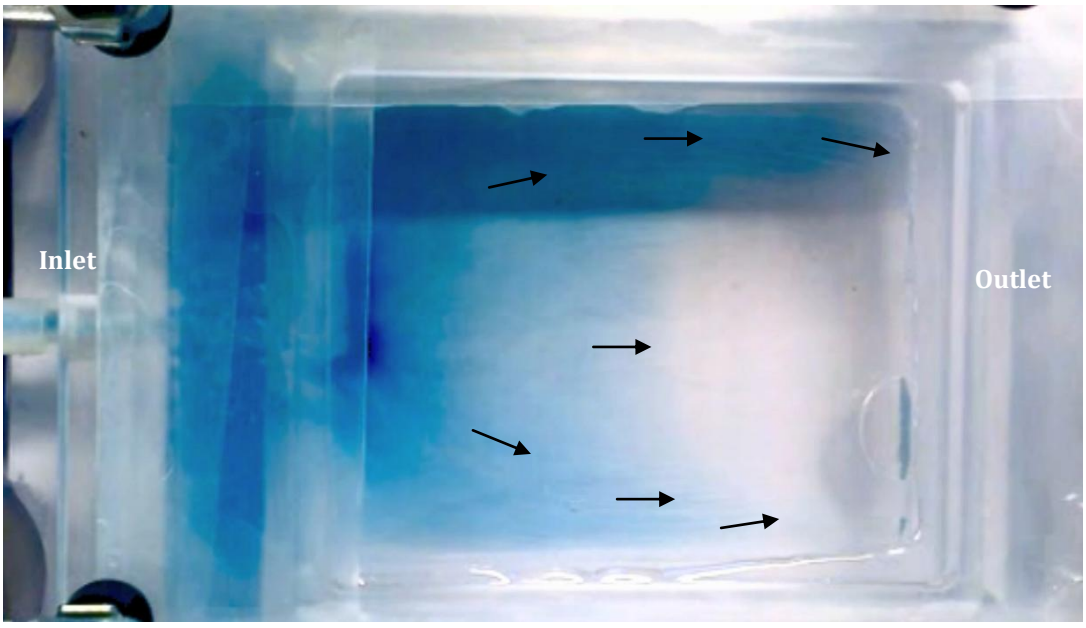


Figure 110: Second image from dye tracing showing streamlines propagating from inlet to outlet (not visible in diagram). Darker horizontal band in upper section artefact created by a shadow cast on the test rig.

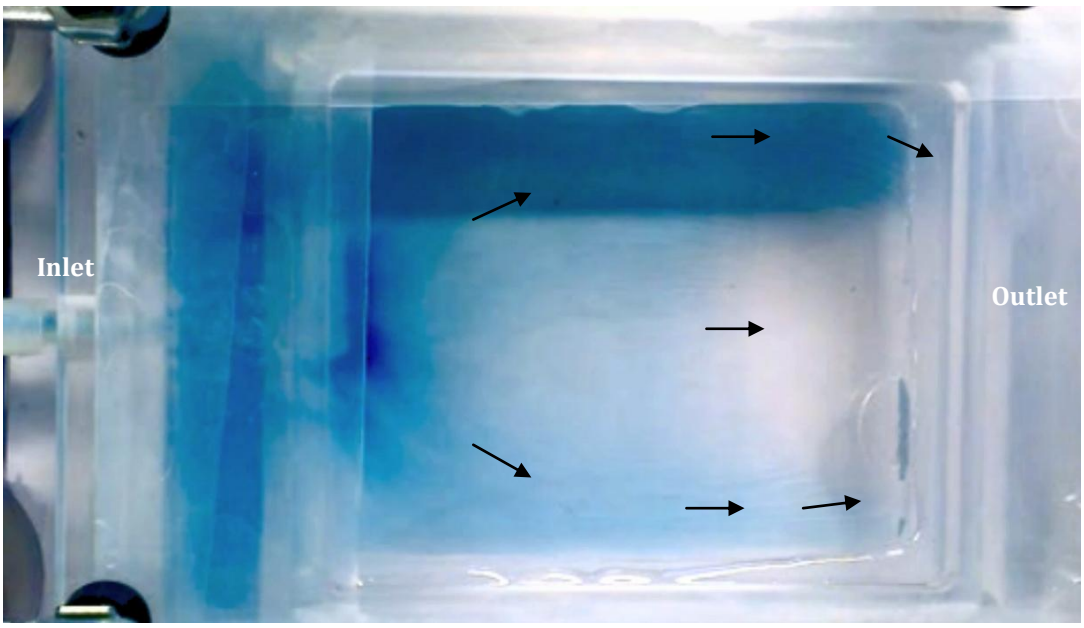


Figure 111: Third image from dye tracing showing streamlines propagating from inlet to outlet (not visible in diagram). Darker horizontal band in upper section artefact created by a shadow cast on the test rig.

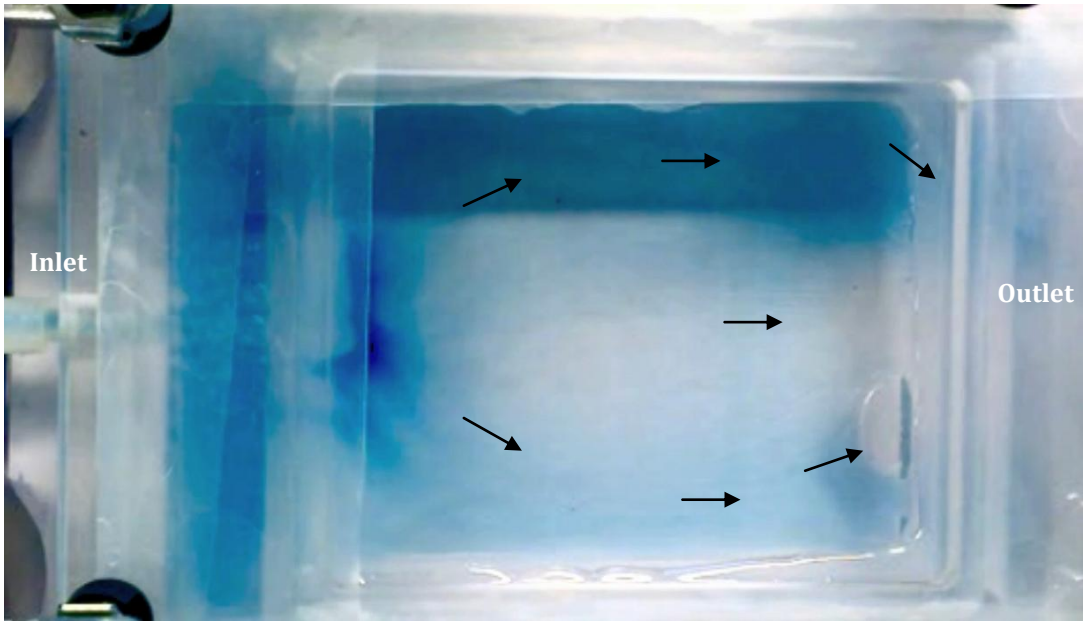


Figure 112: Fourth image from dye tracing showing streamlines propagating from inlet to outlet (not visible in diagram). Darker horizontal band in upper section artefact created by a shadow cast on the test rig.

Clear streamlines can be seen propagating from the inlet to the outlet covering the entire membrane exchange surface. This was a positive first indication that there was good 'coverage' of flow across the membrane exchange surface which should provide good opportunity for plasma absorption by the superabsorber.

3.3.4.3. CFD analysis

As mentioned previously (*3.3.3. Effect of flow on blood*) CFD analysis to assess blood flow in medical devices has been used by several groups and is now seen as part of the 'standard approach' (Sakota et al. 2008). The high complexity of the model as described previously (*3.3.4.1. Difficulties in modelling blood and haemofiltration*) means that certain modelling assumptions must be made.

Pedrizetti et al state that for flow in large and medium-sized vessels blood can be "considered as an isotropic, homogenous, incompressible Newtonian or non-Newtonian inelastic fluid" (Pedrizzetti et al. 2003). Politis et al modelled blood as a Newtonian fluid (i.e. viscosity independent of shear rate, equal to 3.5×10^{-6} m^2/s) which they state is a valid assumption for shear rates greater than $100s^{-1}$ and vessels more than 1mm in diameter and Hct at physiological levels (Politis et al. 2008). The Hct of the blood in the proposed device will be increasing as it is concentrated so this affects the validity of the model somewhat but as mentioned previously (*3.3.4.1. Difficulties in modelling blood and haemofiltration*) it would be highly complex to model the system accounting for this Hct change. Perktold et al studied the shear thinning behaviour of blood in a carotid artery bifurcation and found the average difference wall shear stress between Newtonian and non-Newtonian models was approximately only 10% (Perktold et al. 1998). Similarly, Johnston et al found that in 3-7mm diameter arteries there is no significant difference between Newtonian and non-Newtonian blood models for 70% of the pulse period (Johnston et al. 2006). Selgrade and Truskey used CFD to model blood flow through an LVAD and, due to the high shear stress in the device, modelled blood as a *"Newtonian, incompressible fluid with a density of 1050 kg/m^3 and viscosity of 0.0035 Pa/s"* (Selgrade et al. 2012).

Consequently, blood was modelled as an incompressible Newtonian fluid with a density of $1050kg/m^3$ and a viscosity of $0.0035kg/m^3$ (Pa). CFD analysis was performed using *ANSYS Fluent* 13.0.0 *(ANSYS Inc, Cecil Township, PA)*.

A patch-independent tetrahedral mesh of the fluid volume that would be present in proposed device test rig was generated and is shown in Figure 113.

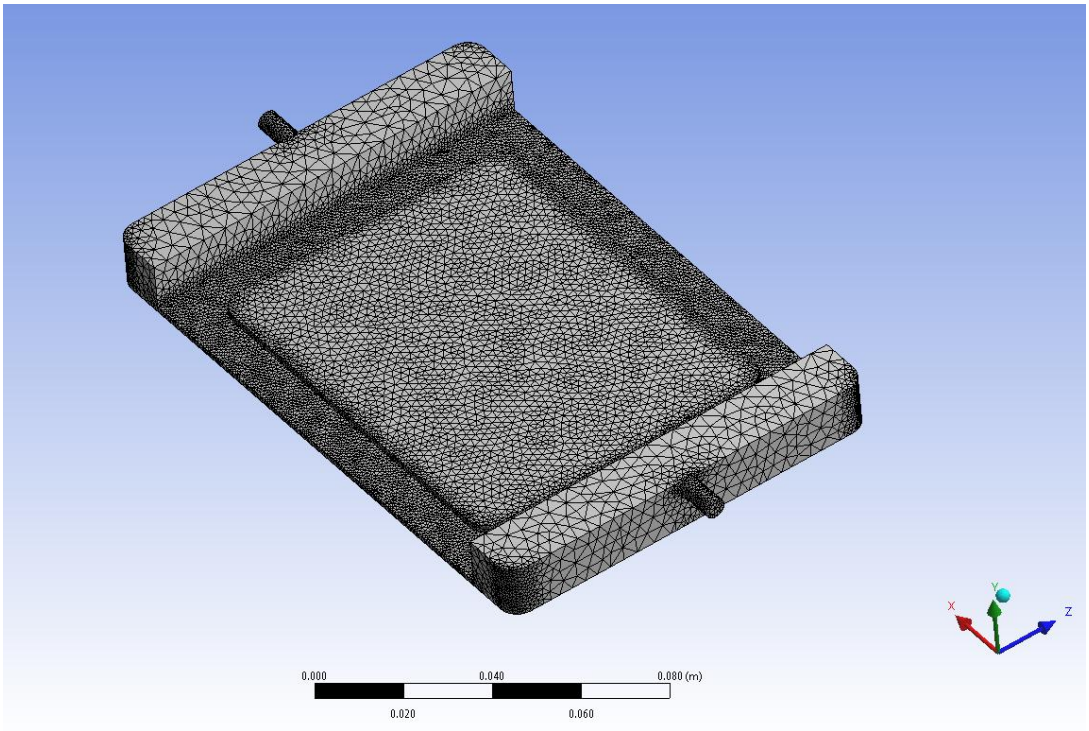


Figure 113: Mesh of the proposed device test rig fluid volume

The inlet boundary condition was set as a mass flow rate of 0.002625kg/s, equal to the proposed flow rate of 150ml/min, whilst the outlet boundary condition was set as an outflow. Pathlines and particle tracks of inert massless particles were used to assess the bulk flow in terms of coverage of the exchange surface, whilst vectors of velocity and contours of wall shear stress were used to highlight any areas of possible shear induced haemolysis.

Pathlines represent lines travelled by neutrally buoyant particles in equilibrium with the fluid motion and help visualise the flow. The pathlines within the proposed device were widely and quite evenly spread across the exchange surface indicating that the blood flow should cover the entire exchange surface area as is shown in the Figure 114 and Figure 115.

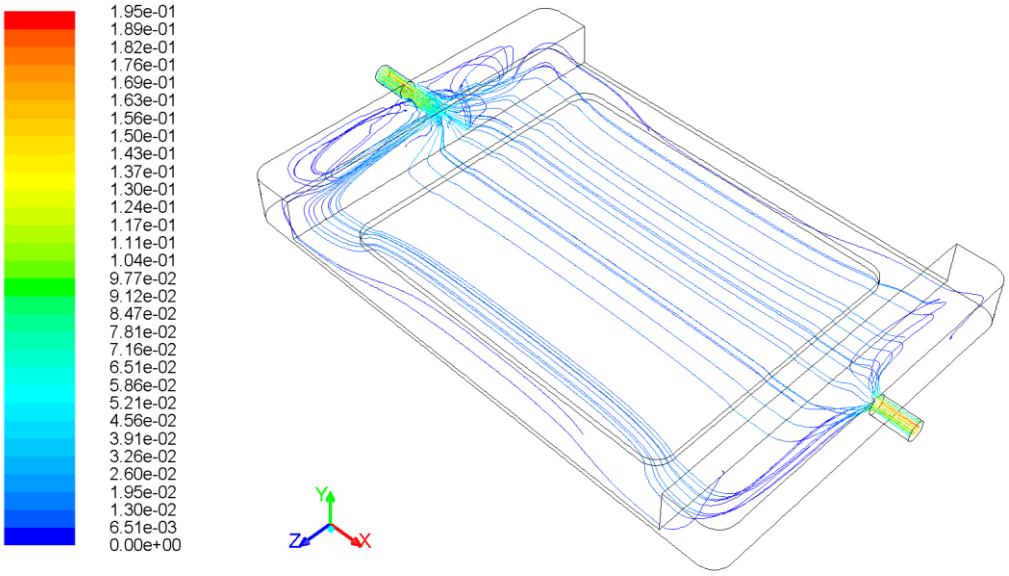


Figure 114: Isometric view of pathlines coloured by velocity magnitude (m/s) according to adjacent legend. Blood is flowing in the positive x direction. Wide spread of pathlines indicates good coverage of exchange surface area.

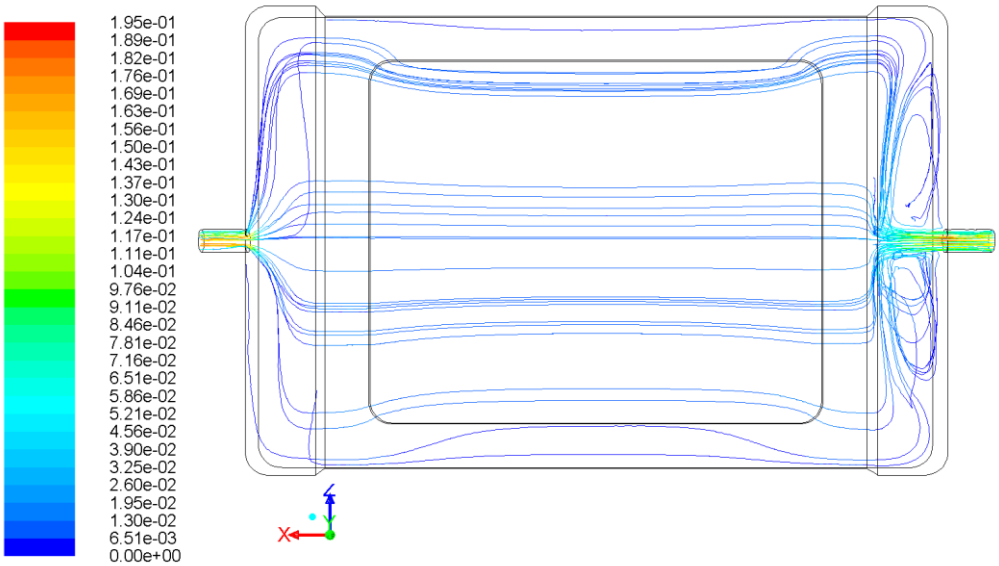


Figure 115: Top-down view of pathlines coloured by velocity magnitude (m/s) according to adjacent legend. Blood is flowing in the positive x direction. Wide spread of pathlines indicates good coverage of exchange surface area.

Similarly, the particle tracking also indicated that there should be good coverage of the exchange surface area by the flowing blood as is shown in Figure 116.

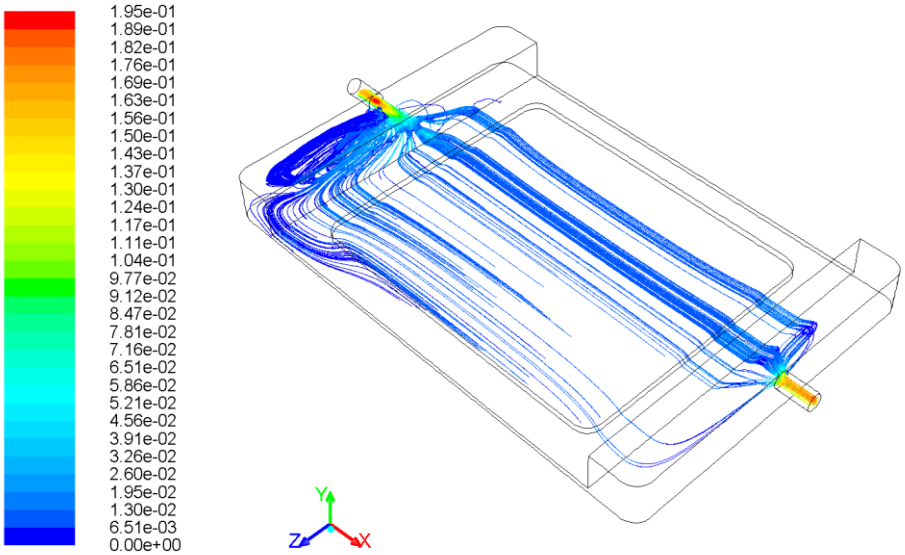


Figure 116: Isometric view of particle tracks coloured by velocity magnitude (m/s) according to adjacent legend. Blood is flowing in the positive x direction. Wide spread of particle tracks indicates good coverage of exchange surface area.

The pathlines showed that there would likely be turbulent flow in the inlet 'filling chamber' as is indicated by the tortuous pathlines in Figure 117.

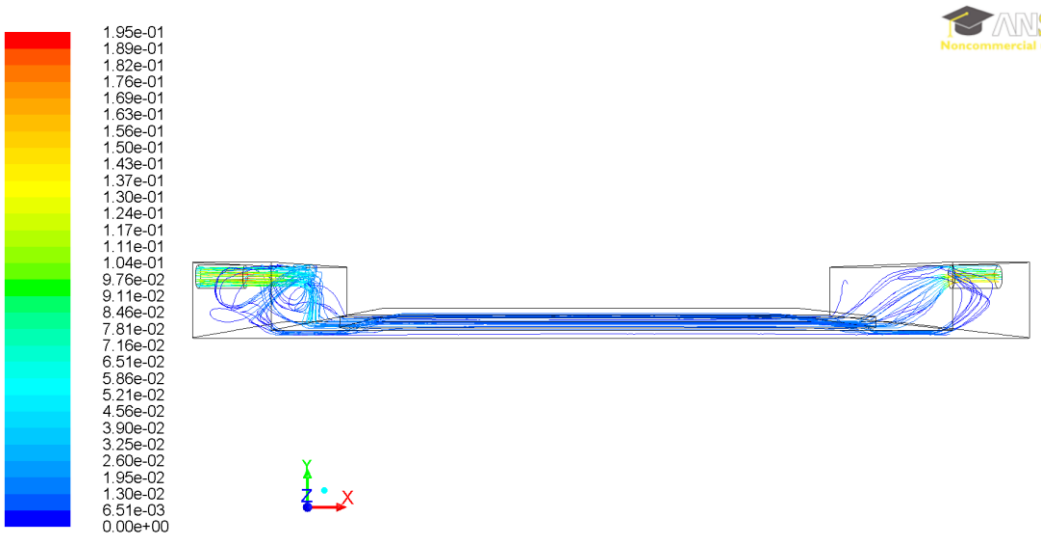


Figure 117: Isometric view of pathlines coloured by velocity magnitude (m/s) according to adjacent legend. Blood is flowing in the positive x direction. Wide spread of particle tracks indicates good coverage of exchange surface area.

However, the vectors of velocity indicated flow velocity would be low across the majority of the device as shown in Figure 118.

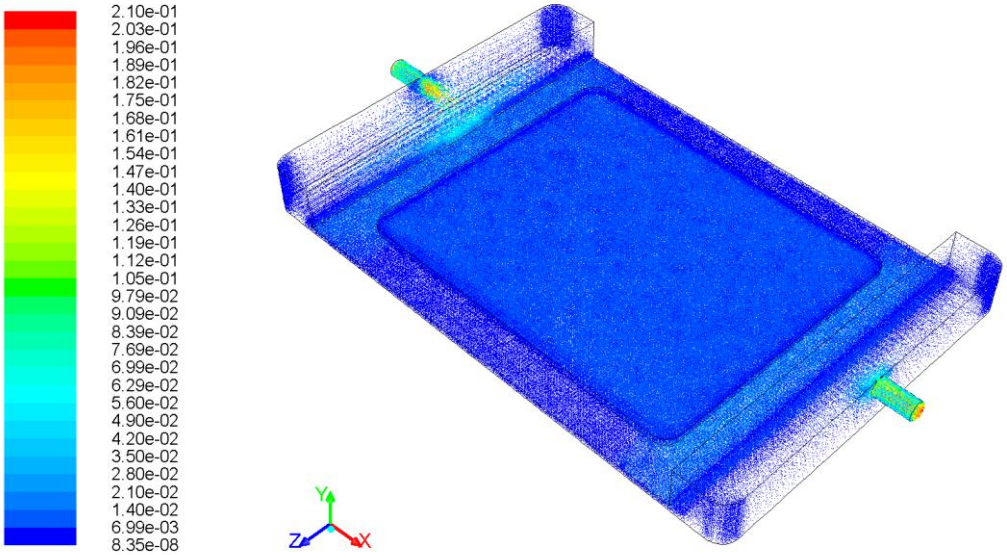


Figure 118: Velocity vectors coloured by velocity magnitude (m/s) according to adjacent legend. Blood is flowing in the positive x direction. Large blue areas indicate low flow velocity across majority of the device.

Additionally the contours of wall shear stress indicated very low shear stresses within the device as thus there would likely be little to no turbulence induced haemolysis in the device as is shown in Figure 119 and Figure 120.

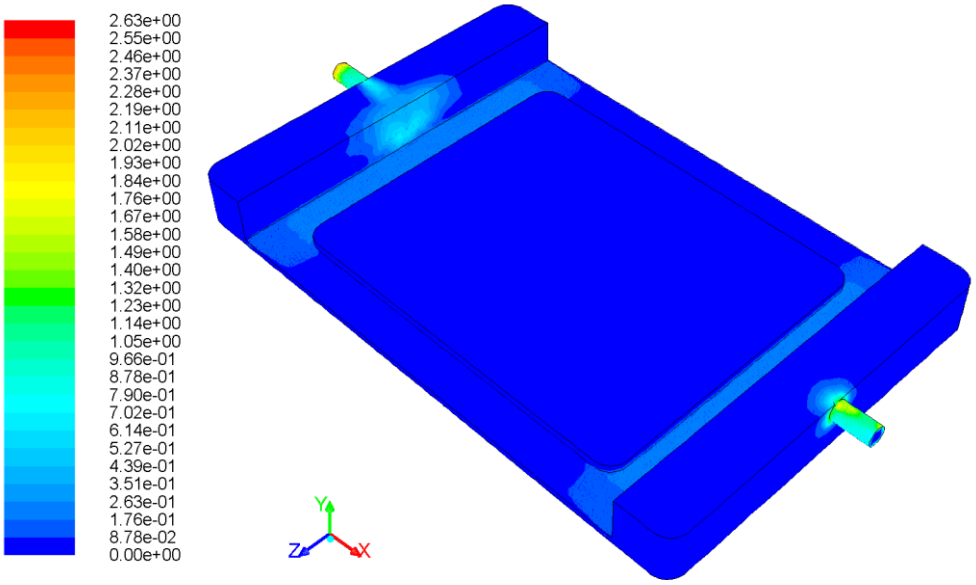


Figure 119: Filled contours of wall shear stress (Pa) according to adjacent legend. Blood is flowing in the positive x direction. Large blue areas indicate low shear stresses within the device, even in the inlet filling chamber.

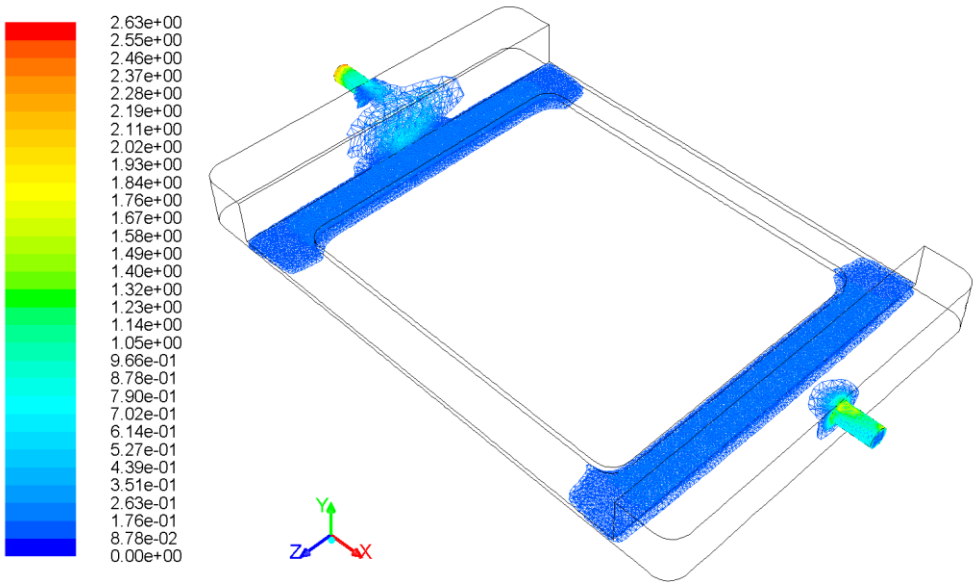


Figure 120: Unfilled contours of wall shear stress (Pa)according to adjacent legend. Blood is flowing in the positive x direction. Large blue areas indicate low shear stresses within the device, even in the inlet filling chamber.

As mentioned previously (*3.3.3. Effect of flow on blood*) a shear stress of 1500 dynes/cm^2 (150 Pa) is generally regarded as the haemolytic threshold for moderate exposure times. The shear stresses presented in the model are far lower than this threshold and thus, shear induced haemolysis should be minimal. Similarly, the shear forces indicated by the model are below the level at which shearing forces alone initiate platelet aggregation, 80 dynes/cm2 (8Pa), suggesting that shear induced platelet aggregation will also be minimal.

The limitation of models such as this one has been discussed at length in this section. The model therefore has only been used as in assessment of bulk flow and as a positive indicator for there being minimal haemolysis induced by the proposed device itself. However, haemolysis should, and will later in this thesis (*4.3.6 Haemolysis*), be properly assessed through subsequent haemolysis analysis with animal blood, as described in the aforementioned 'standard approach' (*3.3.3. Effect of flow on blood*) (Sakota et al. 2008). Since CFD analysis indicated good coverage of flow across the membrane exchange surface, which should provide good opportunity for plasma absorption by the superabsorber, it does not offer an explanation as to why the device failed to haemoconcentrate previously.

3.3.5. Impact on design

It is important to design the proposed device to ensure good bulk flow to adequately 'cover' the exchange surface and give the maximum possible volume of plasma the opportunity to cross the membrane and be absorbed by the superabsorber. However, it is also important to design the

proposed device to avoid shear induced haemolysis arising from flow through the system. It is extremely difficult to accurately model blood flow in the proposed device due to several complexities related to the nature of the system and the fluid itself. Early dye testing and simplified CFD modelling appears to indicate good bulk flow within the device and no shear induced haemolysis but further testing with animal blood is required to make a more informed assessment.

3.4. Surface Mixing

3.4.1. Early Haemoconcentration

Having established that there was suitable bulk flow through the device, and that, whilst membrane fouling may be occurring, similar staining and deposits were seen in Hemosep thus making it unlikely these would *completely* prevent haemoconcentration; a change of approach was required. It was decided that the exchange surface area would be increased in size to dimensions similar to that of Hemosep (two surfaces approximately 130x210 mm). In the initial development stages the proposed device test rig was designed to be small during proof of concept testing in order to make best use of the limited supplies of the costly polycarbonate membrane. It was thought that a 'scaled-down' prototype could be used in early lab development and subsequently 'scale-up' for later testing phases, however this proved not to be the case. A new test rig was designed and manufactured and the exchange surface area was increased from 80x100mm to 110x200mm (and later to 160x200mm). As before CFD analysis was performed to ensure good coverage of the exchange surface by the blood flow and no areas of high shear.

During the first experiment with the exchange surface successful haemoconcentration of 500ml flowing blood was achieved. Figure 121 shows the increase in Hct over time during this experiment which rose from 21.0%Hct to 33.5%Hct in 200mins (mean extraction rate 1.4 ml/min).

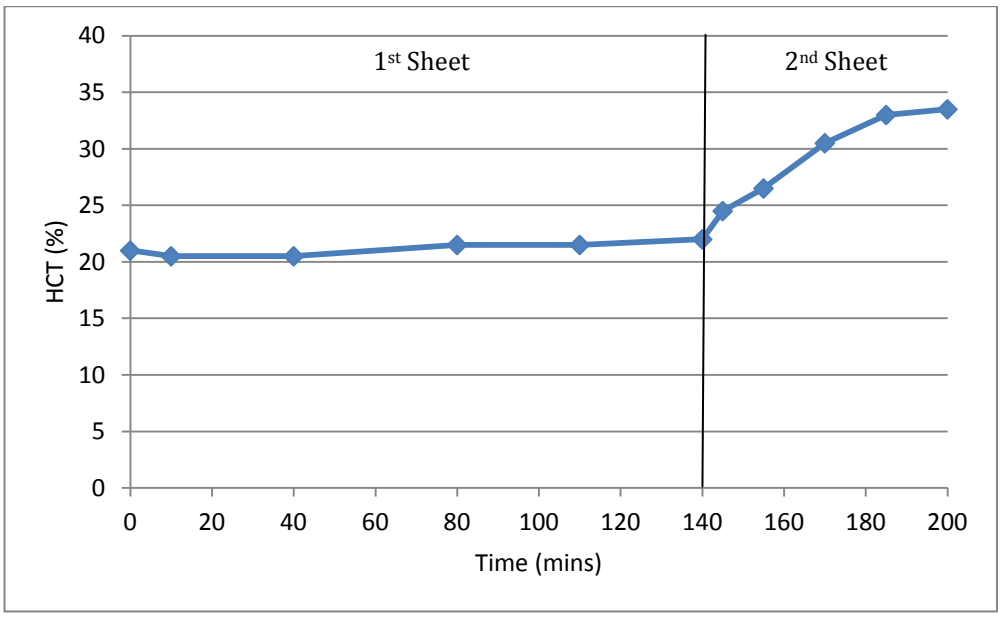


Figure 121: Change in Hct over duration of first experiment (n=1) with larger exchange surface. At 140mins the superabsorber sheet was replaced

There was an initial decrease in Hct, most likely due to the blood mixing with residual saline used to prime to membrane and superabsorber. After 140mins of a very slow rate of haemoconcentration, the superabsorber was changed to a fresh sheet and thereafter haemoconcentration occurred at a fast rate. A possible explanation for this is that there was a loss of contact between the superabsorber and the control membrane, a phenomenon which will be discussed later in this chapter (*3.6. Superabsorber Behaviour*). The superabsorber exhibited a change in colour which is indicative of the absorption of plasma as is shown in Figure 122.

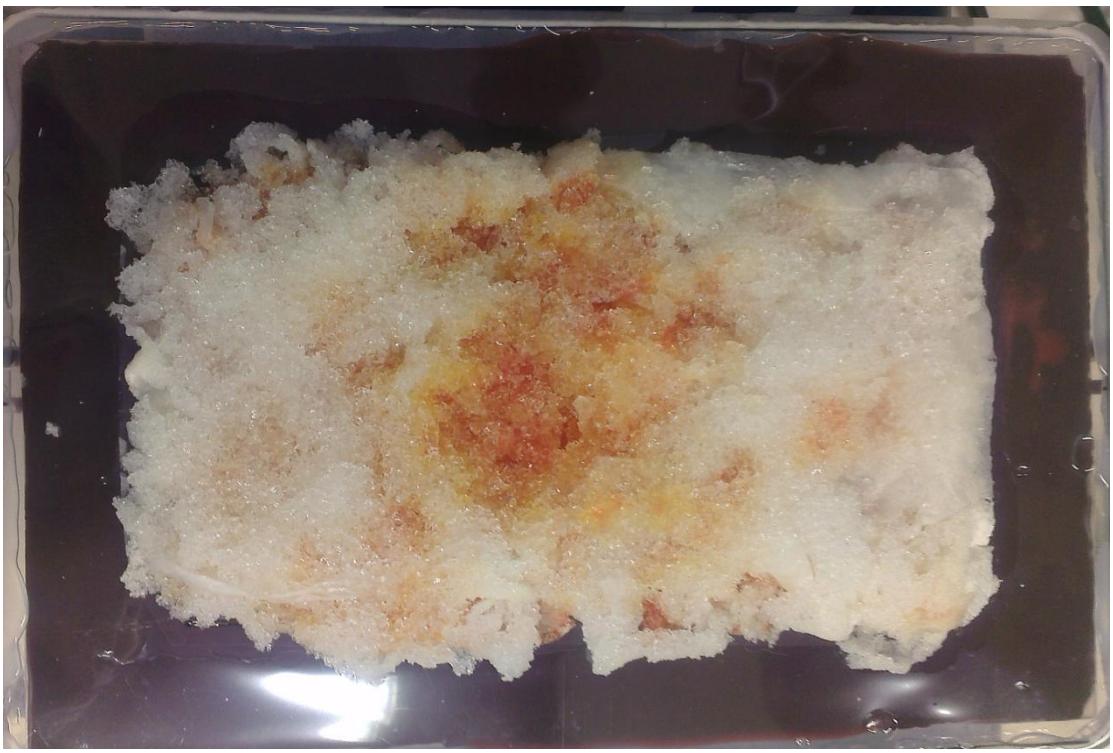


Figure 122: Colour change of the superabsorber indicating plasma absorption

The TMP pressure profile over the duration of the experiment is shown in Figure 123 and shown that the plasma transfer across the control membrane occurred at a low TMP.

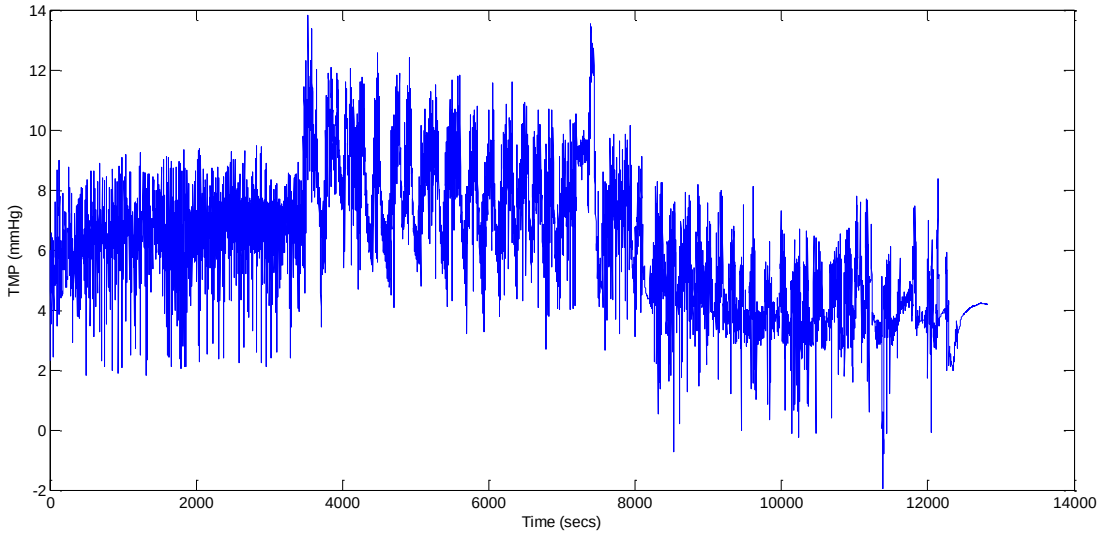


Figure 123: TMP profile for the duration of the experiment showing plasma transfer occurred at a low TMP

Interestingly, at the end of the experiment the control membrane exhibited the same staining as was seen previously (Figure 58) when the system failed to haemoconcentrate. This is shown in Figure 124, the degree of staining is somewhat exaggerated in this image as the test rig is yet to be disassembled.

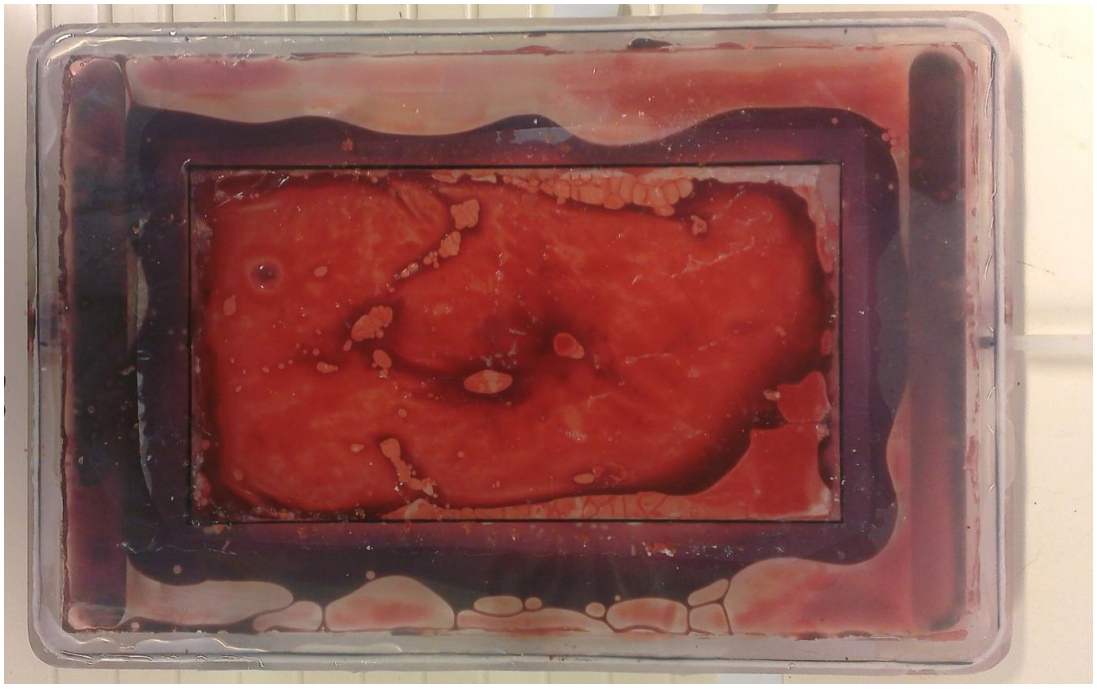


Figure 124: Membrane after successful haemoconcentration exhibits same staining as was seen previously

The presence of this staining confirmed the notion that the presence of the staining itself and the implied fouling was not sufficient to completely prevent haemoconcentration from occurring although it was still possible that it severely hindered plasma transfer. When the membrane was observed under the SEM after washing it showed much of the same blurry light-in-colour 'deposit' as can be seen in Figure 125.

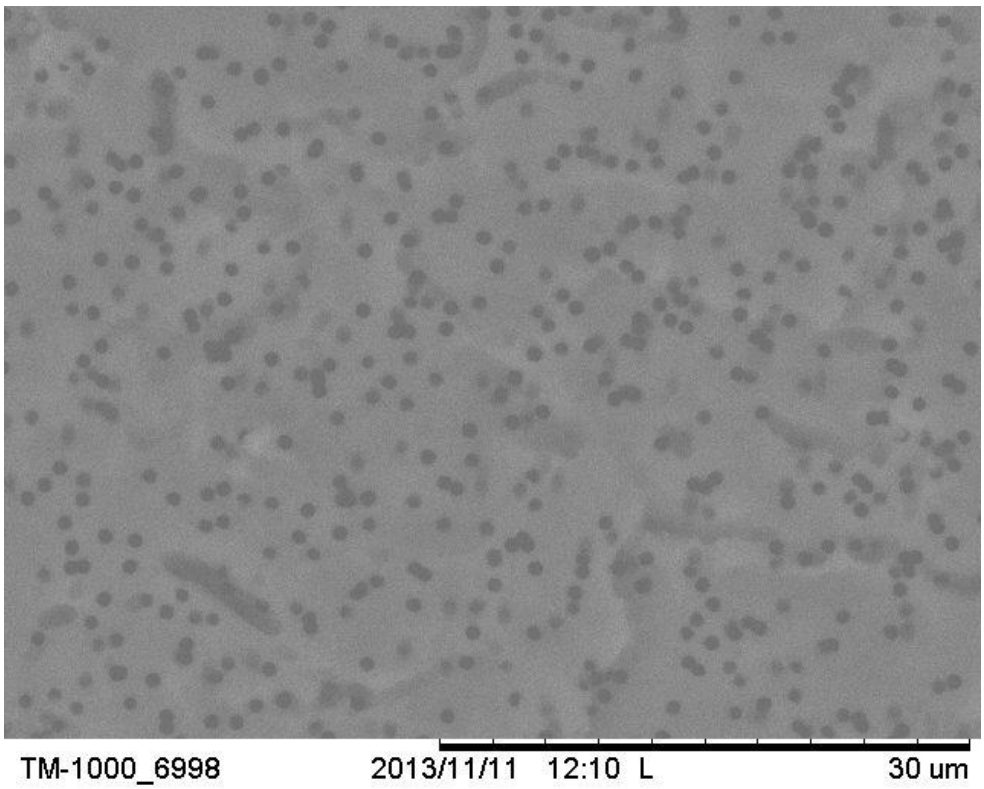


Figure 125: SEM image at x3000 magnification of membrane from first occurrence of successful haemoconcentration with the proposed device test system.

This 'deposit' had been seen previously (Figure 60) in membranes when haemoconcentration was unsuccessful and was deemed a potential indicator of protein deposit. If this were indeed the case, it would again suggest that the deposits observed previously were not *completely* preventing haemoconcentration, although they may still be greatly hindering it.

This initial result was a positive indication that haemoconcentration of flowing blood by this method could be achieved; however at this stage the rate of haemoconcentration was far slower than what would be expected of the proposed device.

During a repeat of this initial experiment an interesting observation was made. The experiment began with the superabsorber in place as usual, however after 150min with only a 1% increase in Hct the superabsorber was removed to determine if any plasma transfer could be achieved without it. The superabsorber sheet itself exhibited some signs of having absorbed a very small amount of plasma as is shown in Figure 126.

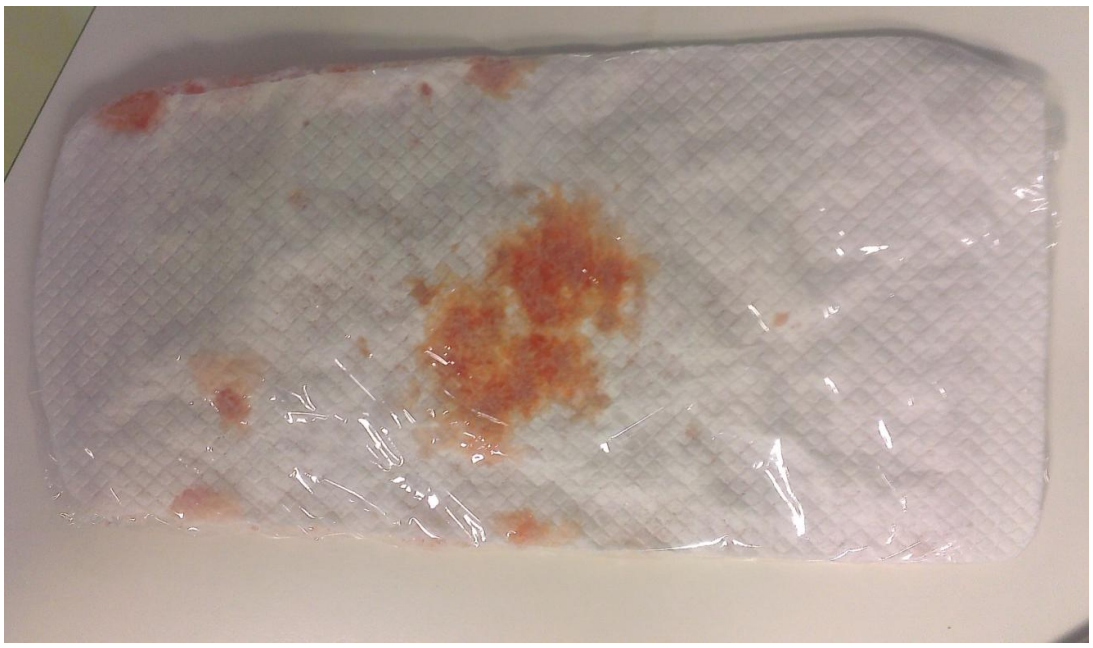


Figure 126: Superabsorber sheet showing signs of very limited plasma absorption

Upon inspection of the control membrane itself it could be seen that a very small number of small air bubbles were present at the membrane surface at that these bubbles moved in an orbital path with the motion of the orbital agitator. The blood which was in these pathways appeared different to the surrounding blood, which had a 'marbled' appearance, as it had a consistent solid colour, seemingly being 'stirred' by the motion of the bubbles. Additionally, the area of control membrane immediately above the bubble pathways on the open air side was wet with plasma, whereas the remaining areas of the membrane were largely dry. Each of these observations can be seen in Figure 127.

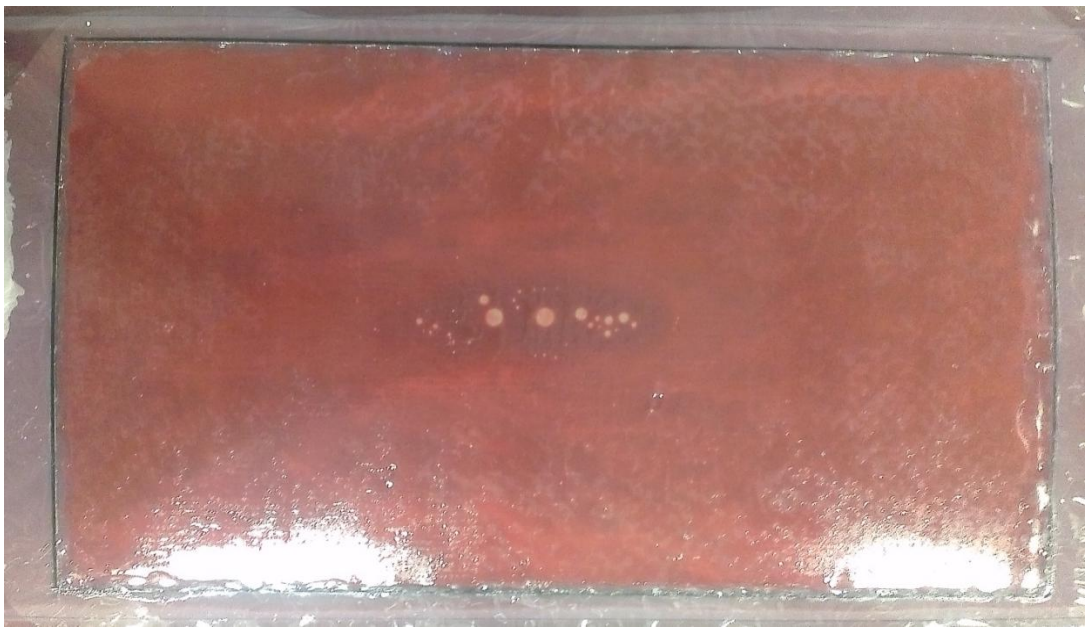


Figure 127: Open air side of the control membrane. The bubbles and their pathway can be seen as well as the 'stirred' section of the blood at the membrane surface. Additionally the difference in 'wetness' on the air side of the control membrane can be seen.

A paper towel was used to make a 'relief' of the wet and dry areas of the air side of the control membrane which would indicate in which areas of the exchange surface plasma transfer was occurring. Figure 128 shows the 'relief'.

Figure 128: Relief of the wet and dry areas of the air side of the control membrane which would indicate in which areas of the exchange surface plasma transfer was occurring, indicated by the yellow staining on the paper towel.

It can be seen from the relief that the area of bubble stirring was also the area of best plasma transfer, indicated by the yellow staining of the paper towel. The stirring of the blood by the bubbles appeared to improve conditions for plasma transfer, in order to further test this concept more bubbles were introduced into the blood channel. The larger number of bubbles increased the area of 'stirred' blood and, as could be seen from the subsequent relief taken, the area of exchange surface in which plasma transfer was occurring as can be seen in Figure 129 and Figure 130.

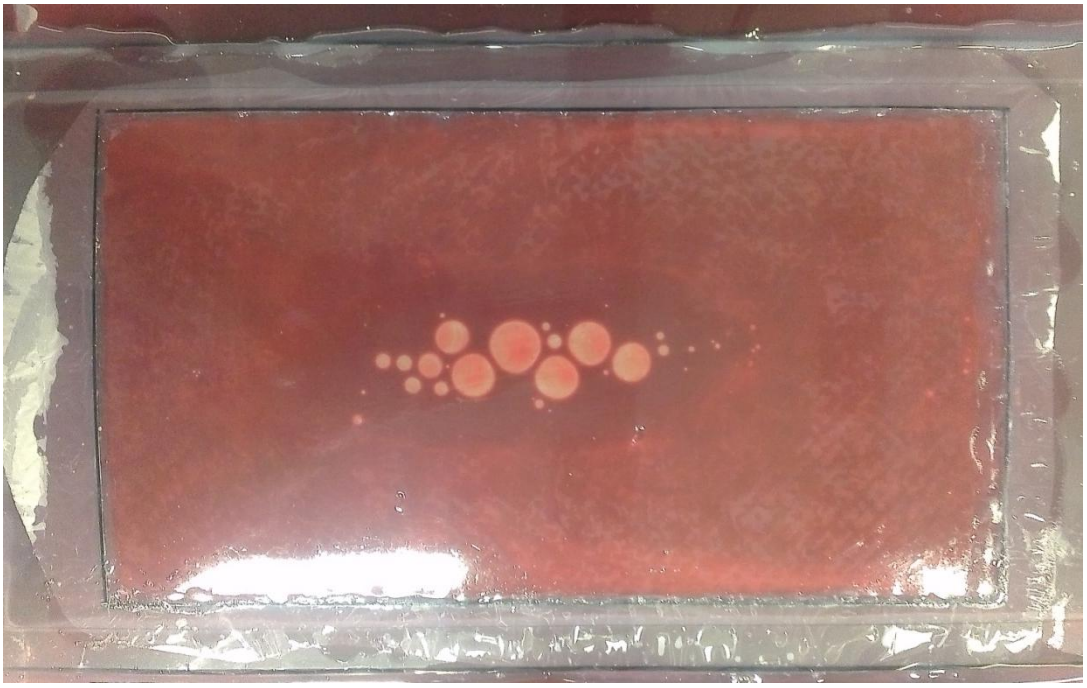


Figure 129: Open air side of the control membrane. An increased number of bubbles at the membrane surface resulting in a larger 'stirred' region.

Figure 130: Subsequent relief of the wet and dry areas of the air side of the control membrane showing an increased area of plasma transfer corresponding to the increased 'stirred' region as indicated by the larger area of yellow staining on the new piece of paper towel.

When this process was repeated the area of plasma transfer increased further as can be seen in Figure 131 and Figure 132.

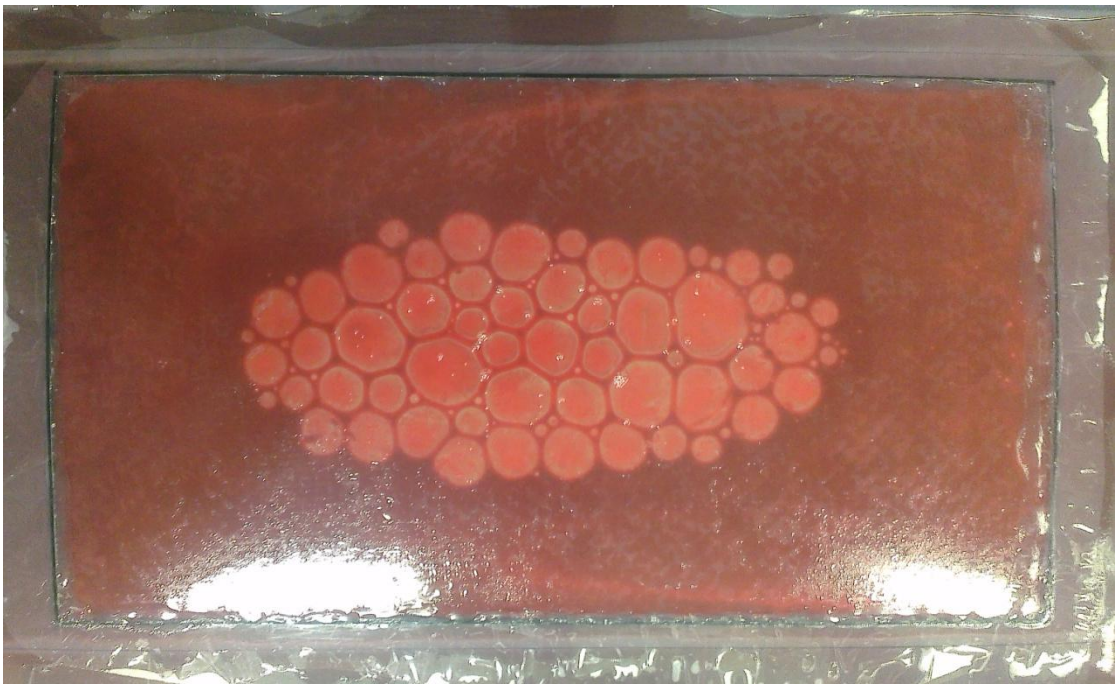


Figure 131: Open air side of the control membrane. A further increased number of bubbles at the membrane surface resulting in an even larger 'stirred' region.

Figure 132: Subsequent relief of the wet and dry areas of the air side of the control membrane showing a further increased area of plasma transfer corresponding to the further increased 'stirred' region as indicated by the larger area of yellow staining on the new piece of paper towel.

In order to further confirm this observation a subsequent experiment was conducted in which no superabsorber was used, but instead bubbles were allowed in the blood channel in order to determine if their presence would improve haemoconcentration rate if utilised from the outset. Any plasma which crossed the membrane would be left to sit on the membrane surface and periodically absorbed with a paper towel.

During the experiment a large amount of plasma was seen 'sitting' on the air side of the control membrane indicating a superior rate of plasma extraction to what had been observed previously with this system as can be seen in Figure 133.

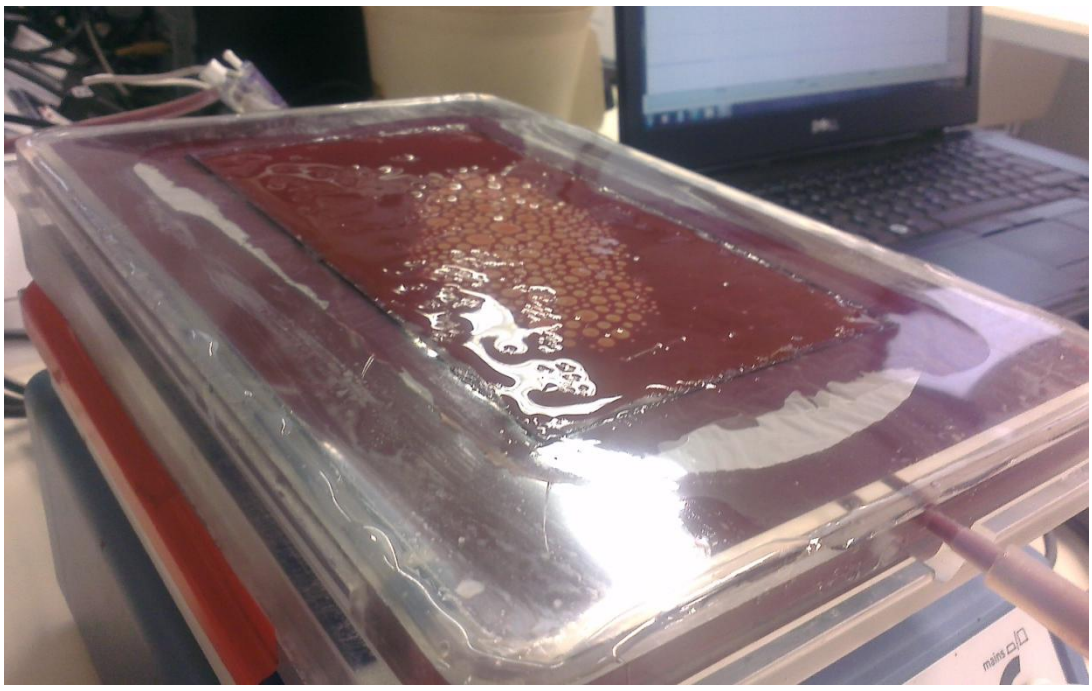


Figure 133: Plasma 'sitting' on the air side of the control membrane

When this plasma was extracted it was seen to be bright yellow in colour indicating very little to no haemolysis as is shown in Figure 134.

Figure 134: Bright yellow plasma recovered from the surface of the air side of the control membrane

Without the superabsorber in position the behaviour of the bubbles at the membrane surface could be observed clearly. The orbital motion of the agitator appeared to have two effects on the bubbles at the membrane surface. Firstly it induced the orbital motion of the bubbles which acted to stir the blood and appeared to improve plasma transfer, and secondly, during agitation the bubbles appeared to sink below the blood surface into the middle of the channel depth.

When there was no agitation the bubbles rose to, and came in contact with, the membrane surface as can be seen in Figure 135.

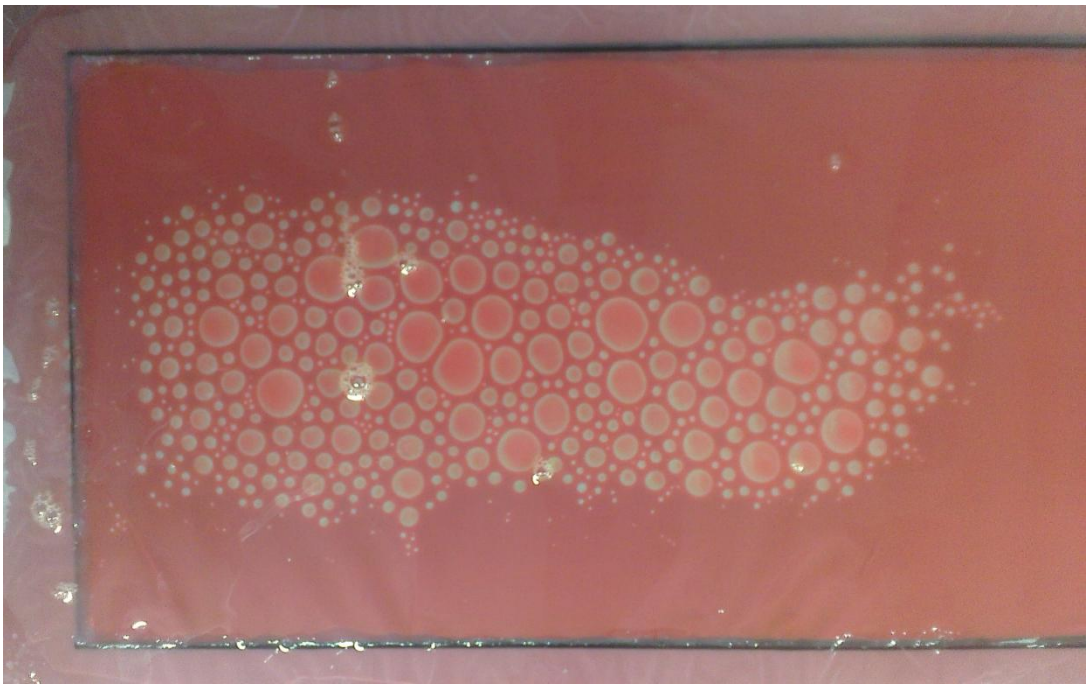


Figure 135: With no agitation the buoyant bubble rise to, and come in contact with, the membrane surface.

However, during agitation the bubbles appeared to sink below the blood surface into the middle of the channel depth, indicated in Figure 136 by their more reddish colour.

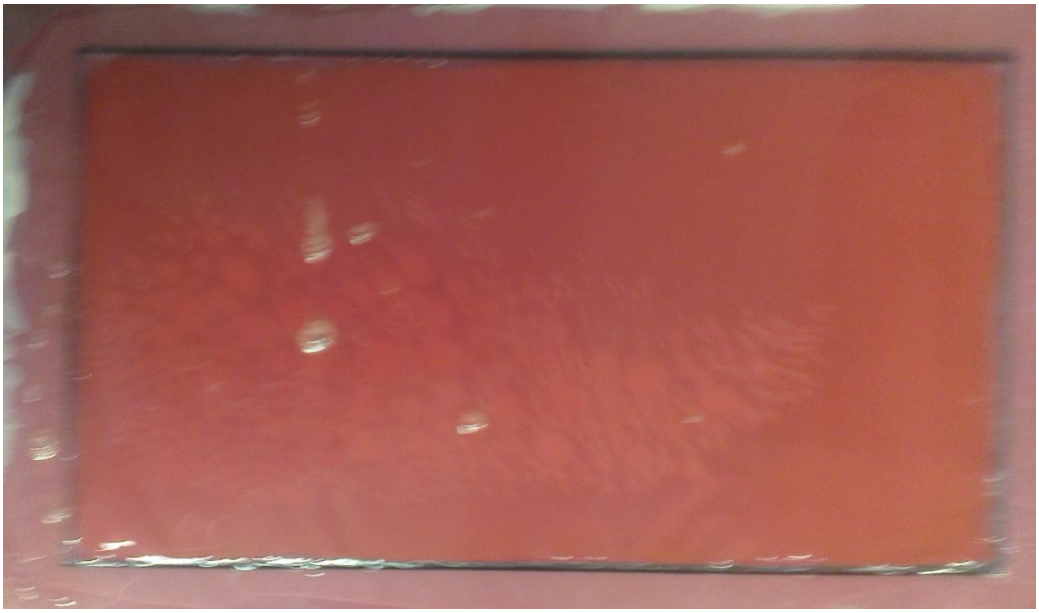


Figure 136: During agitation the bubbles appear to sink below the blood surface into the middle of the channel depth, indicated by their more reddish colour

Utilising this surface bubble mixing phenomenon resulted in a much improved rate of haemoconcentration as can be seen in Figure 137 where 500ml of bovine blood rose from 19.0% Hct to 36.0% Hct in 100mins (mean extraction rate 2.6ml/min).

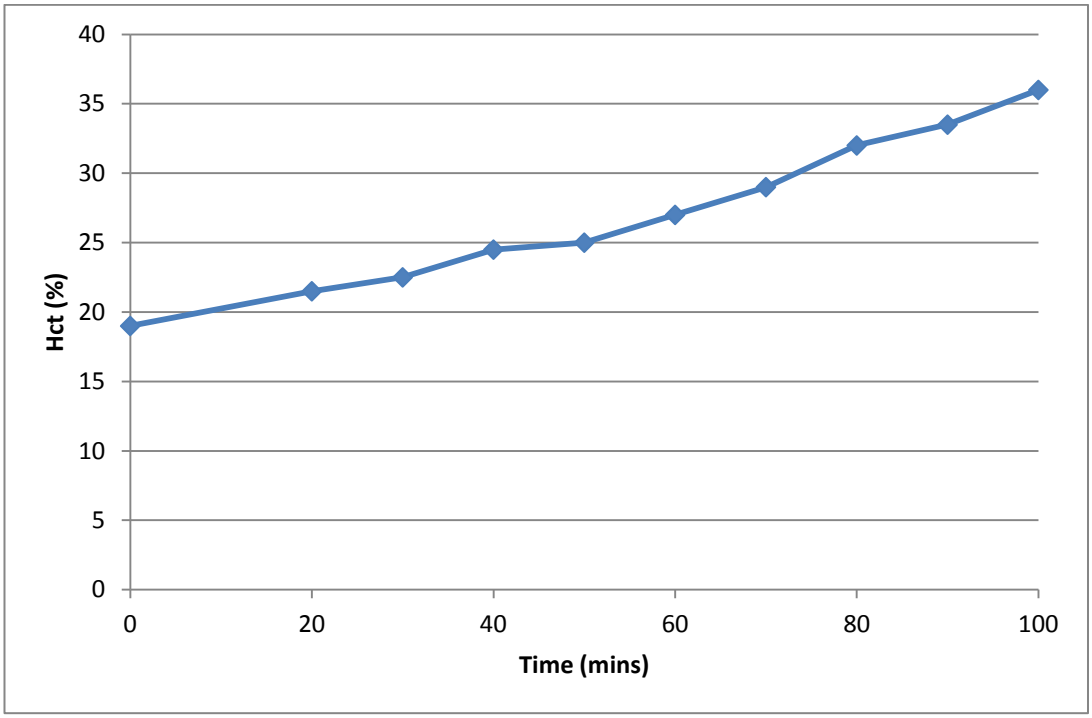


Figure 137: Rise in Hct over the duration of the experiment when utilising surface bubble mixing without superabsorber (n=1)

Additionally, at the end of the experiment there appeared to be less staining of the membrane than was seen previously (Figure 124). This is shown in Figure 138, again, the degree of staining is somewhat exaggerated in this image as the rig is yet to be disassembled.

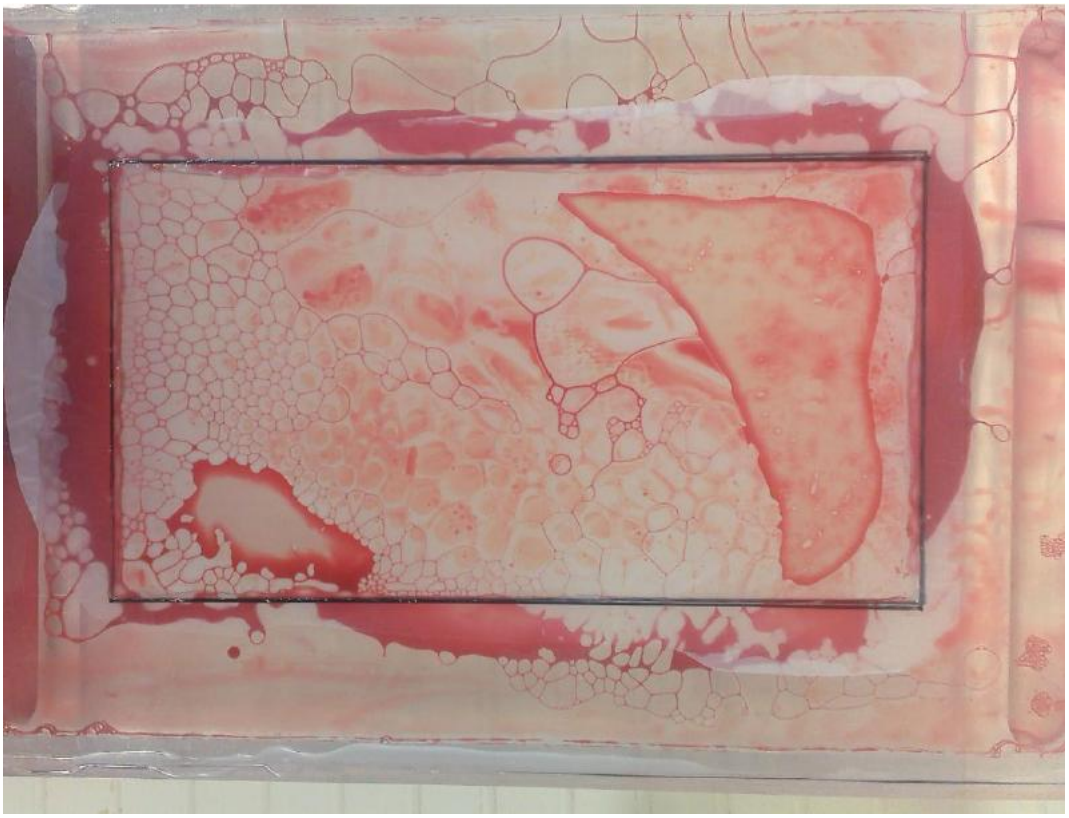


Figure 138: Appeared to be reduced staining of control membrane when utilising surface bubble mixing

Again, as is shown Figure 139, the plasma transfer occurred at very low TMP.

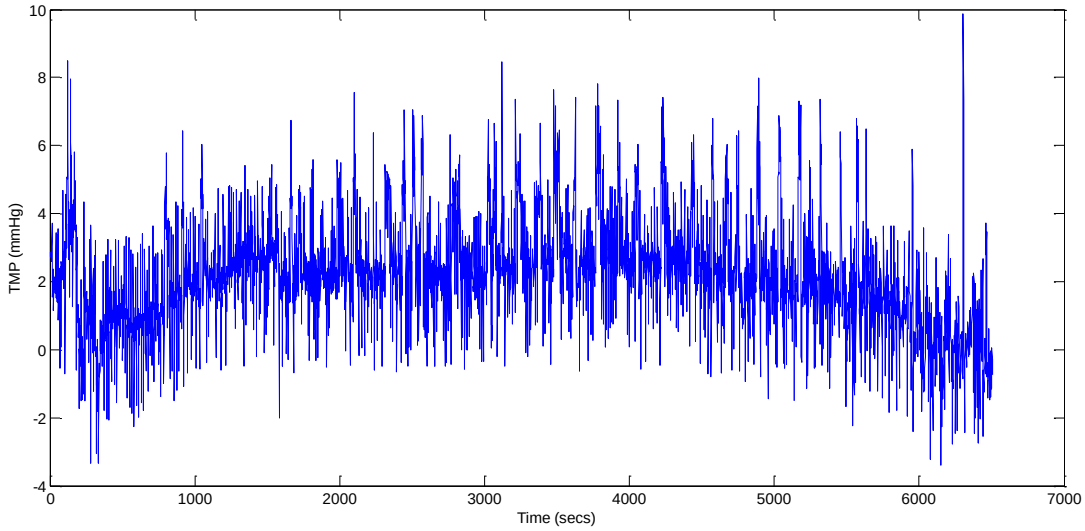


Figure 139: TMP profile over duration of the experiment when utilising surface bubble mixing.

The motion of the bubbles at the membrane surface appears to locally improve plasma transfer across the control membrane. It has been seen previously in this chapter that the development of a concentration polarisation layer at the membrane surface and subsequent fouling can

reduce the performance of filtration systems (*3.2.3. Membrane fouling*). It is possible therefore that both the increase in rate of haemoconcentration and reduction in membrane staining observed could be the result of a reduction in membrane fouling. The observed surface bubble mixing could therefore be a means of controlling the development of a concentration polarisation layer and membrane fouling. Direct observation of the occurrence or prevention of these phenomena is extremely difficult to achieve and thus in order to better ascertain the likelihood that surface bubble mixing is indeed means of fouling control, it is important to understand the nature of other fouling control strategies to identify possible similarities in mechanism.

3.4.2. Fouling Control Strategies

Previously in this chapter the concepts of concentration polarisation and membrane fouling, the impact they can have on haemofiltration, and the importance of controlling these phenomena were discussed at length (*3.2.3. Membrane fouling*). The various methods employed to prevent or reduce these phenomena were alluded to and will now be discussed in detail. It was mentioned that flow manipulation strategies (such as increased flow rates, backflushing, pulsatile and reversed flow) are often employed to control concentration polarisation and membrane fouling, and additionally how some groups have sought to exploit secondary flow, flow instabilities or vortices to reduce the concentration polarisation layer and increase mixing with the bulk solution (Kroner et al. 1988; Moulin et al. 1996; Mallubhotla et al. 1997; Hilal et al. 2005; Jaffrin 2012).

As mentioned previously (*3.3.2. Effect of flow on transfer rate*), improvement of hydrodynamic conditions (i.e. shear stress and cross-flow velocity) at the membrane surface is one important fouling control strategy and has been applied to the filtration of blood (Jaffrin 1989; Mohanty et al. 2011; Jaffrin 2012). Improvement of these hydrodynamic conditions leads to an increase in turbulence and a weakening of concentration polarisation, resulting in improved mass transfer (Mohanty et al. 2011; Jaffrin 2012). Several groups have highlighted the importance of shear in controlling concentration polarisation and removing the fouling cake layer in order to sustain a permeate flux (Jaffrin 1989; Bellhouse et al. 1994; Valette et al. 1999; Hong et al. 2002; Cui et al. 2003; Jaffrin 2008). Vassiliev et al were one of the first groups to postulate that the growth of particle-rich layer at the membrane surface is controlled by a shear induced erosion mechanism and it was later observed that plasma filtration rate is proportional to wall shear rate (Jaffrin et al. 1992). Bian et al found that in the nano- and micro-filtration of river water, concentration polarisation and membrane fouling can be controlled by increasing shear rate which induces transport of fouling particles away from the membrane surface; larger particles by diffusion, and smaller particles by diffusion and Brownian motion (Bian et al. 2000).

3.4.2.1. Membrane Surface Modifications

One approach to controlling membrane fouling is to make modifications to the membrane surface (Hilal et al. 2005). Surface modifications are generally used to prevent adhesive fouling (Hilal et al. 2005). The fact that hydrophilic membranes are less susceptible to fouling has been discussed previously (*2.2.4. Membrane materials*) and consequently many research groups have sought to impart hydrophilic properties in membranes to reduce fouling (Courtney et al. 1996; Mockel et al. 1999; Hilal et al. 2005; Xu et al. 2009; Hollinger 2011; Su et al. 2011). Surface modifications allow for changes to be made to the surface properties of a given membrane without severely altering its porous structure (Hilal et al. 2005). There are two techniques of surface modification; coating, which involves dipping the membrane in a solution containing antifouling polymers, and grafting, which involves covalently bonding hydrophilic species to the membrane surface (Wang et al. 2002). However, the coatings and grafts are susceptible to erosion which diminished reliability and durability, moreover these processes are time consuming and add expense to the system (Park 2002; Wang et al. 2002).

3.4.2.2. Additional Force Fields

A number of alternate methods of fouling control rely on charge interactions between the solutes and the membrane (Hilal et al. 2005). Electroultrafiltration involves the application of an electric field to impose an electrophoretic force on particles and limit their accumulation on the membrane surface (Huotari et al. 1999; Iritani et al. 2000; Wakeman et al. 2002). Sarkar et al investigated the effect of an applied DC field in protein fractionation of BSA and lysozyme and found it significantly improved permeate flux (Sarkar et al. 2009). Ultrasonic fields as a method for fouling control have also been investigated and utilise ultrasonic waves to disperse particles, although flux increases by this method have been found to be minimal and some groups have reported membrane damage as a result of ultrasound (Wakeman et al. 2002; Kyllonen et al. 2005).

As mentioned previously (*3.3.2. Effect of flow on transfer rate*), a major method of controlling membrane fouling is flow manipulation which includes; inserts, turbulence promoters, increased flow rates, mixers, backflushing, pulsing, backflushing, high shear, pulsatile and reversal flow, vortex generation, gas sparging and use of force fields (Winzeler et al. 1993).

3.4.2.3. Backflushing/Backpulsing

Backflushing (also termed backwashing) is a common method of fouling control used in cross-flow filtration processes in which filtrate is periodically pumped back through the membrane to the feed side to lift deposits from the membrane surface which are then swept away by the cross-flow (Hilal et al. 2005; Jaffrin 2012). It is particularly effective with strongly adhered deposits and pore-fouling (Wakeman et al. 2002). It is necessary for the backflush pressure to be greater than the normal operating TMP. If backflushing is performed at fast rate it is often called backpulsing and is continual cyclical forward and reverse filtration. Backpulsing is particularly effective for filtration of colloidal suspensions and protein filtration applications (Wenten 1995; Hilal et al. 2005). Several groups have studied the use of backpulsing and found it to be an effective method of fouling control, including in cross-flow microfiltration systems (Rodgers et al. 1992; Redkar et al. 1995; Wenten 1995). Ma et al found that twice as much filtrate could be extracted when using water or gas backpulsing, however, after a long period backpulsed membranes performed worse due to the fact that, whilst backpulsing effective removed external fouling, it actually exacerbated internal fouling (Ma et al. 2001).

Backflushing/backpulsing would not be particularly suitable for use in the proposed device for two reasons; firstly, the nature of the superabsorber means that any fluid which crosses the membrane will be absorbed by it and thus there would be little to no fluid available for backflushing, and secondly, if any fluid that had only just crossed the membrane and was yet to be absorbed were backflushed to the blood side of the membrane there is a risk of carrying harmful substances from the superabsorber into the blood.

3.4.2.4. Pulsatile Flow

Pulsatile flow of the feed fluid encourages mixing and has been shown to improve performance of both tubular and flat sheet membranes (Jaffrin 2012). In certain applications the filtration flux has been reported at up to seven times greater when utilising pulsatile flow (Howell et al. 1993). The use of pulsatile blood flow in plasmapheresis was first proposed by Galetti et al, who found a 50-100% improvement in filtration rate compared to steady flow in a single layer flat-sheet polycarbonate membrane similar to that to be used in the proposed device (Galletti et al. 1983). Similarly, Jaffrin et al found a 50% improvement in filtrate output with pulsatile flow in a hollow fibre filter (Jaffrin 1989). The improvement in plasma filtration rate found by Jaffrin et al is shown in Figure 140.

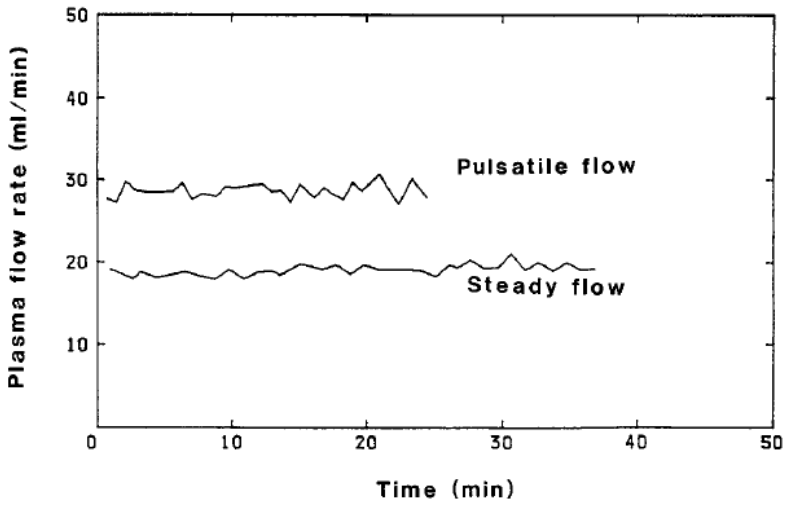


Figure 140: The beneficial impact of pulsatile flow on plasma filtration rate in a hollow fibre filtration system at 2Hz using a 0.1m² polypropylene hollow fibre for bovine blood in acid citrate dextrose, inlet blood flow = 80ml/min, Hct = 38%, ΔV = 1.5ml, P_{OUT} = 20mmHg (Jaffrin 1989)

Jaffrin et al suggest that the acceleration and deceleration of flow causes a tumbling motion in the RBCs which assists mixing and filtrate flux.

A roller pump from a Stockert pulsatile roller pump system *(Stockert, Munich, Germany)* without the control system will be used in the testing procedures in this study and consequently oscillatory, not pulsatile flow, will be induced. A comparison between the blood pressure profiles from the radial artery of a patient undergoing open heart surgery generated by a roller pump (A) and the heart (B) is shown in Figure 141.

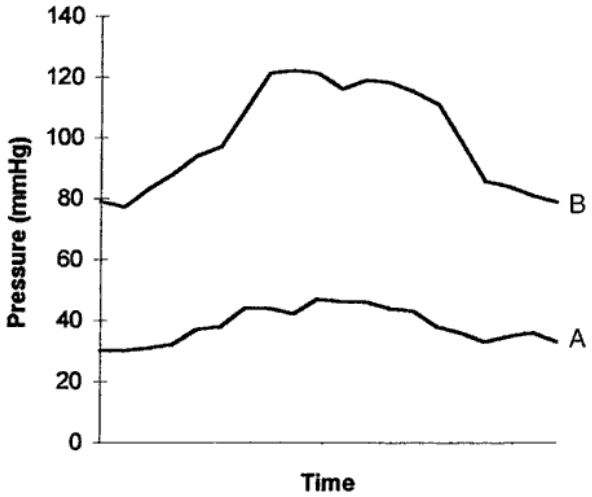


Figure 141: Comparison between the blood pressure profiles from the radial artery of a patient undergoing open heart surgery generated by a roller pump (A) and the heart (B) (Gourlay et al. 2000)

However due to the difficulties of inducing pulsatile flow artificially it is unlikely that true physiological pulsatile flow was used in aforementioned papers which reported its benefits to filtration (Gourlay et al. 2000). The physiological importance of pulsatile flow is well recognised but its use in CPB remains controversial due to a range of reported benefits and disadvantages confounded by the technical difficulty in actually reproducing true physiological pulsatile flow (Hornick et al. 1997; Gourlay et al. 2000). Therefore it is unclear if it is reasonable to expect that the proposed device would typically be implemented in a MUF circuit utilising pulsatile flow.

3.4.2.5. Turbulence

As mentioned previously (*3.3.2.2. Turbulence and Vortices*), inducing turbulence is a common technique for reducing fouling at the membrane surface. Inserts are perhaps the most basic turbulence promoter and work by disrupting the flow path and causing instabilities and vortices which promote fluid mixing, minimised the effects of concentration polarisation and increase mass transfer away from the membrane surface to the bulk (Thomas 1973; Jeffree et al. 1981; Ahmad et al. 2005; Hilal et al. 2005). For example, in tubular membrane systems, baffles such as an axial corkscrew rod will introduce the secondary flow and fluid instabilities have been used to create turbulence (Gupta et al. 1995; Ahmad et al. 2005). Additionally, turbulence promoters have been shown to enhance mass transfer from the membrane surface back to the bulk solution, again leading to an increase in filtrate flux, as well as reducing concentration polarisation (Auddy et al. 2004). These turbulence promoters rely to some extent on the feed fluid being pumped at a high velocity to induce the shear stresses required to control fouling, but a similar effect can be achieved by rotating the membrane surface at high speed (Hilal et al. 2005). Subsequently, a wide range of rotating disk and vibrating membrane systems have emerged for use in a variety of applications and have demonstrated good performance (Jaffrin 2008). The *Plasmacell* system from *Hemascience (Santa Ana CA, USA)*, and later commercialised by *Baxter (Deerfield, IL, USA)*, developed a disposable haemofiltration device incorporating a single rotating tubular polycarbonate membrane which utilised the combination of the centrifugal force pulling formed elements away from the membrane, and the high shear rates and vortices produced, to generate a high flux per unit area (Jaffrin 1989). Schooneman et al found the system could extract 600ml of cell-free plasma in 30mins without haemolysis or complement activation (Schooneman et al. 1986; Jaffrin 2008). It was later investigated in-vitro by Beaudoin and Jaffrin who found the system could extract plasma at a high rate even at low TMP (Beaudoin et al. 1986; Jaffrin 2008).

As discussed previously (*3.3.2.2. Turbulence and Vortices*), Bellhouse et al utilised pulsatile flow coupled with dimpling on the surface of a flat membrane to develop vortices at the membrane

surface and reported a filtration velocity of 1.75L/min/m^2, five times higher than hollow-fibre or flat bed membrane filters at the time (Bellhouse et al. 1988).

In the early development stages a flow disrupting system was tested in the proposed device and consisted of a series of parallel 'ladder-like' cellulose acetate baffles adjacent to the membrane surface as shown in Figure 142.

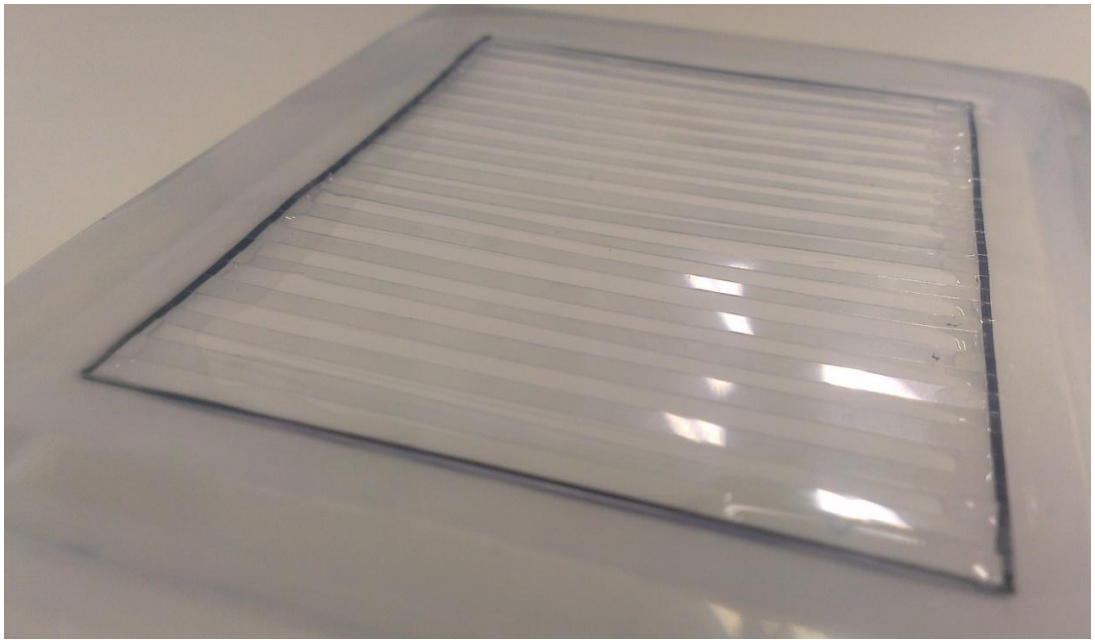


Figure 142: Ladder-like cellulose acetate baffles adjacent to the membrane surface to act as turbulence promoters

Although the system failed to haemoconcentrate and there was a small leak in the system, there was evidence of good plasma transfer indicated by the bright yellow plasma absorbed by the superabsorber sheet as is shown in Figure 143.

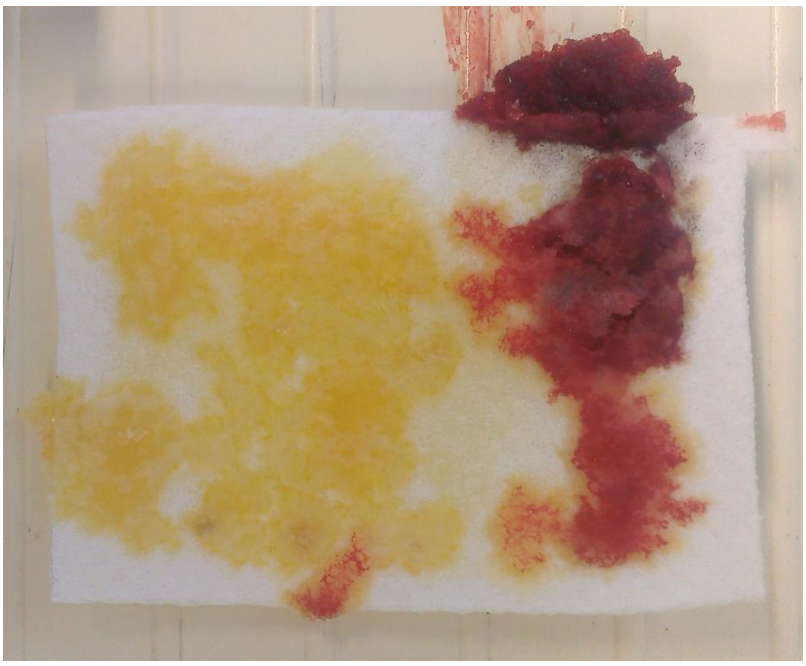


Figure 143: Bright yellow plasma absorbed by superabsorber in system with turbulence promoters suggesting that some good plasma exchange was achieved. The dark red colour of the superabsorber was from blood absorbed as a result of a small leak in the system.

The impact of turbulence upon haemolysis has been discussed previously (*3.3.3. Effect of flow on blood*). If turbulence is used as a fouling control strategy to improve filtration flux then care must be taken to avoid doing so at the expense of adversely affecting the blood quality by inducing haemolysis.

3.4.2.6. Gas Sparging

Gas-sparging, in which gas bubbles are injected into the feed flow to create an intermittent gas/liquid two-phase flow and scour away deposits and reduce concentration polarisation, is a technique which has been shown to significantly improve the performance of some membrane processes due to the beneficial effect it has on controlling concentration polarisation and cake deposition (Cui et al. 1996; Cui et al. 2003; Gupta et al. 2005; Hilal et al. 2005; Tiranuntakul 2011). The motion of the bubbles can generate a variety of flow patterns which can be manipulated to control fouling, and are dependent upon on several factors including the distribution of the gas phase, bubble size, and distance between the bubbles, which in turn are dependent upon the ratio of gas and liquid flow rates and the module geometry (Mohanty et al. 2011).

Gas sparging in pipes can take two broad forms depending on the 'void fraction' in the pipe which, in turn, is directly dependent upon the gas and liquid phase velocities: bubble flow, in

which air bubbles are dispersed in the liquid phase, and slug flow, in which a 'slug' of gas intermittently flows through the pipe (Cabassud et al. 2001; Chang et al. 2002; Mohanty et al. 2011). Slug flow induced shear stress which removes the fouling layer, and has been shown to be the more effective technique in tubular membrane systems (Mercier et al. 1997; Li et al. 1997a; Psoch et al. 2005). Additionally, slug flow induces turbulence which strongly reduces the concentration polarisation and can, to some extent, remove foulant from the membrane itself (Bellara et al. 1996; Ducom et al. 2003; Mohanty et al. 2011).

In gas sparging and air scouring systems, the several mechanisms are known to be involved in increasing filtration flux, but their relative contribution to the overall effect depends upon the configuration of the individual membrane and system in question. The first mechanism is the generation of secondary flow and wakes by movement of bubbles which disrupt the boundary layer as well as promote local mixing at the membrane surface. Another mechanism is the physical displacement of concentration polarisation layer by the gas slugs themselves as they penetrate the concentration polarisation layer, as demonstrated by Bellara et al who observed that in hollow fibre membrane systems the thickness of the film separating the bubbles from the membrane wall during sparging (<10um) was less than the thickness of the concentration polarisation layer in single phase flow (10-20um). A third mechanism is pressure pulsing as a consequence of the movement of gas slugs which is similar to the effect generated by pulsatile flow but may also increase the mean pressure in the system, and a further mechanism is the increased superficial cross-flow velocity induced by high gas flow rates (Bellara et al. 1996; Cui et al. 1996).

Cui and Wright investigated the use of gas sparging to break up the cake layer on the membrane surface and prevent concentration polarisation in a cross-flow tubular membrane system and found a 320% increase in flux (Cui et al. 1996). The flux improvement was more pronounced with severe concentration polarisation and Cui and Wright suggest that the bubbles in the system could be used as stationary or slow-moving baffles (Cui et al. 1996). However, Wakeman et al suggest that gas sparging is not as effective as using turbulence promoters due to the problem of handling the gas (Wakeman et al. 2002). The effectiveness of gas sparging in flat sheet membrane modules has been investigated. Mercier-Bonin et al found that gas sparging doubled permeate flux in yeast microfiltration with flat-sheet ceramic membranes, but perhaps more importantly also found that the improvement was more pronounced in horizontal membrane modules such as the one used in testing of the proposed device (Mercier-Bonin et al. 2000). The reason for this may be that the bubbles are more evenly distributed in horizontal systems which do not rely so much on well designed preferential flow paths for bubbles (Cui et al. 2003). Li et al found that gas sparging reduced concentration polarisation and increased permeate flux by up to 50% in a flat sheet membrane module used to filter protein solutions

including HSA, BSA, immunoglobulin, and lysozyme (Li et al. 1998). Ghosh et al found the gas sparging increase permeate flux and enhance protein fractionation of BSA and lysozyme by ultrafiltration in flat sheet polysulfone membrane modules even at low TMP (50kPa) (Ghosh et al. 1998). The ability of Ghosh et al to fractionate two similarly sized proteins demonstrates why protein fractionation of a protein mixture is an example of a filtration technique which benefits greatly from controlling concentration polarisation through improving hydrodynamics at the membrane surface (e.g. through gas sparging), and moreover, protein fractionation is very similar in this regard to haemofiltration (Cui et al. 2003). However, as will be discussed later (*3.4.3.4. Potential problems with bubble mixing*), the benefits of gas sparging in protein fractionation must be weighed against the potential damage to proteins inflicted by the gas-liquid environment (Cui et al. 2003).

Recently, CFD analysis has been used to better assess the mechanism and impact of gas-sparging fouling control strategies (Ahmad et al. 2005; Li et al. 2006; Mohanty et al. 2011). Ndinisa et al assessed gas-sparging in a flat-bed membrane system using CFD and found that the increased flux seen with increased bubble size was due to the an increase in overall shear stress and turbulence at the membrane surface, whilst Rios et al found that increased gas velocity resulted in a broader stress distribution as well as increased shear stress (Ndinisa et al. 2006b; Ratkovich et al. 2009; Mohanty et al. 2011).

3.4.2.7. Air Scouring and Bubbling

For membrane filter modules submerged in the feed solution, another method for control fouling is air scouring, in which an uplifting flow of coarse air bubbles (2-5mm diameter) is supplied by diffusers at the bottom of the membrane which disrupt flow and induce shear at the membrane surface leading to improved filtration flux (Bellhouse et al. 1994; Ueda et al. 1997; Bouhabila et al. 1998; Chua et al. 2002; Hong et al. 2002; Wicaksana et al. 2006; Tiranuntakul 2011). Air scouring largely works by the same mechanisms as gas sparging.

Prieske et al found that larger bubbles are more efficient at scouring the membrane surface since they result in higher lift and drag forces on the membrane (Prieske et al. 2008), whilst Ndinisa et al report that fouling reduction by air scouring increases with increased flow below a certain threshold (Ndinisa et al. 2006a). It has also been suggested that, under certain conditions, intermittent flow results in better fouling control than continuous filtration, as well as reducing energy requirements (Mohanty et al. 2011). Additionally, back-transport of fouling particles into the bulk phase is reported to be enhanced by air scouring (EUROMBRA 2006).

3.4.2.8. Dispersed phase

Utilising solid media in place of bubbles as a form of two-phase flow to disrupt the boundary layer have been studied by several groups and a patent on the use of micro-scale particles to control concentration polarisation was published as early as 1970 by Bixler and Rappe (Bixler et al. 1970; Rios et al. 1987; Parvatiyar et al. 1995; Parvatiyar 1996; Kim et al. 2002; Cui et al. 2003; Hilal et al. 2005).

Parvatiyar et al and Rios et al both investigated the use of a 'dispersed phase' to disrupt concentration polarisation and it was found that the 'dispersed phase' acted as a turbulence promoter and could control the formation and size of eddies as well as the rate of energy dissipation in the fluid which both impact mass transfer (Rios et al. 1987; Parvatiyar et al. 1995; Parvatiyar 1996). Parvatiyar et al and Rios et al both utilised stainless steel balls, diameter 3mm, as a means of fouling control in the filtration of a gelatin solution with Rios et al reporting significantly increased permeate flux (Rios et al. 1987; Parvatiyar 1996). As Figure 144 shows, Parvatiyar and Govind demonstrated that the use of a dispersed phase is very effective at reducing concentration polarisation, particularly at low Reynold's number (Parvatiyar et al. 1995).

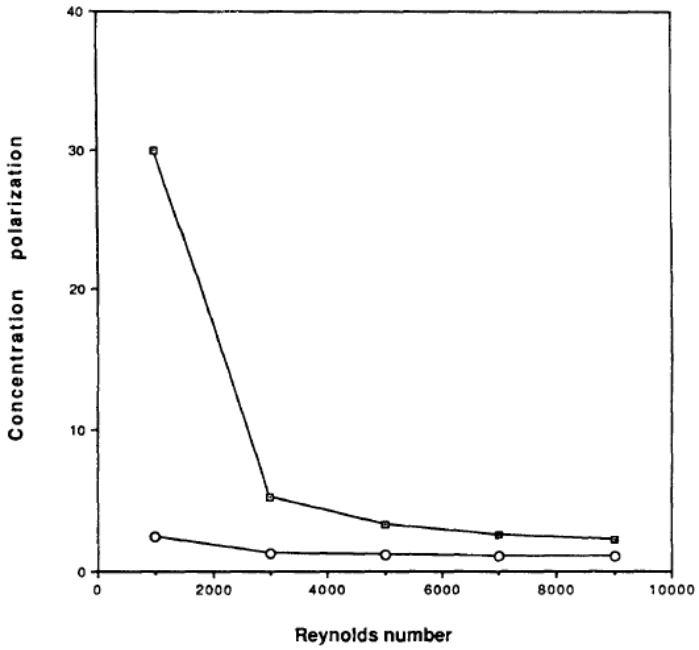


Fig. 4. Variation of concentration polarization with Reynolds number. $\phi = 0.5$, $d/D = 0.5$, $Pe = 200$, $W = 1$: ○, with dispersed phase; □, without dispersed phase.

Figure 144: The beneficial impact of a dispersed phase on concentration polarisation (Parvatiyar et al. 1995)

As Hilal et al state "Concentration polarization in a membrane tube can be controlled by the placement of inert solid particles as a dispersed phase in UF experiments" (Hilal et al. 2005).

Kim et al utilised 4mm diameter glass balls to disrupt the concentration polarisation and reduce fouling in a rotating flat-sheet membrane system which resulted in a significant filtrate flux increase (Kim et al. 2002).

Basu and Huck studied the use of 10mm diameter polyethylene media for fouling control in a hollow fibre membrane system for biofiltration of organic carbon and found that the fouling rate was reduced by half with the support media (Basu et al. 2005). Siembida et al investigated the use of 2.5-3mm diameter polypropylene granular material for fouling control in a membrane bioreactor and found that filtrate flux increased by 20% and fouling was significantly reduced which prolonged the uninterrupted operation of the membrane since the need for chemical cleaning was reduced (Siembida et al. 2010). Similarly, Johir et al found that the use of 300-600µm activated carbon in a flat sheet membrane system prevented the sudden rise in TMP seen with membrane fouling *"by producing extra shearing effect on to membrane surface and reducing deposition of particles on to membrane surface by scouring"* (Johir et al. 2011). Pradhan et al combined gas sparging and the use of a solid granular medium to mechanically disrupt the fouling layer in a submerged vertical flat sheet membrane system and found that with gas sparging alone fouling reduction increased with increased flow rate, but that the addition of the granular medium allow the system to achieve the same fouling reduction at lower flow rates (Pradhan et al. 2012).

However, solids have the potential to damage the membrane, whilst gas bubbles pose lesser risks and can be more easily separated from the feed stream and consequently are described by Hilal et al as "ideal as a second phase" (Cui et al. 2003; Basu et al. 2005; Hilal et al. 2005; Siembida et al. 2010). The SEM images in Figure 145 from Siembida et al illustrates the damage that can occur to membranes with long term solid material fouling control (Siembida et al. 2010).

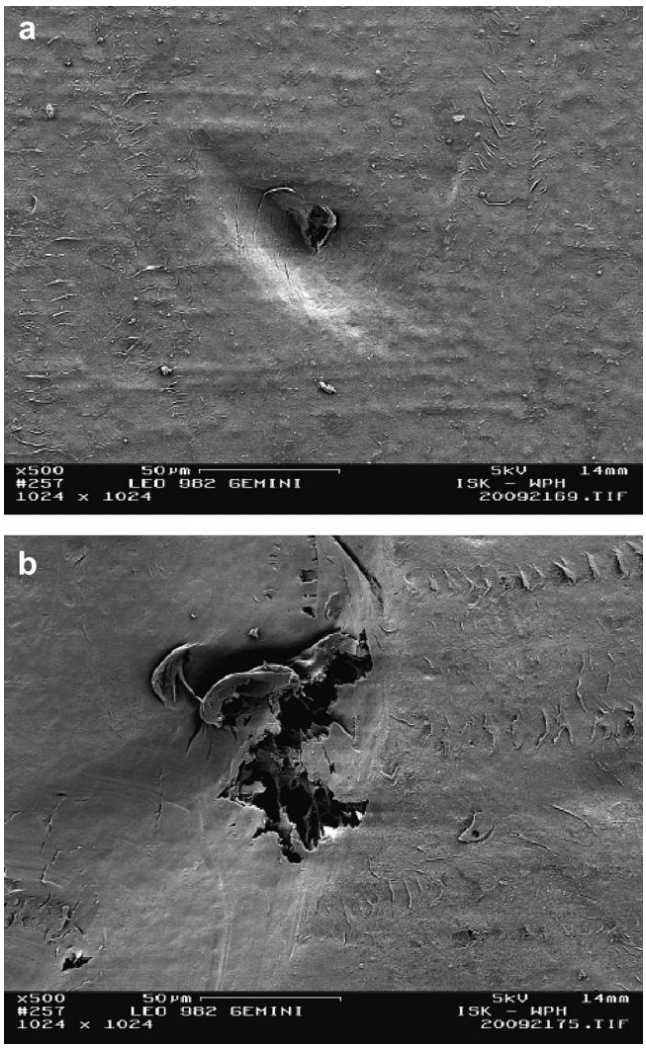


Figure 145: SEM images at 500x magnification of polyethersulfone flat sheet membrane surface after 25 months operation a) with granular medium b) without granular material (Siembida et al. 2010)

As well as being easy to remove from the feed stream, bubbles are similarly easy to introduce into the feed stream and thus the number of stirring elements in system can be continuously adjusted. Also, bubbles are deformable in the fluid and thus their movement within the feed stream may invoke less damage to formed elements in the blood compared to if solid media or a rigid stirrer were used. Additionally, Cui and Wright suggested that the bubbles in a filtration system could be used as stationary or slow-moving baffles (Cui et al. 1996).

Lee et al studied the use of entrapped large 'slug' air bubbles in improving filtration of bacterial suspension in flat sheet membranes (Lee et al. 1993). A peristaltic pump was used to introduce air slugs into the cross-flow flat sheet membrane module to increase the shear rate in the flow channel and reduce concentration polarisation (Lee et al. 1993). The group found that filtrate flux improved by 100% in a 300kDa polysulfone membrane and 30% in a 0.2µm PVDF microfiltration membrane (Lee et al. 1993). This demonstrates that large bubbles at low flow

rates can be used to disrupt concentration polarisation in horizontal cross-flow flat sheet membrane modules similar to that of the proposed device.

3.4.3. Surface bubble mixing

3.4.3.1. Surface bubble mixing as a fouling control strategy

Surface bubble mixing appears to be a combination of gas sparging, air scouring, dispersed phase disruption and more conventional mechanical stirring.

It has been seen in the previous chapter how stirring at the membrane surface is commonly employed in 'stirred cell' dead end filtration modules (Li et al. 2011; Foley 2013). This form of mechanical stirring has also been utilised in blood filtration systems. Ye et al utilised a stirring bar in order to reduce concentration polarisation in their assessment of membrane for a blood filtration system (Ye et al. 2003). Lameire et al describe how resistance to mass transfer in haemodialysers *"is primarily due to the unstirred boundary layer just adjacent to the membrane. Minimising the thickness of these unstirred layers is primarily dependent on achieving relatively high shear rates, particularly in the blood compartment" (Lameire et al. 2000)*. Stirring to increase flux is also utilised in nature where it is known that motility of the rumen in sheep occurs in the main to help stir the contents to encourage movement of solute towards the epithelium and promote absorption and transfer from the lumen to the blood (Dobson 1984).

More advanced fouling control strategies have been discussed (*3.4.2. Fouling Control Strategies*) including how in gas sparging and air scouring concentration polarisation and membrane fouling are successfully controlled by the introduction of air bubbles into the feed flow. Similarly, solid particles of up to a few mm in diameter used as a 'dispersed phase' in the feed fluid can also induce turbulence at the membrane surface and improve filtration flux, however these materials have been seen to cause damage to the membrane and thus utilising larger bubble or 'slugs' may be a better approach. Gas bubbles have been described as ideal as a second phase due to the lesser risks they pose to the membrane and the ease at which they can be separated from the feed fluid (Hilal et al. 2005). Similarly, bubbles are easy to introduce to the feed fluid and thus the number of mixing elements is adjustable during the process, and moreover the deformability of bubbles may invoke less damage to blood cells.

Additionally, Mercier-Bonin et al found that gas sparging was more effective in horizontal systems (such as that used in the proposed device) compared to vertical systems, most likely due to a better distribution over the membrane surface (Mercier-Bonin et al. 2000; Cui et al. 2003). Cui and Wright postulated that slow moving or stationary bubbles could be used as

baffles and Lee et al demonstrated that the use of air slugs moving at low flow rates can be used to disrupt concentration polarisation in horizontal cross-flow flat sheet membrane modules similar to that of the proposed device (Lee et al. 1993; Cui et al. 1996). The motion of the bubbles in surface bubble mixing is the driving factor for controlling concentration polarisation and membrane fouling and thus it is important to understand the behaviour of bubbles as currently used in filtration systems.

3.4.3.2. Bubble behaviour

The behaviour of bubbles rising in air scouring membrane systems has been discussed (*3.4.2.7. Air Scouring and Bubbling*) and is highly relevant to the movement of bubbles in the surface mixing phenomena described (Miyahara et al. 1988; Tsuchiya et al. 1988; Cui et al. 2003). As the bubbles move a secondary flow is generated in the wake region behind the bubble, the strength and extent of which is determined by the shape of the bubble which in turn is influenced by the size of the bubble (Cui et al. 2003). As bubble size increases the bubble shape changes from spherical to ellipsoidal, to a spherical cap shape, and is dependent upon surface tension forces and body forces characterised by Reynold's number, Eotvos number, and Morton number as is shown in Figure 146 (Cui et al. 2003).

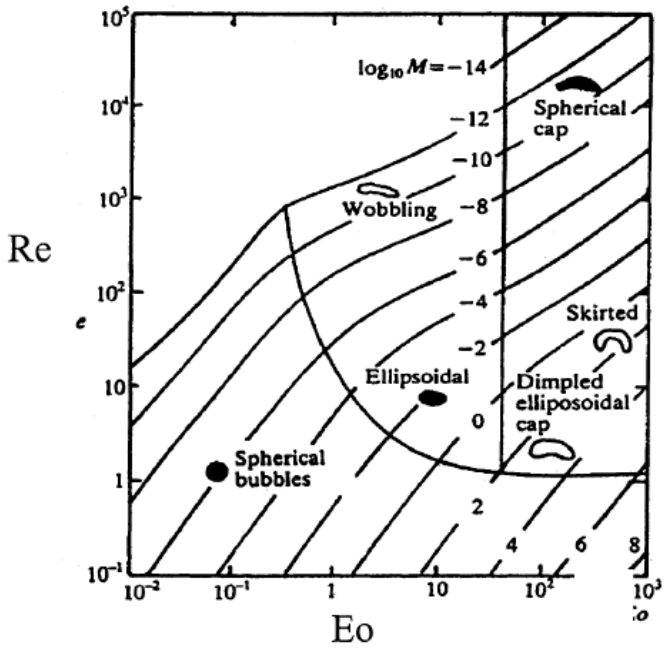


Fig. 1. Bubble characteristics chart (after Clift et al. [9]). Eotvos number, $Eo = g(\rho_l - \rho_g)d_e^2/\sigma$; Reynolds number, $Re = \rho_l d_e u_b/\mu_l$; Morton number, $M = g\mu_l^4(\rho_l - \rho_g)/\rho_l^2\sigma^3$.

Figure 146: Relationship between bubble size and shape change as characterised by Reynold's number, Eotvos number, and Morton number (Cui et al. 2003)

Spherical bubbles are usually <1mm in diameter and can be regarded as a solid particle, they have no boundary layer separation surrounding them and thus no bubble wake (Cui et al. 2003). Ellipsoidal bubbles are generally between 1.5mm and 15mm in diameter and move like a 'rocking disc', the boundary layer separates at a movable point along the bubble rim from which a helical vortex emanates (Cui et al. 2003). Spherical cap bubbles are large bubbles (>15mm) and produce a wake approximately 4.5 times their size, the boundary layer separation occurs at the circular rim behind which vortex rings develop (Cui et al. 2003). Spherical cap bubbles are known to create strong secondary flows and enhance local mixing (Cui et al. 2003). These bubble shapes and their associated streamlines and wakes are shown in Figure 147.

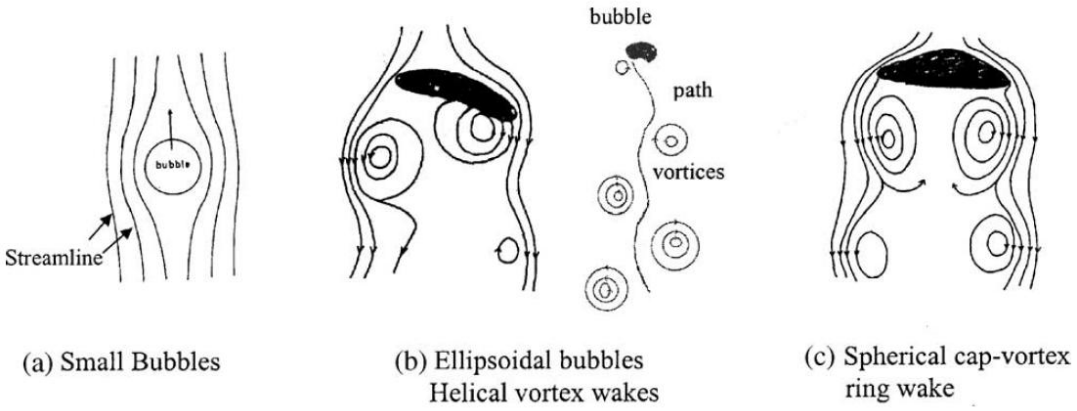


Figure 147: Streamlines and wakes associated with different bubble types (Cui et al. 2003)

The streamlines and wakes related to ellipsoidal and spherical cap bubbles were studied and photographed by Miyahara et al and are shown in Figure 148 and Figure 149.

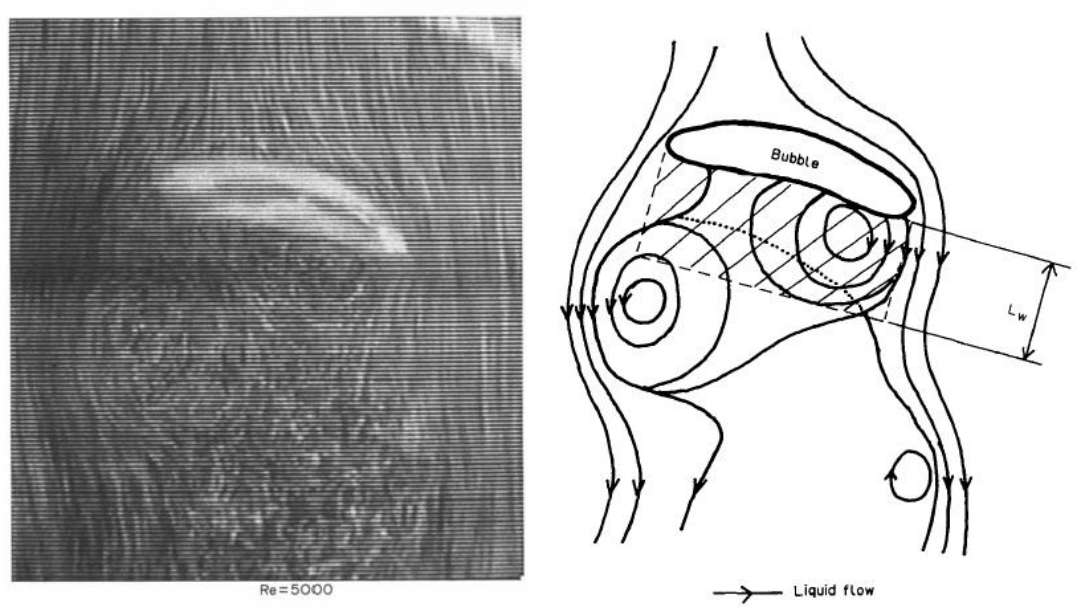


Figure 148: Streamlines and wakes related to movement of an ellipsoidal bubble (Miyahara et al. 1988)

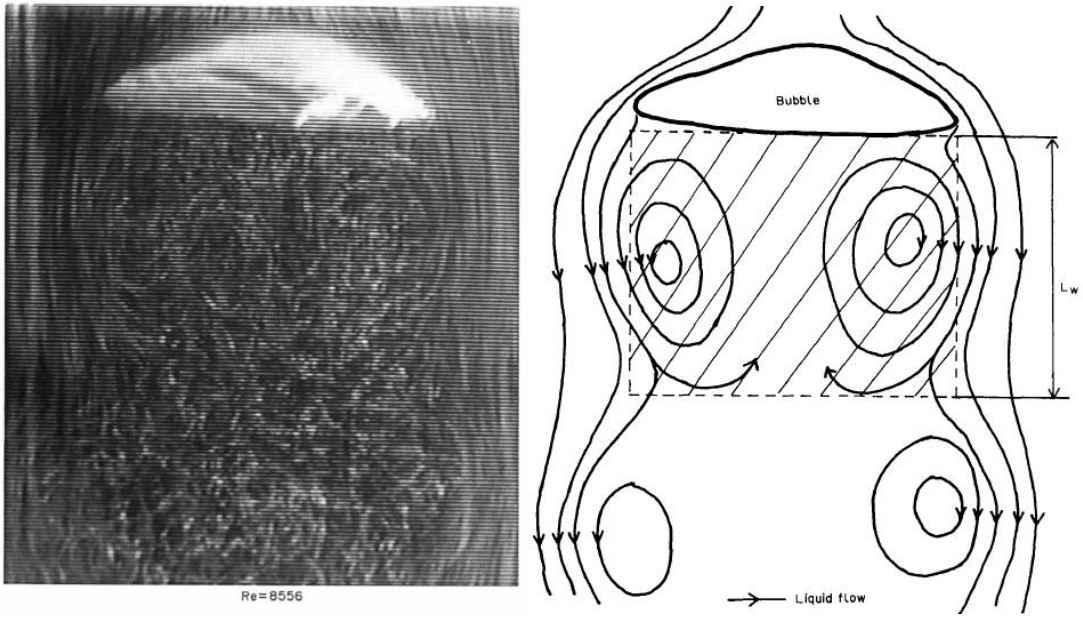


Figure 149: Streamlines and wakes related to movement of a spherical cap bubble (Miyahara et al. 1988)

More recently, CFD modelling has been used to better understand and predict the hydrodynamic impact of bubble motion in filtration systems. Essemiani et al used CFD to generate a two-dimensional model of the movement of spherical cap bubbles in a vertical flat sheet membrane module and were able to determine bubble area, direction and velocity (Essemiani et al. 2001). Subsequently, Wei et al used CFD to model the shear stresses invoked in the wake of spherical cap bubbles in a vertical flat sheet membrane system (Wei et al. 2013). Figure 150 shows the distribution of shear stress on the membrane surface as calculated by Wei et al for bubbles of volume a) 25ml b) 60ml and c) 200ml and the corresponding shape and position of the bubbles (Wei et al. 2013).

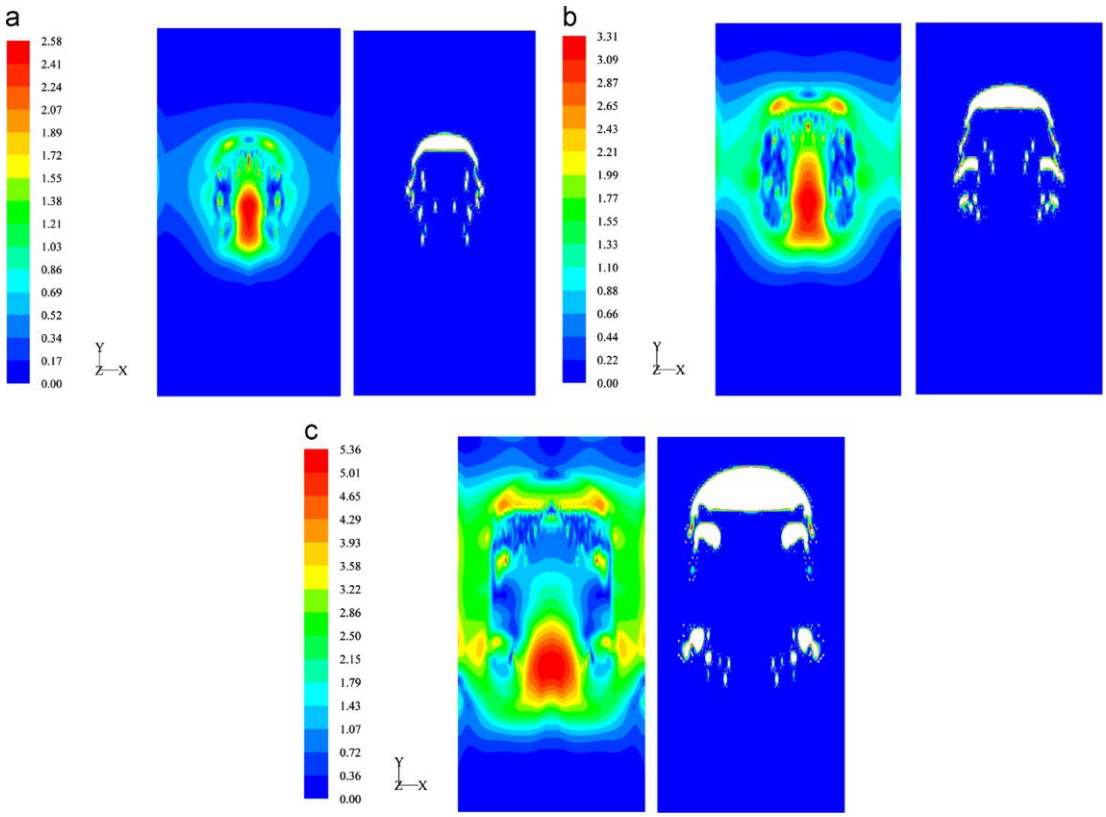


Figure 150: Distribution of shear stress on the membrane surface as calculated by Wei et al for bubbles of volume a) 25ml b) 60ml and c) 200ml and the corresponding shape and position of the bubbles (Wei et al. 2013)

The above descriptions and models relate to the movement of bubbles as they rise in vertical flat sheet membrane systems where the movement is driven by the buoyancy of the bubble as opposed to the proposed device where the membrane is horizontal and the bubble motion is driven by the orbital agitator. However, the work demonstrates how bubble motion can generate turbulence and secondary flow, and strengthens the notion that similar movement of bubbles at membrane surface aids filtration flux in the previously described surface mixing phenomena (*3.4.3.1. Surface bubble mixing as a fouling control strategy*).

3.4.3.3. Impact of surface bubble mixing on filtration

Based on the understanding of the mechanisms and reported results of current fouling control strategies, particularly pertaining to the use of bubbles, coupled with the previous observations of improved plasma transfer area, it appears that the use of surface bubble mixing would be of benefit to the proposed device. In order to test this, the performance of proposed device in testing with and without surface bubbles was compared. Experiments in both groups were conducted without the use of the superabsorber at an agitation speed of 120rpm and were repeated three times. Each experiment was ended after 90 minutes or once a Hct of 40% had

been reached. The mean Hct change over time with and without surface bubble mixing is shown in Figure 151.

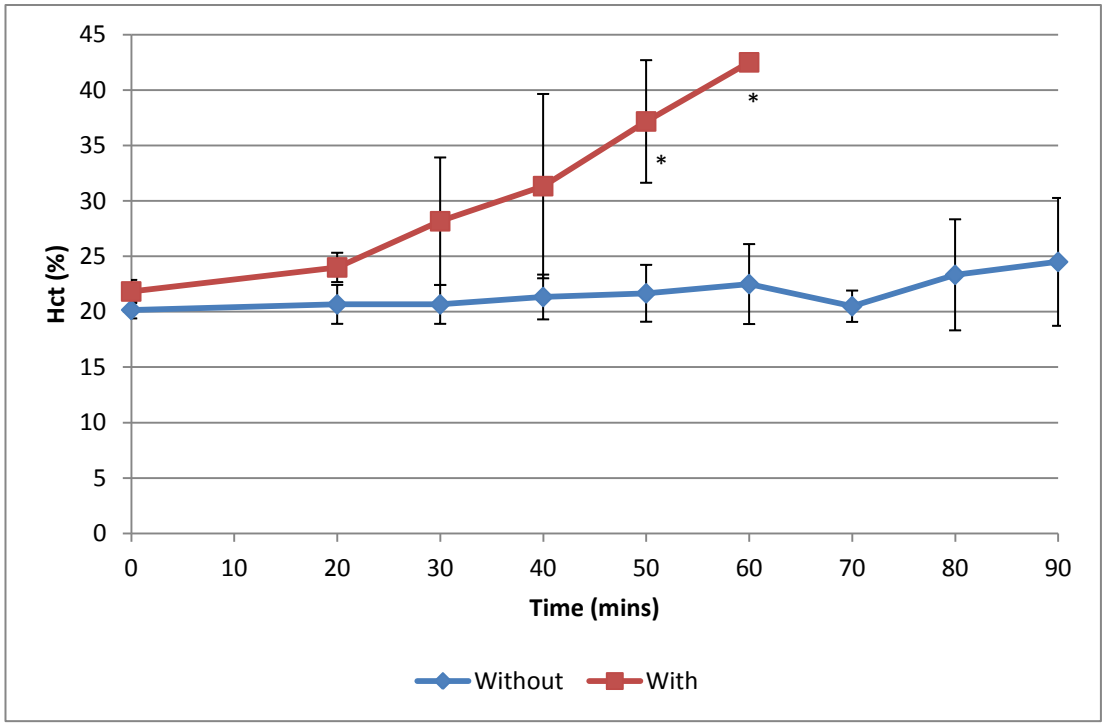


*Figure 151: Mean change in Hct over time with and without the use of surface bubble mixing, n=3, significance calculated by unpaired (two sample) t-test ($p<0.05$). * denotes significant difference between groups at this timepoint.*

It can be seen from Figure 151 that the use of surface bubble mixing noticeably improves the rate of haemoconcentration, resulting in significantly higher Hct beyond 40mins ($p<0.05$, unpaired (two sample) t-test). In every experiment conducted with surface bubble mixing the target Hct of 40% was reached within 60mins, whereas in every experiment without surface bubble mixing the target Hct was not reached.

Despite the positive results observed, there are potential problems associated with the use of surface bubble mixing which are discussed in the following section (*3.4.3.4. Potential problems with bubble mixing*).

As an aside, the use of 3mm diameter AISI 316 stainless steel ball bearings as a mixing media was also tested but a number of disadvantages became immediately apparent. Firstly, the fact that the balls were not buoyant meant that good disruption of the concentration polarisation layer immediately adjacent to the membrane surface could not be guaranteed, secondly, the balls had a tendency to group together and move as one mass reducing the 'mixed area', thirdly in order to assure equal distribution of the balls the proposed device had to be positioned

perfectly level on the workbench, and fourthly, care had to be taken to prevent one of the balls occluding the channel outlet. These observations along with the aforementioned drawbacks of using solid mixing media meant that their use in the proposed device was not investigated further.

3.4.3.4. Potential problems with bubble mixing

The bubble mixing system described, like the air- scouring and gas sparging techniques, introduces a blood-air interface to the haemofiltration system. A number of groups have highlighted the potential risks associated with a blood-air interface.

Leverett et al studied sources of haemolysis in a rotational viscometer with one variable of interest being the haemolysis arising from the air-blood interface (Leverett et al. 1972). The group found that "substantial amounts of haemolysis may occur at the air interface". Sowemimo-Coker et al list as an example of determinant of haemolysis in filtration systems the presence of trapped air pockets in the filter or in foam generated by blood mixing (Sowemimo-Coker 2002).

Malinauskas et al found that during extracorporeal circulation, air entrained during cardiotomy suction damages the blood (Malinauskas et al. 1988). Later, Waters et al confirmed this observation in their own study into suction induced haemolysis (Waters et al. 2007). Additionally, it has been reported that blood in bubble oxygenators is damaged by air as a result of "interfacial denaturation of plasma proteins" which causes sublethal RBC damage and haemolysis (Waters et al. 2007). In fact, one factor which drove the work of Bellhouse on the development of oxygenators was the knowledge that the systems available at the time involved direct injection of oxygen bubbles into the blood reservoir which was damaging to the blood, thus reducing the time these 'bubble oxygenators' could be used for (Bellhouse 1992).

Damage to proteins is known to occur in gas-liquid two-phase systems, predominantly at the gas-liquid interface, and may occur as a result of bubbles and foaming in the fluid (Clarkson et al. 1999a; Cui et al. 2003; Hilal et al. 2005). At high gas flow rates, the propensity for foaming is higher and can lead to conformational changes in proteins in the bulk (Clarkson et al. 1999b). Clarkson et al found that the damage to proteins in foam is mainly due to denaturation of the protein surface and that the damage can be minimised by optimising ionic strength and pH (as discussed previously in *3.2.3.1 Protein Adsorption* this is also the case for protein adsorption) (Clarkson et al. 2000). Bubble induced damage to proteins can also occur due to changes in the tertiary structure, subsequent aggregation, whereas high shear as a result of bursting bubbles and damage through oxidation were found to be insignificant by Clarkson et al (Clarkson et al.

1999a; Clarkson et al. 1999b; Cui et al. 2003). Despite this, provided gas sparging and the associated bubble movement occurs at a low flow rate, damage to proteins can be negligible whilst still significantly improving filtration performance (Cui et al. 2003). It is important to point out that the bubbles used in surface bubble mixing are designed to remain at the membrane exchange surface, however in cardiovascular surgery it is typical that filters/bubble traps are used to prevent any gas emboli generated which could also act as a safety mechanism in the event that any mixing bubbles escape the proposed device (Semrad et al. 2015). The impact of surface bubble mixing on the blood in the proposed device will be assessed in the next chapter as part of the blood quality analysis.

3.4.4. Impact on design

Based on the demonstrated beneficial effect of utilising surface bubble mixing coupled with the ease at which this fouling control strategy could be implemented, subsequent testing of the proposed device incorporated the introduction of a small number of air bubbles into the device to reduce concentration polarisation and membrane fouling as described previously (*3.4.3.1. Surface bubble mixing as a fouling control strategy*). As mentioned (*3.4.3.4. Potential problems with bubble mixing*), the impact of surface bubble mixing on the blood in the proposed device will be assessed and would be abandoned if results demonstrated a deleterious effect on blood quality.

3.5. Agitation and Wave motion

3.5.1. Agitation

In surface bubble mixing the movement of the bubbles across the membrane surface is driven by the agitation of the entire membrane system. Agitation is used in Hemosep and results in a faster rate of haemoconcentration as was shown in Figure 32.

The increased rate of haemoconcentration shows that there is an increased filtration flux with agitation. The Hemosep system operates without flow across the membrane surface and thus the improved filtration flux is most likely due to the movement of blood across the membrane surface caused by the orbital agitator inducing shear, leading to a reduction in concentration polarisation. Since the Hemosep is not a rigid system, the orbital agitator results in movement of the entire Hemosep bag including the membrane itself and thus it is possible that mass transfer is also assisted in this way.

Membrane motion is a phenomenon, which, like turbulence and shear stress at the membrane surface, is known to alter filtration performances (EUROMBRA 2006). In hollow fibre membrane systems, aside from bubble induced surface shear and lateral flow, lateral fibre movement is one mechanism that acts to limit membrane fouling (Cui et al. 2003). A number of plasma separation systems have relied on mechanical agitation to control membrane fouling by RBCs, and, as mentioned previously (*3.4.2.5. Turbulence*), a wide range of rotating disk and vibrating membrane systems have emerged for use in a variety of applications and have demonstrated good performance (Gupta 1996; Lin et al. 2000; Jaffrin 2008). Wicaksana et al studied the impact of hollow fibre movement on filtration of a yeast suspension and found that direct mechanical movement of the fibres was able to reduce fouling rate by up to a factor of three, whilst bubble induced shear and fibre movement was more effective and able to reduce fouling rate by up to a factor of ten (Wicaksana et al. 2006). Similarly, Berube et al found that air slug induced lateral sway of fibres led to physical contact between fibres and aided foulant erosion and enhanced permeate flux (Berube et al. 2006).

Agitation in Hemosep and in the proposed device also acts to prevent areas of stasis in the bag or exchange channel respectively. The negative impact of stasis in blood flow has been discussed previously (*3.3.3. Effect of flow on blood*), specifically, how stasis contributes to haemolysis by increasing contact time between RBCs and foreign surfaces, and how areas of stasis and low shear allow the formation of rouleaux and RBC aggregation (Bacher et al. 1970; Pedrizzetti et al. 2003; Kameneva et al. 2004; Connes 2011). The agitation utilised in Hemosep and the proposed system should ensure distribution of flow across the entire channel, preventing stasis induced problems and helping to ensure good coverage of the membrane exchange surface.

The benefits of agitation have been demonstrated in Hemosep and consequently agitation is utilised in the proposed device. Moreover, agitation is used as the driving force for bubble surface mixing which has been demonstrated to be beneficial to plasma filtration rates in the proposed device. However, by observing the membrane and the rate of plasma extraction during testing of the proposed device it is possible that agitation may also aid haemoconcentration in another way by inducing a 'wave motion' as is discussed in the next section.

3.5.2. Wave motion

The physical flexibility of the Hemosep system may also assist mass transfer by another mechanism. The motion of orbital agitator in the Hemosep system moves at a fixed rotational speed of 120rpm for a period of 90 seconds in alternating clockwise and anticlockwise directions generates a 'wave motion' along the membrane which was also observed during

testing of the proposed device. Figure 152 comprises stills taken from a video depicting this wave motion.

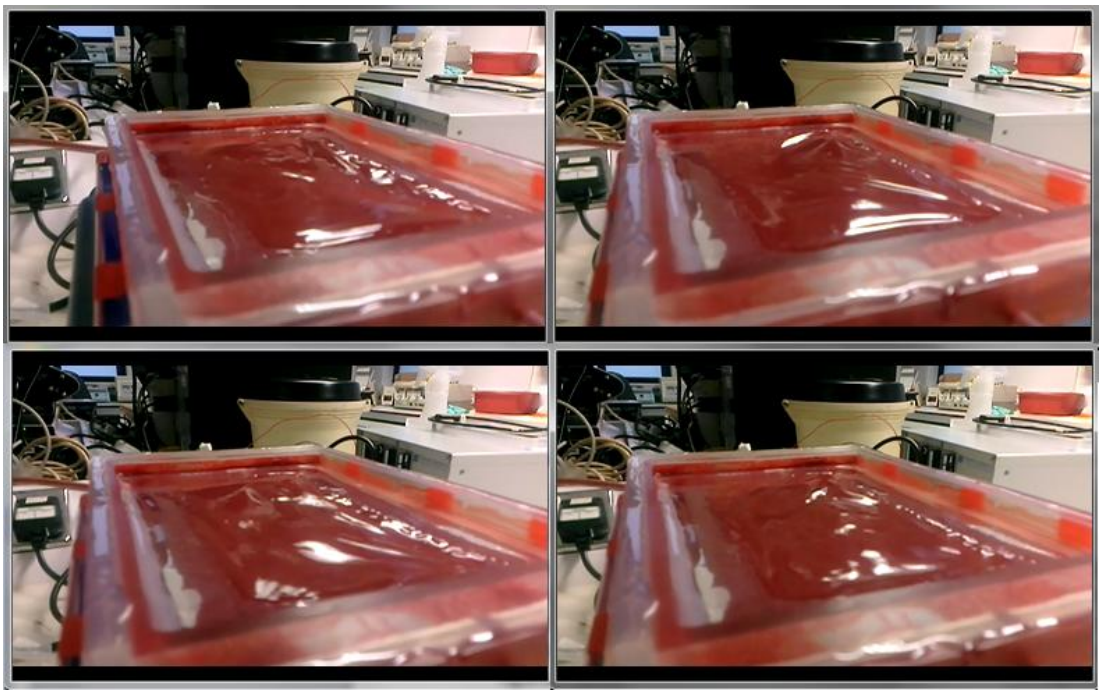


Figure 152: Stills captured from a video showing the wave motion of the membrane under the influence of orbital agitation

The nature of the motion of waves generates two possible mechanisms for improved mass transfer across the membrane. The agitator supplies a 'disturbing force' in the blood which generates the waves seen, which is counteracted by a 'restoring force' which acts to return the blood surface to a 'flat' state at the 'still water level' and is the result of a combination of gravity and surface tension (Trujillo et al. 2002; Garrison 2006). One component of this restoring force is an uplift force which acts to move the fluid below the 'still water level' upwards as illustrated in Figure 153 (Trujillo et al. 2002; Gaeta et al. 2012).

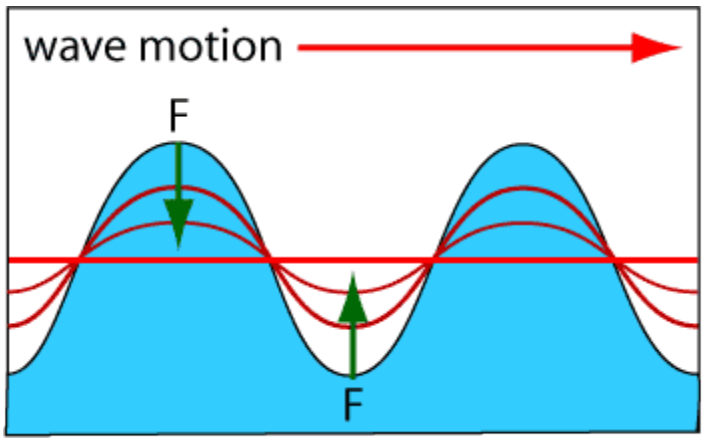


Figure 153: Upward and downward restoring forces in waves (Konrad 2015)

In the proposed device, the membrane is in direct contact with the blood surface during this 'wave motion' and this upward acting force may act to generate a locally elevated TMP at the membrane similar to the pressure pulsing induced by gas slug movement (Cui et al. 2003). This locally elevated TMP could be a mechanism to improve filtration flux by acting as the driving force for plasma transfer across the membrane.

Another mechanism through which wave motion may assist filtration is orbital motion of particles at the surface of a wave. The movement of a waveform across the surface of a fluid at the interface between two fluids of different densities (e.g. air and water) involves the transfer of energy from particle to particle in circular or orbital paths (Garrison 2006). This orbital motion continues downwards below the surface, diminishing in magnitude, to a depth approximately equal to half the wavelength as shown in Figure 154 (Trujillo et al. 2002; Garrison 2006).

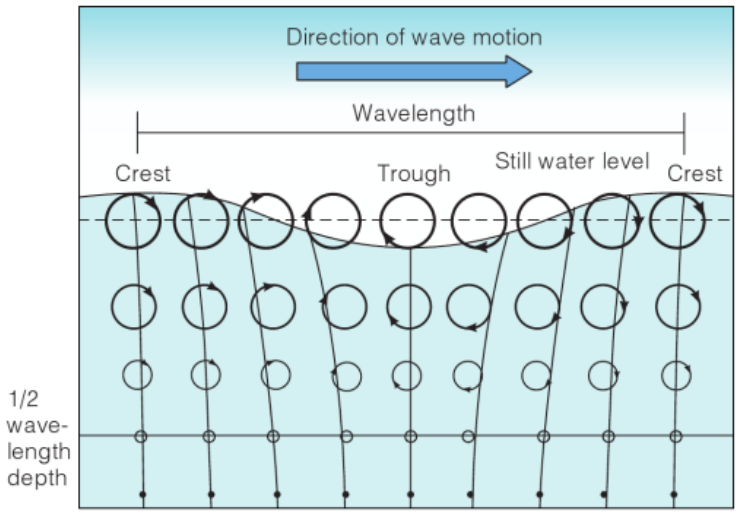


Figure 154: Orbital motion below fluid surface as wave propagates (Garrison 2006)

Below the wave base the orbital motion is negligible and wave motion no longer stirs any sediments which may be present (Trujillo et al. 2002; Nittrouer et al. 2009). It is clear therefore that the circular motion of particles at, and just below, the wave surface could act to induce turbulence at the membrane surface and thereby control concentration polarisation and fouling. One way in which the benefits of wave motion could be investigated whilst retaining the flow distribution supplied by the agitator was to alter the tautness of the control membrane. A taut membrane would prevent wave motion at the membrane surface whilst a slack membrane would allow propagation of waves across its surface. A slack membrane may also be beneficial by increasing the exchange surface area but also by keeping the surface mixing bubbles in the rig and close to the membrane surface. This is illustrated in Figure 155 where the membrane can be seen to be slightly bowed by the volume of blood beneath it meaning that, due to their buoyancy, the bubbles in the blood channel rise to the highest point and are kept there, in close contact with the membrane and unable to leave the device via the inlet which is at a lower elevation.

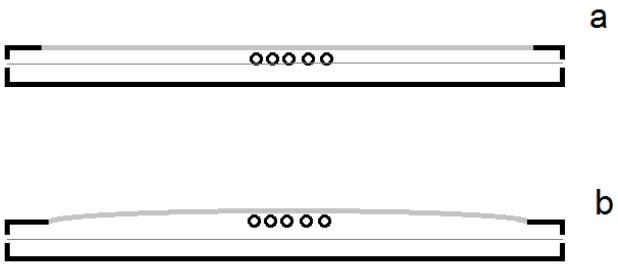


Figure 155: Sketch to demonstrate of how a slack membrane (b) may keep bubbles in device better than a taut membrane (a) by allowing them to rise to a plan higher than the inlet and outlet.

This can also be seen in Figure 156.

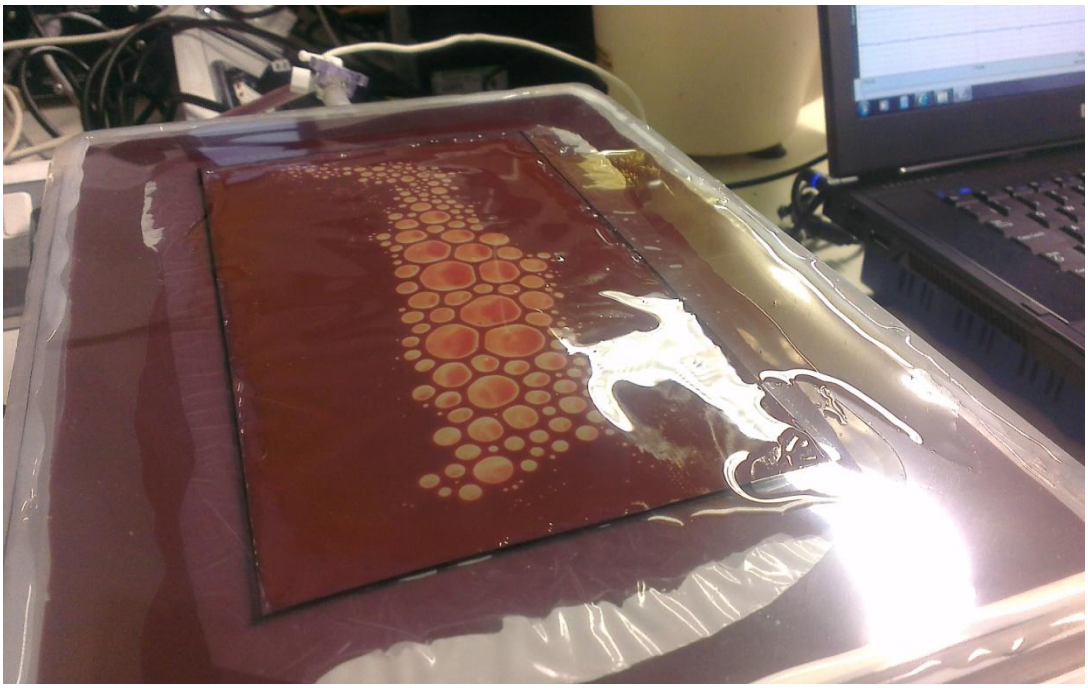


Figure 156: Slack membrane keeping bubbles inside test rig and close to the membrane surface due to their buoyancy

The rise in Hct with either a taut or a slack membrane was compared. Experiments in both groups were conducted without the use of the superabsorber at an agitation speed of 120rpm and were repeated three times. Each experiment was ended after 90 minutes or once a Hct of 40% had been reached. The mean Hct change over time with either a taut or a slack membrane is shown in Figure 157.

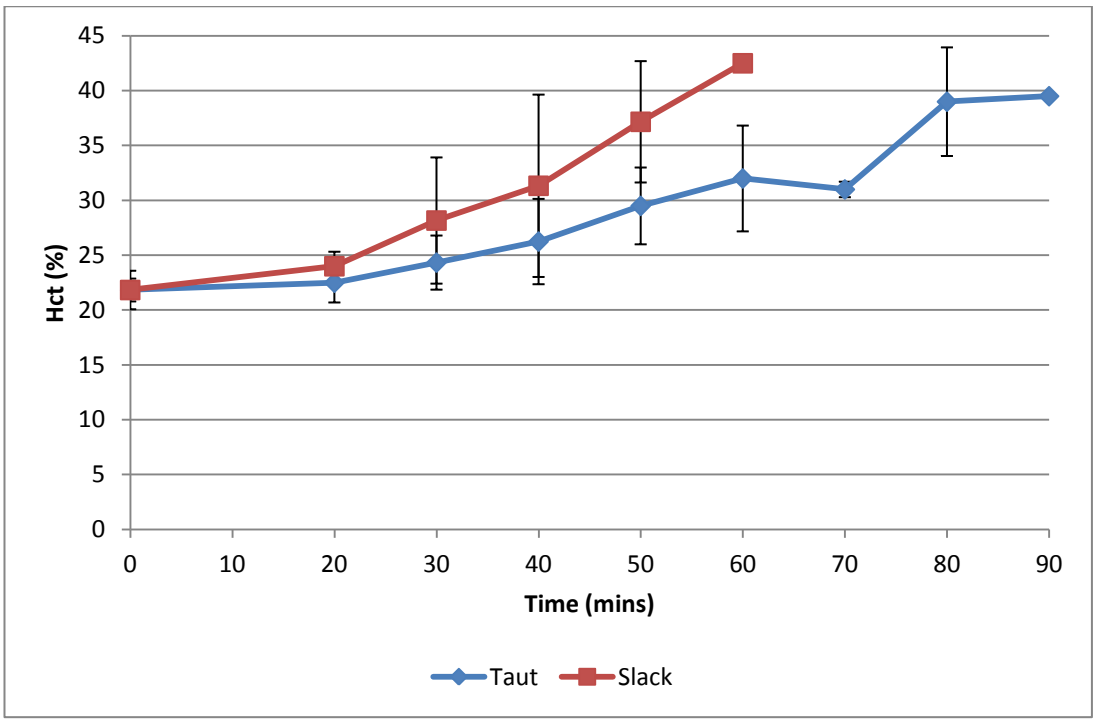


Figure 157: Mean Hct change over time with either a taut or a slack membrane, n=3, significance calculated by unpaired (two sample) equal variance t-test ($p<0.05$). In the slack group at 60mins n=1 as two of the three experiments were discontinued at 50mins having already reached the target 40% Hct.

As can be seen from Figure 157 a slack membrane appears to be slightly beneficial to the haemoconcentration in the proposed device. There was no significant difference between the two methods at any interval between 0mins and 60mins, although at 60mins it was close to significance ($p=0.06$, unpaired (two sample) equal variance t-test). It should be noted, however, that every experiment with the slack membrane reached the target Hct of 40% before 60mins whereas when a taut membrane was used the target was reached at 60mins in one experiment, 80mins in the second experiment and was not reached before 90mins in the third experiment. It is possible therefore than wave motion is mainly beneficial at higher Hcts and that since experiments were discontinued once 40% Hct was reached this was not fully reflected in the results, for example, at 60mins the best performing experiment in the slack group had been discontinued and had it not been may have skewed the mean Hct for this time interval higher resulting in a significant difference between the groups at this time interval.

3.5.3. Impact on design

The benefit of agitation has been demonstrated in Hemosep and is required to drive the movement of the surface bubble mixing fouling control strategy. Based on the small improvement in haemofiltration seen when wave motion in a slack membrane was employed,

the use of a slack membrane in the proposed device was continued in subsequent designs of the proposed device.

3.6. Superabsorber Behaviour

The superabsorber is the key functional element of the proposed device and consequently its behaviour can have a considerable impact upon successful haemoconcentration.

3.6.1 Response to water vs saline

The general behaviour of superabsorbent polymers has been discussed in detail previously (*2.3.1. Superabsorbent Polymers*). For technical purposes the swelling capacity of superabsorbent polymers (SAPs) is defined as the amount of 0.9 wt% saline solution that a SAP can absorb under unrestricted swelling conditions and after surface water has been removed by centrifugation (Elliott 2004). The reason saline is specified as the test solution is that the capacity of SAPs varies according to the content of the fluid, typically, SAPs can absorb 1000 times their own weight in distilled water, 300 times their own weight in tap water, and 50 times their own weight in saline (Bullinger et al. 2009). As discussed previously (*2.3.2.3. Other factors which influence SAP capacity*), the reduced capacity with different solutions is due to the reduced osmotic pressure between the gel phase and solution phase as a result of the higher salt concentration in the solution (Chatterjee et al. 2002; Peppas et al. 2010).

The specific swelling phenomena of sodium polyacrylate in a sodium chloride solution and the impact of the presence of multivalent ions in the solution were described in detail in *2.3.2.3. Other factors which influence SAP capacity*.

Saline is one of several possible priming solutions used in cardiopulmonary bypass and is the priming solution used in the priming phase of the Hemosep procedure and thus these factors, in conjunction with its low cost, meant that it was used during testing of the proposed device to both dilute blood and prime the superabsorber (Gu et al. 2006; Ghosh et al. 2009). The response of the sodium polyacrylate superabsorber to be used in the proposed device to both distilled water and saline was observed. Figure 158 and Figure 159 show the different response of 2g sodium polyacrylate superabsorber in 100ml distilled water and saline, left to stand for 2mins. The solutions were tinted with food colouring to make it easier to see the difference in response, the food colouring does not impact the response seen and the test has been replicated without it. Figure 158 shows 2g Sodium Polyacrylate superabsorber in 100ml saline (left) and distilled water (right) left to stand for 2mins.

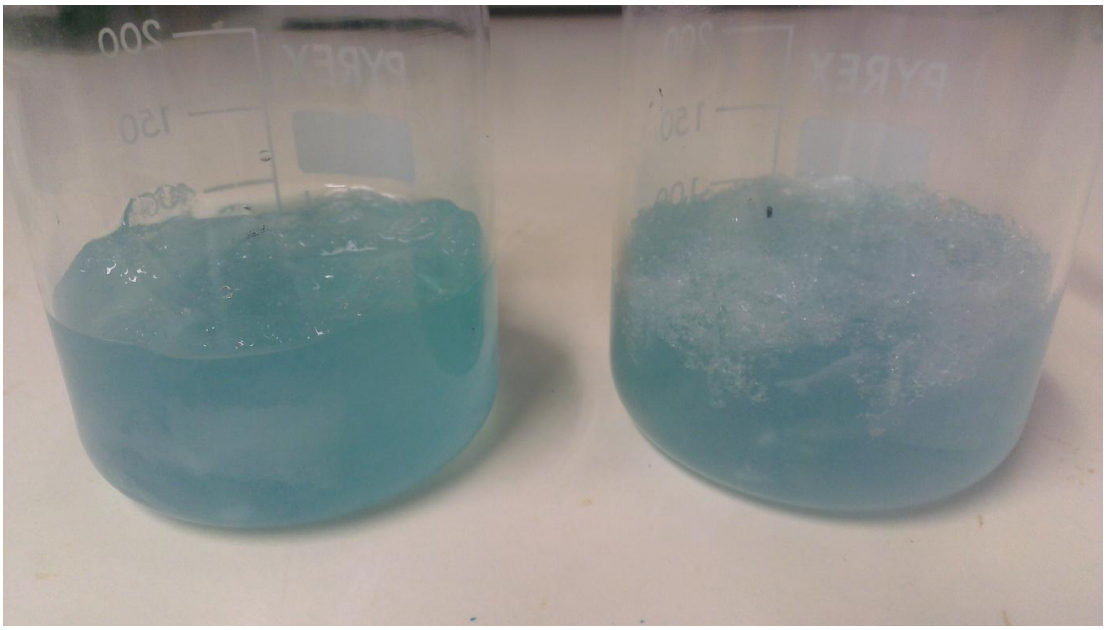


Figure 158: 2g Sodium Polyacrylate superabsorber in 100ml saline (left) and distilled water (right). Tinting of solutions with food colouring was for visualisation only.

It can be seen that whilst the superabsorber has completely absorbed the distilled water, it has been unable to completely absorber the saline, leaving a large fraction of the saline free as 'un-captured' liquid. This 'un-captured' fluid could be poured from the beaker as is shown in Figure 159.

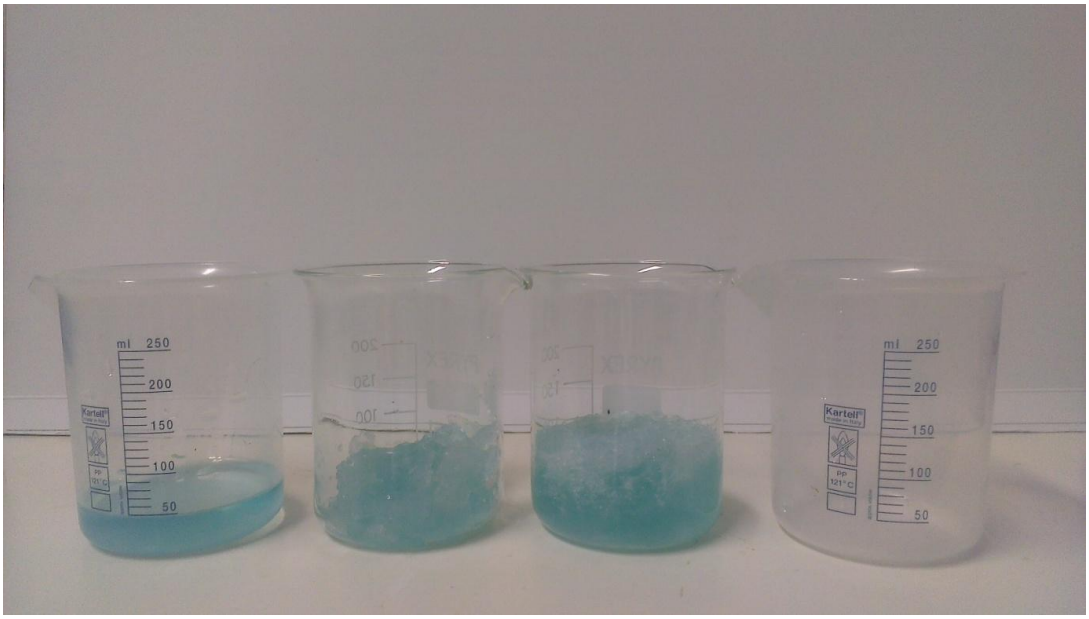


Figure 159: 50ml residual fluid drained from superabsorber in saline, no residual fluid drained from superabsorber in distilled water

50ml residual saline was drained from the beaker, whereas no distilled water could be drained. This demonstrates that there is a clear difference in sodium polyacrylate superabsorber capacity between saline and distilled water.

The clear disparity between the response of the superabsorber to saline and distilled water has an unfortunate detrimental impact upon the potential performance of the proposed device. Priming is a necessary step in order to establish good contact between the superabsorber and the membrane but the difficulty in eliminating residual priming fluid from the blood flow channel means that in order to prevent damage to the RBCs an isotonic solution must be used. However, the ion concentration of the available isotonic solutions will reduce the overall capacity of the superabsorber. There are several other solutions which are used for priming of the CPB circuit and could therefore be used as a priming solution for the proposed device but, as Figure 160 shows, their ionic composition is similar (Ghosh et al. 2009).

	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	HCO_3^-	pH	Other	mosmol/l
Dextrose 5%	0	0	0	0	0	0	4.2	Glucose 50 g/l	279
Saline 0.9%	154	0	154	0	0	0	5.0	–	308
Hartmann's	131	5.0	111	2.0	0	29 (lactate)	6.5	–	280
Plasmalyte A	140	5.0	98	0	3	27 (acetate)	7.4	–	294
						29 (gluconate)			
Normasol R	140	5.0	98	0	3	27 (acetate)	7.4	–	294
						29 (gluconate)			
Bicarbonate 1.26%	150	0	0	0	0	150	7.0	–	300
Gelofusine	154	0.4	120	0.4	0	0	7.1–7.7	Gelatine 40 g/l	274
Starch	154	0	154	0	0	0	4.5–5.5	Starch	308
Human Albumin 4.5	100–160	<2	100–160	0	0	<0.1 citrate	7.1	Albumin 40–50 g/l	300

Figure 160: Table of possible priming solutions for CPB and their ion content (Ghosh et al. 2009)

Moreover, the use of priming solutions which contain multivalent ions such as Hartmann's solution should also be avoided. As mentioned previously (*2.3.2.3. Other factors which influence SAP capacity*), the sodium polyacrylate superabsorber is known to react with multivalent cations such as calcium and iron resulting in increased cross-link density in the polymer network (Peppas et al. 2010). This results in an increased crosslink density in the hydrogel

network and consequently the swollen polymer can precipitate in the swelling medium with little to no fluid absorption (Peppas et al. 2010).

On initial inspection, Dextrose 5% appears to be a suitable alternative since it is isotonic but does not contain any of the listed ions (Corrigan et al. 2011); however there are a number of disadvantages with using Dextrose 5% as a priming solution. Firstly, the metabolism of dextrose invokes a dilutional effect on plasma bicarbonate which may cause metabolic acidosis, secondly, the metabolism of glucose leads to a hypotonic solution, and thirdly, dextrose use in conjunction with elevated serum glucose and insulin concentrations after CPB may result in hyperglycaemia which has been linked with worsened neurological outcomes and is especially dangerous in diabetic patients (Obel et al. 1967; Chin et al. 2006; Gu et al. 2006; Ghosh et al. 2009). The use of dextrose as a priming solution for Hemosep has previously been tested but has no beneficial impact on performance.

Nevertheless, the capacity of the superabsorber after priming with saline should still be sufficient to absorb a considerable amount of plasma as the success of the Hemosep system demonstrates.

3.6.2. Swelling and Disintegration

It was observed that when the superabsorber swelled as it took up fluid, it often did so in a way that was non-uniform resulted in highly swelled areas adjacent to completely 'dry' areas. This can be seen in Figure 161.

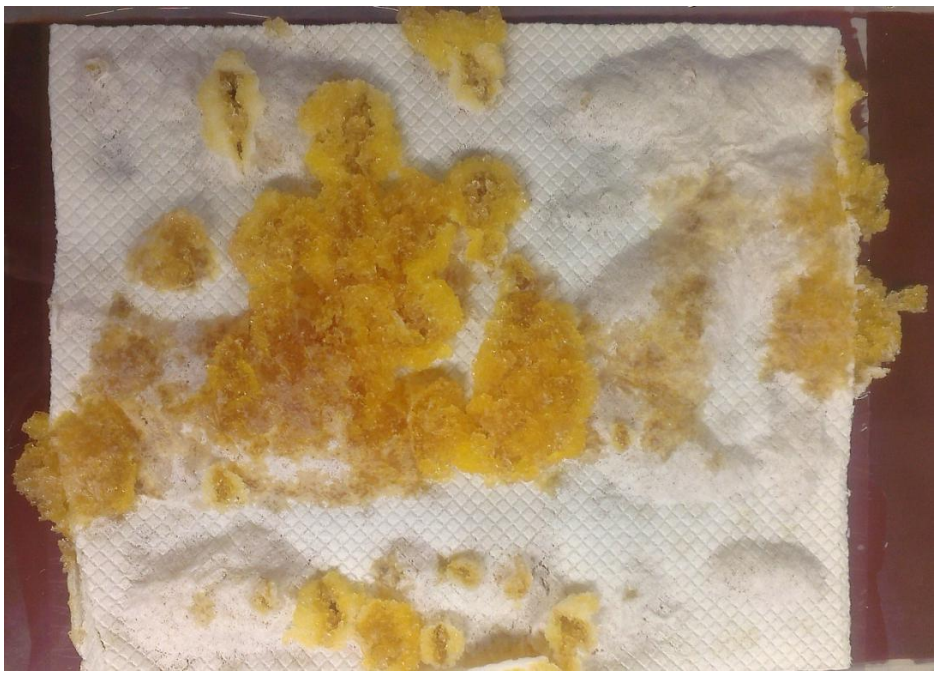


Figure 161: Non-uniform swelling of superabsorber. Swelled elements which have absorbed plasma and appear yellow can be seen adjacent to dry regions still displaying the 'hatching' pattern seen on the dry manufactured sheets of the superabsorber.

This non-uniform swelling can result in 'disintegration' and 'furling' of the superabsorbent sheet as different elements swell to different degrees, as can be seen Figure 162.

Figure 162: Furling and disintegration of superabsorber as it swells in a non-uniform manner.

This can have a number of consequences for the performance of the device which will be discussed in *3.6.3. Loss of contact with the membrane*. In order to understand why this occurs, it is important to first understand the kinetics of superabsorber swelling.

3.6.2.1. Kinetics of superabsorbent hydrogel swelling

During the swelling of superabsorbent hydrogels the surface layers swell first since they are in direct contact with the fluid, but thereafter the fluid must diffuse through these swollen layers to reach dry regions, which restricts the subsequent swelling rate of the superabsorber (Peppas et al. 2010). This is also true for individual particles of the superabsorber in which fluid must pass through a swollen shell to reach the 'dry' core, and consequently the speed of diffusion through the swollen regions of the superabsorber will be a determining factor in the swelling rate (Peppas et al. 2010).

Hydrogel swelling rate can be increased by increasing the contact area with the fluid, highlighting the importance of the large exchange surface area in the proposed device. This is generally achieved by either reducing the particle size by grinding or making superporous monolithic hydrogels (Chen et al. 1999; Omidian et al. 2007; Peppas et al. 2010).

The classical Stefan Problem is a mathematical problem which describes the temperature distribution in a homogenous medium undergoing a phase change e.g. the melting of ice into water (Gupta 2003). Similarly, superabsorbent hydrogel swelling from its initial dry state can be considered a 'Stefan Problem' in which there is a time-dependent moving boundary between three states: dry superabsorber, partially swollen superabsorber, and swollen superabsorber (Peppas et al. 2010). The speed at which this boundary propagates depends on several material and environmental properties (e.g. particle size, pore size, pore distribution), for example in super-porous hydrogels the boundary will propagate rapidly as the pores allow for several pathways for diffusion, whilst conversely, in non-porous hydrogels the boundary will move very slowly as the swelling rate is entirely dependent on fluid diffusion through the material (Peppas et al. 2010).

Figure 163 shows the three stages of superabsorbent swelling which broadly speaking begins with a diffusion process and end with a relaxation process (Omidian et al. 1998; Peppas et al. 2010).

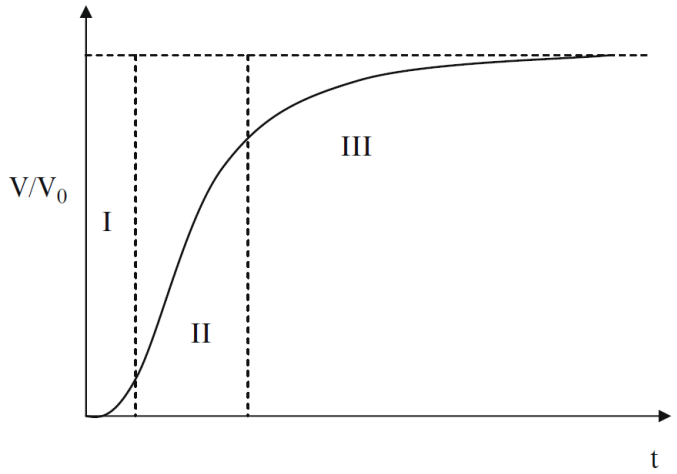


Figure 163: Generalised profile of superabsorbent swelling over time and the phases associated with this phenomenon (Peppas et al. 2010)

In phase I the dry layer of superabsorber comes in contact with the fluid and becomes wet which involves the plasticisation of the solid dry superabsorber particles, this takes time since the glass transition temperature of the superabsorber (230°C for sodium acrylate) is much higher than room temperature (Peppas et al. 2010; Sigma-Aldrich 2014). Phases II encompasses the diffusion of water into the mass of the superabsorber immediately after initial plasticisation (Peppas et al. 2010). In phase III, the equilibrium swelling capacity is reached after which there is a slow relaxation of the polymer chains (Peppas et al. 2010).

It can be seen therefore that initial swelling is rapid, but that the subsequent propagation of fluid through the superabsorber is dependent upon the speed of diffusion through swollen areas and the speed of relaxation of polymer chains. The diffusion through these swollen areas refers to both the superabsorbent as a whole and the individual elements of the superabsorber. If supply of fluid is slow enough, and/or distribution is not sufficient, then it is likely that the fluid will be absorbed by diffusing to the dry core of the partially swollen elements at the point of initial absorption rather than diffuse through the swollen shell to completely dry elements and the boundary propagation will be very slow. In this way non-uniform swelling will occur and will result in the highly swollen elements immediately adjacent to dry elements as was observed.

It is possible another phenomena, known as gel blocking, which related to the swelling of superabsorbent polymers (SAPs) is occurring.

3.6.2.2. Gel Blocking

When there is limited space between the SAP particles the swelling process can lead to a 'gel blocking' phenomenon in which the swollen particles form a barrier between the fluid and the still dry particles (Chatterjee et al. 2002; Masoodi et al. 2012). This is illustrated in Figure 164.

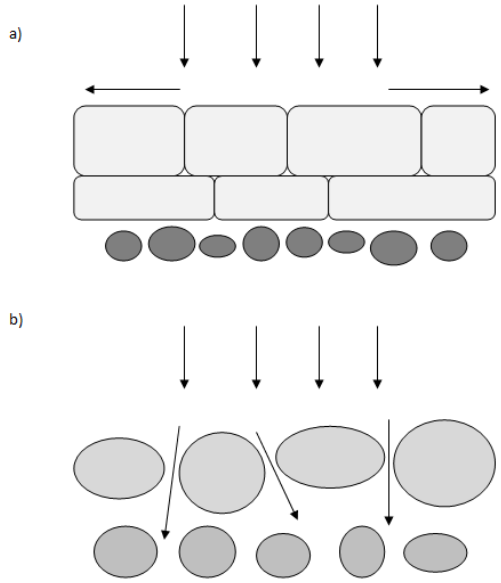


Figure 164: Gel blocking phenomenon in which if there is limited space between SAP particles then their swelling can a) create a barrier to fluid propagation, rather than b) allowing fluid to reach dry elements

This phenomenon is desirable in applications such as cable sheathing when the SAP is designed to prevent fluid propagation, but in applications such as nappies, where the intention is to retain as much fluid is possible steps must be taken to avoid gel blocking, specifically the introduction of non-swelling elements which distribute fluid evenly to the dry particles (Chatterjee et al. 2002; Masoodi et al. 2012). This further highlights the importance of good distribution of the fluid directly to the dry areas, rather than relying on distribution within the superabsorber material. As described previously (*2.3.1. Superabsorbent Polymers*), reduced cross-link density in SAPs will lead to an increased absorption capacity but will reduce the gel strength meaning that under load absorption capacity is reduced and the problem of gel-blocking is exacerbated (Chatterjee et al. 2002; Elliott 2004; Jockusch et al. 2009). During the testing and operation of the proposed device there should be no load applied to polymer network and so gel blocking by this method should be minimal.

Based on the understanding of the kinetics of SAPs, in particular the diffusion of fluid through swollen elements and potential gel blocking, it was thought that the non-uniform swelling and subsequent furling and disintegration of the superabsorber may be related to the rate of supply of, and contact with, the fluid to be absorbed. In order to ascertain if there was any observable

difference in the swelling behaviour of the superabsorbent under different rates of fluid supply two identical pieces of the superabsorber measuring 50mm x 50mm were fed 20ml of water, one by slowly dripping over its surface from above using a syringe, and the other by pouring from above using a beaker. Figure 165 depict what was observed.

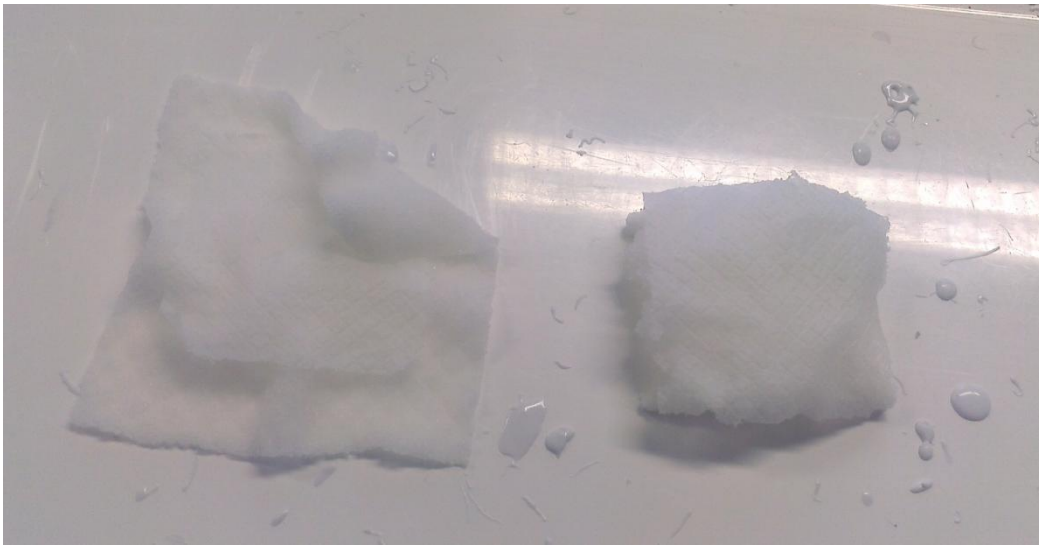


Figure 165: Identical superabsorber sheets supplied with water as different rates: quickly pouring from above (left) and slowly dripping from above (right).

As can be seen in Figure 165 the superabsorber sheet which was provided with a more rapid supply of fluid to absorber has done so in a more uniform manner, retaining its shape better by expanding proportionally in every plane (the original dimensions of the sheet can be seen by looking at the support material which is used to retain the sodium polyacrylate particles and has become separated from the swollen gel), whereas the superabsorber sheet which was slowly supplied with fluid to absorb has expanded mostly in one direction by increasing its thickness. This is the result of more pronounced 'furling' as can be seen more clearly in Figure 166 when the experiment was repeated with water tinted with food colouring.

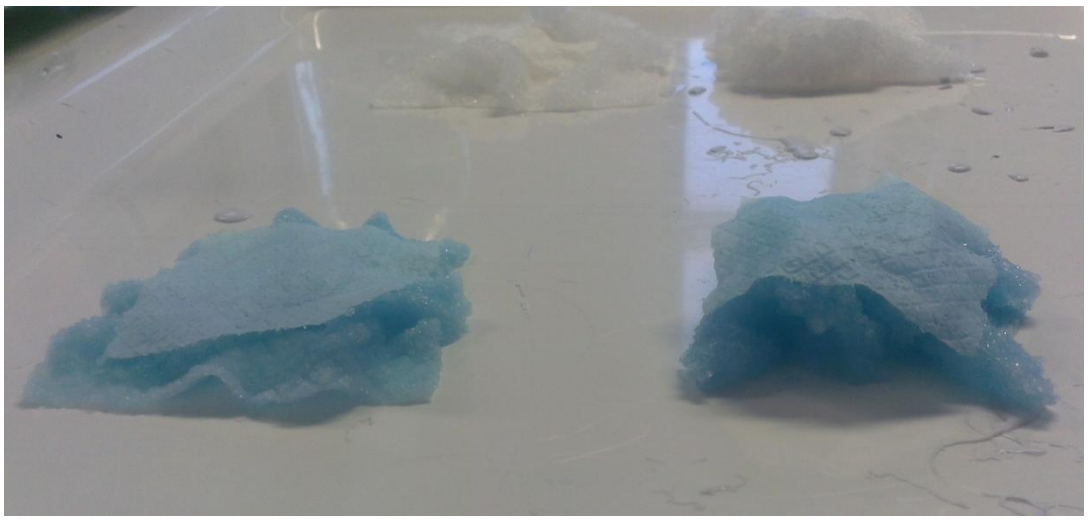


Figure 166: Identical superabsorber sheets supplied with tinted water as different rates: quickly pouring from above (left) and slowly dripping from above (right). 'Furling' is more pronounced in sheet supplied with fluid more slowly by dripping from above.

The furling can be seen yet more clearly when the support material is removed as is shown in Figure 167.

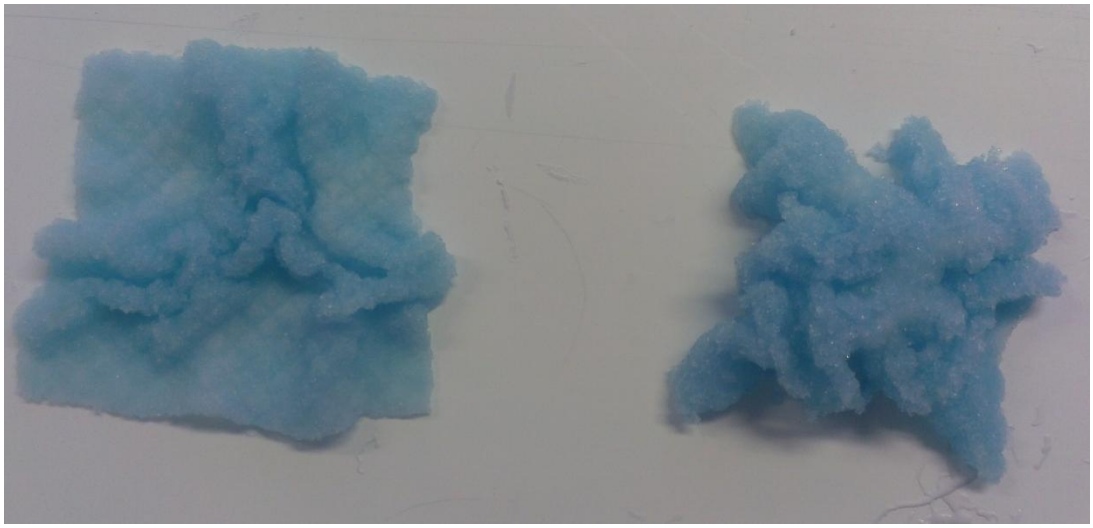


Figure 167: Identical superabsorber sheets supplied with tinted water as different rates: quickly pouring from above (left) and slowly dripping from above (right). With the support material removed furling can be seen more clearly.

Again this further highlights the importance of good distribution of the fluid directly to the dry areas, rather than relying on distribution within the superabsorber material by diffusion through swollen areas. The role of 'fluff' in SAPs and its function as a fluid distributor has been discussed previously (*2.3.1. Superabsorbent Polymers*), and thus one way to achieve better distribution may be to alter the design of the superabsorber sheet to incorporate a higher fraction or different configuration of the support material (fluff) or to change the material itself to one which better distributes the fluid.

3.6.3. Loss of contact with the membrane

One major consequence of the non-uniform swelling and disintegration of the superabsorber is that it results in loss of contact with the membrane. If two non-adjacent areas of the superabsorber swell, the non-swollen region between will be lifted clear of the membrane surface as can be seen in Figure 168.

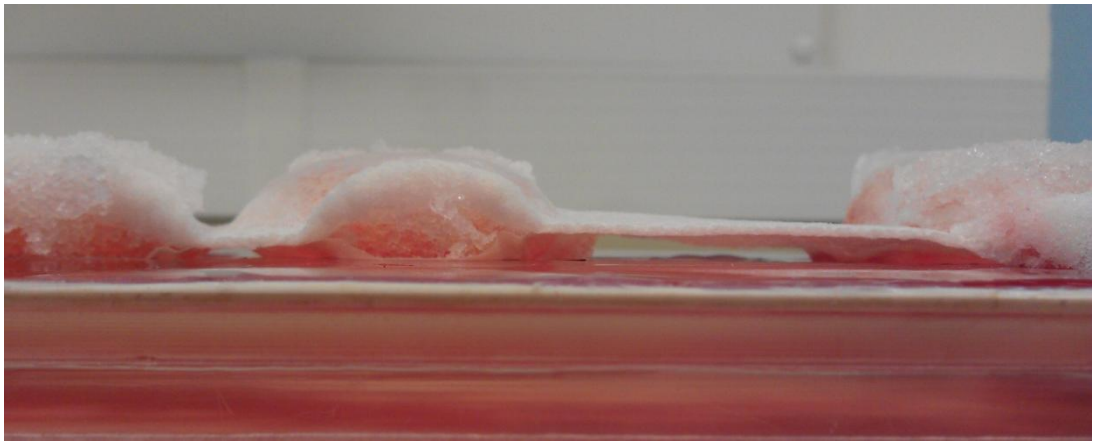


Figure 168: Lifting of un-swollen regions of the superabsorber by the swelling of neighbouring regions

This 'bridging' phenomenon renders large areas of the superabsorber incapable of directly absorbing plasma from the blood, vastly reducing the capabilities of the proposed device. In order for a given region of the superabsorber to absorb plasma there must be a consistent uptake path from the plasma in the blood, through the membrane pores, to the structure of the superabsorber. Without this contact, these regions will only swell by diffusion of fluid through the previously swollen areas which, as has been discussed (*3.6.2.1. Kinetics of superabsorbent hydrogel swelling*), is a far slower pathway. Good contact between the superabsorber and the membrane must be maintained at all times to ensure good performance of the proposed device. The priming step in the Hemosep procedure is for precisely this reason, to create good contact between the superabsorber and the control membrane to ensure a large area for plasma uptake.

3.6.4. Potential impact of patient condition

It was mentioned in the previous chapter that one factor which can impact absorption capacity is pH. Low pH solutions have a high concentration of H_+ ions which will protonate the polymer network and essentially cause it to no longer be a polyelectrolyte (Chatterjee et al. 2002). Blood pH is known to change depending on its gaseous content, e.g. an increase in blood CO_2 will result in an increase in blood acidity (respiratory acidosis) by the disassociation of carbonic acid into

bicarbonate and hydrogen ions ($H_2O + CO_2 \leftrightarrow H2CO3 \leftrightarrow HCO_3^- + H^+$) (Schrier 2010). Since blood CO_2 can increase as a result of poor pulmonary function, a known morbidity associated with CPB, it follows that the blood of a patient who exhibited poor pulmonary function may be more difficult to concentrate with the proposed device as the higher concentration of H^+ ions present could protonate the superabsorber resulting in a decrease in its capacity (Butler et al. 1993; Asimakopoulos et al. 1999; Huang et al. 2003).

3.6.5. Impact on design

It was stated previously (*2.3.1. Superabsorbent Polymers*) that a requirement of SAPs is that when an excess of fluid is encountered, the particles must swell to their full capacity but not degrade or dissolve beyond that (Brannon-Peppas et al. 2012). Although this refers to individual SAP particles, it should also be the case for the material as a whole. Ideally, the superabsorber would swell in a uniform manner so that there would be a uniform osmotic potential across the exchange surface. However, since the rate of fluid supply to the superabsorber is controlled by the membrane and its complex operating behaviour with regards to fouling, it is difficult to ensure uniform swelling. With a rapid rate of plasma extraction and thus a good supply of fluid to be absorbed, uniform swelling should follow, however paradoxically, the rapid rate of plasma extraction is reliant on good contact between the superabsorbent and the membrane which, as has been shown, can be lost with non-uniform swelling. Certain measures may have to be put into place to ensure the superabsorber has a tendency to swell in a more uniform manner. One way to achieve this would be inhibit expansion perpendicular to the control membrane and the associated disintegration physically by utilising a retaining material. In later experiments a retaining material was use to hold the superabsorber in contact with the membrane but allow some expansion perpendicular to the membrane surface. Consequently the retaining material needed to be able to stretch and expand with the superabsorber so that it did not hamper absorption but still exerted a force which maintained good contact between the superabsorber and the membrane. Initially a bandage mesh material was used but later a thin PVC film was utilised.

3.7. Value of design improvements

The combined benefit of the design improvements can be assessed by comparing the rate of haemoconcentration achieved with the first successful system against the rate achieved by a later system incorporating all the design alterations mentioned in this chapter. The early system incorporated an exchange surface area of 110x200mm with and 2mm channel depth and an

internal priming volume of approximately 172.8ml. The technical drawing of this early test rig is shown in Figure 169.

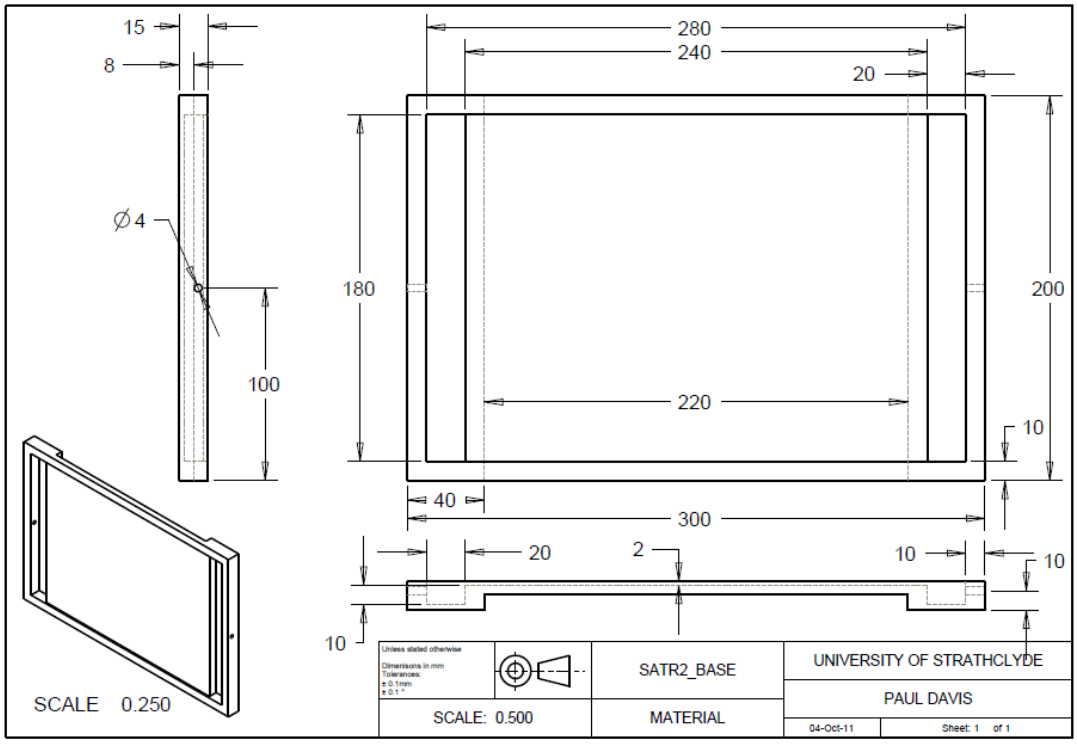


Figure 169: Technical drawing of an early test rig

Blood was pumped at 150ml/min and the device was agitated at 120rpm but the polycarbonate membrane was taut, there was no surface bubble mixing and no retaining film for the superabsorber. The device concentrated 500ml of 17.5%Hct bovine blood to an Hct of 30.5% in 150mins removing approximately 250ml of plasma in that time resulting in a mean plasma extraction rate of 1.7ml/min.

The improved system had a larger exchange surface area of 155 x 200mm with a 2mm channel depth and a greatly reduced internal priming volume of approximately 76.3ml. This was achieved mainly by removing the filling chambers which were present in previous prototypes and allowing blood to enter the exchange channel from below via a 'scooped' inlet. A comparison of these two inlet systems can be seen in Figure 170 and Figure 171.

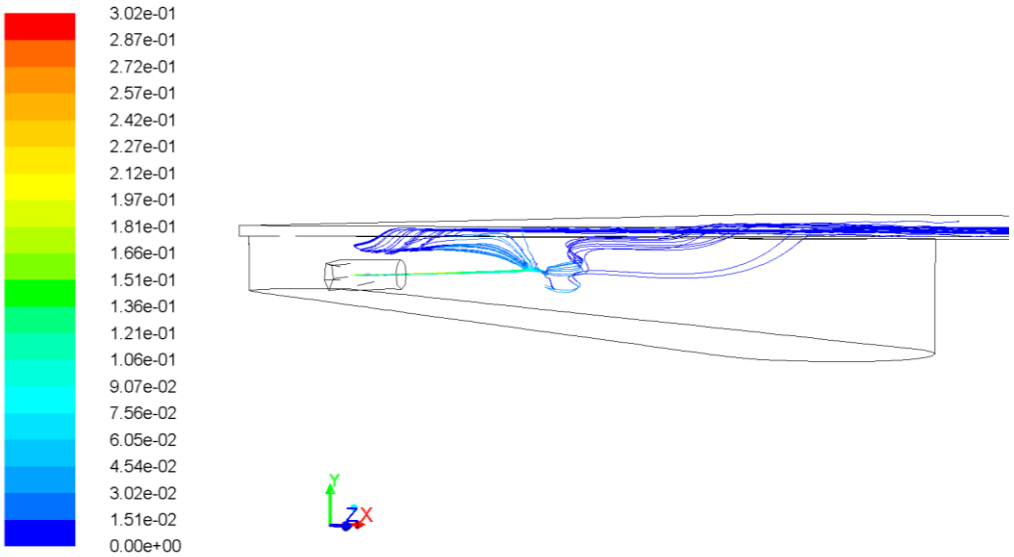


Figure 170: Particle tracks coloured by velocity magnitude (m/s) according to adjacent legend, of blood entering filling chamber of early test rig with large internal prime volume

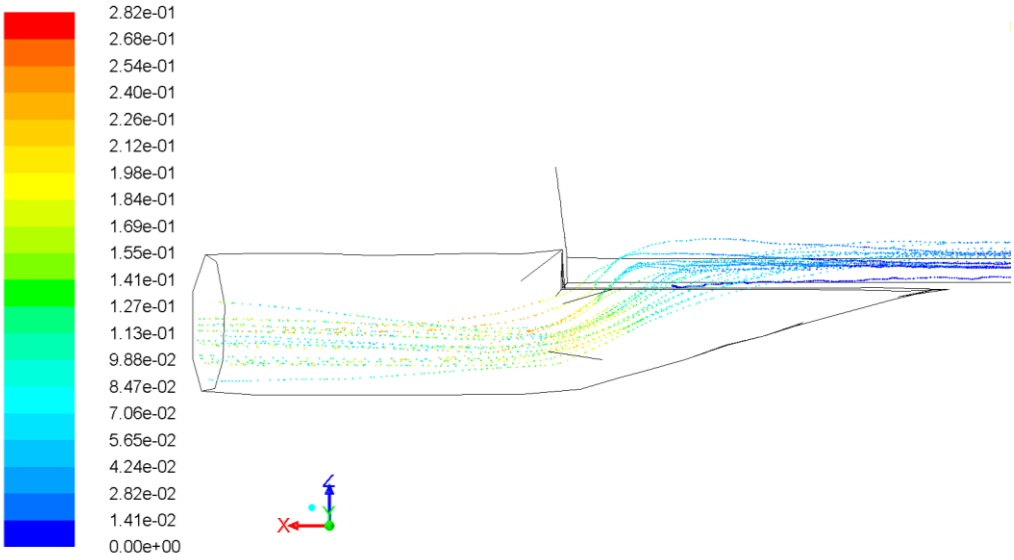


Figure 171: Particle tracks coloured by velocity magnitude (m/s) according to adjacent legend, of blood passing through scooped inlet of improved test rig with small internal prime volume

The technical drawing of the improved test rig is shown in Figure 172.

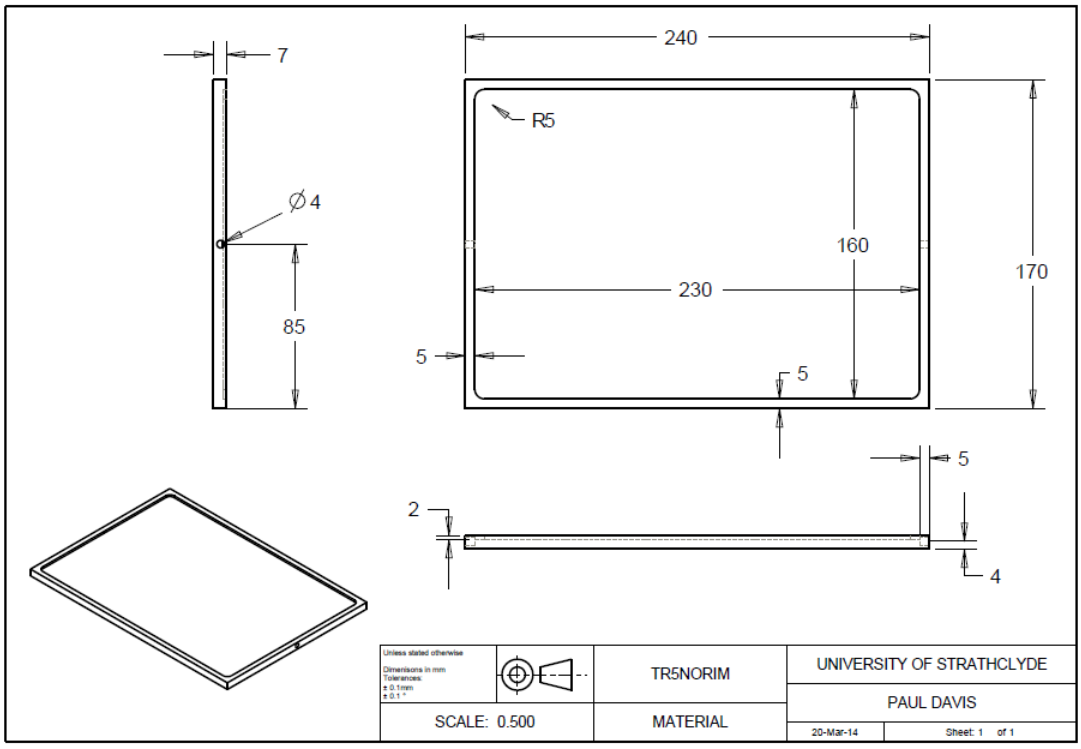


Figure 172: Technical drawing of the improved test rig

Again blood was pumped at 150ml/min but the device was agitated at slower 100rpm and the polycarbonate membrane was slack. Surface bubble mixing was incorporated and a retaining film was in place above the superabsorber. The device concentrated a larger volume of blood, 800ml of 21.5%Hct bovine blood, to an Hct of 39.0% in 40mins removing approximately 360ml of plasma in that time resulting in a mean plasma extraction rate of 9ml/min, over five times greater than the initial device.

Figure 173 shows the comparison between the rates of haemoconcentration for these two experiments.

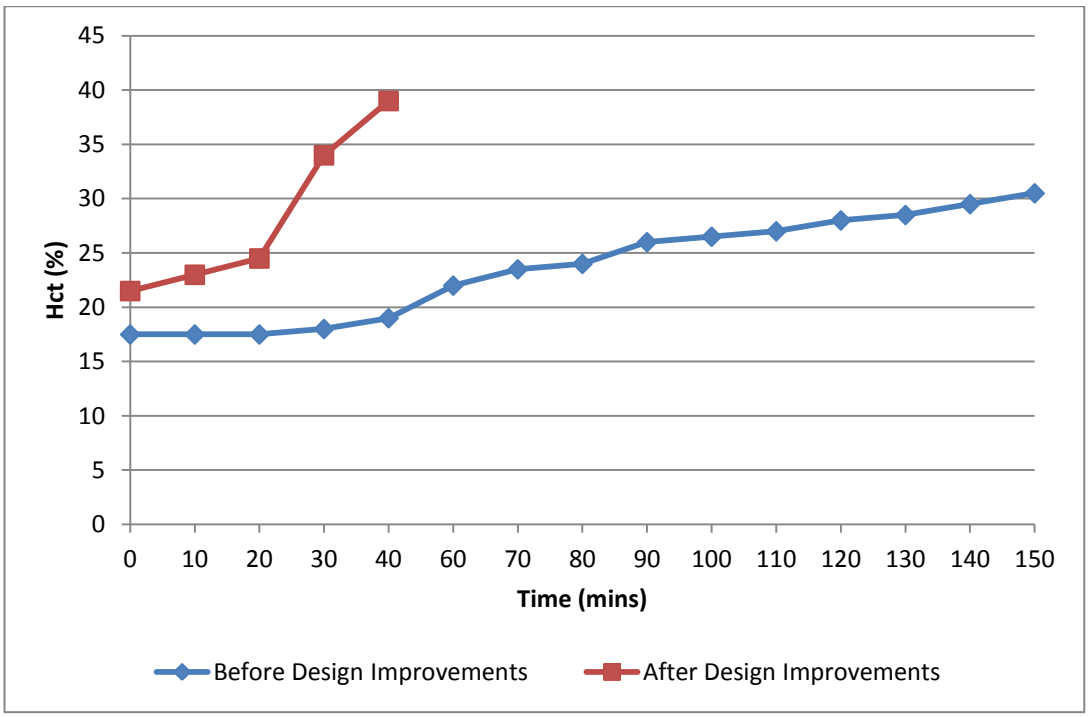


Figure 173: Comparison of the rate of haemoconcentration before and after the design improvements described in this chapter, n=1.

There is a clear improvement in the rate of haemoconcentration achieved by the proposed device. The MUF time of 40mins is now close to what would be expected of a device in a clinical setting and thus is close to meeting the objective set in 1.4. Thesis Objective:

"Haemoconcentration should occur in a timely manner, comparable to current modified ultrafiltration procedures, measured by achieving a suitably rapid rise in haematocrit."

The next objective pertained to the quality of the blood:

"The haemoconcentrated blood should be of good quality with minimal damage to the constituent parts of the blood, specifically maintaining as much as possible the populations of red blood cells and platelets. In addition, demonstration of removal of some pro-inflammatory mediators would be advantageous."

The next chapter will therefore focus on assessment of blood quality as outlined in this objective.

Chapter 4

Device impact on blood quality

4. Device Impact on Blood Quality

The quality of the haemoconcentrated blood which leaves the proposed device is a highly important parameter. Ideally, cell population and morphology should be preserved as considerable loss of functioning cells through use of the device would be harmful to the patient and the poor clinical outcome could far outweigh the benefits of the MUF procedure. The proposed device must demonstrate minimal damage to the blood. This aspect is assessed in this chapter through analysis of experiments with animal blood. If the proposed device can demonstrate successful removal of pro-inflammatory mediators by filtration this would be an additional benefit. This is also assessed in this chapter.

4.1 Initial Blood Observations

4.1.1 Erythrocyte morphology

The appearance of red blood cells can be seen as a measure of blood quality since abnormal morphology can indicate the presence and potential source of a patient's haematological problems (Ciesla 2011; Rosenfeld et al. 2011). A normal RBC will have a circular, biconcave shape with a diameter between 6.5µm and 8.5µm (average 7.3µm) and thickness of 1.2-1.5µm at the centre and 2-2.4µm at the periphery as shown in Figure 174 (Brujan 2010; Tripathi 2011). A typical RBC will have a lifespan of approximately 120 days (Tripathi 2011).

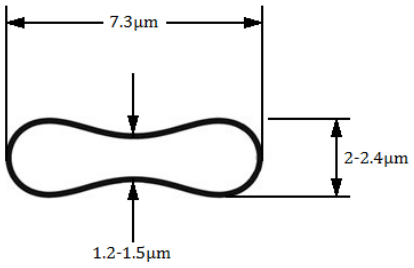


Figure 174: Dimensions of a typical RBC

There are several morphological aberrations of this typical RBC shape which can be observed in the blood, such cells are called poikilocytes (Rosenfeld et al. 2011). The most common poikilocytes are echinocytes, crenated erythrocytes, acanthocytes, schistocytes, spherocytes, keratocytes, ovaloechinocyte (burr cells), eccentrocytes and torocyte (target cells), whilst stomatocytes and ovalocytes are less common (Rosenfeld et al. 2011). Figure 175 shows these cells as they appear in a blood smear under the microscope.

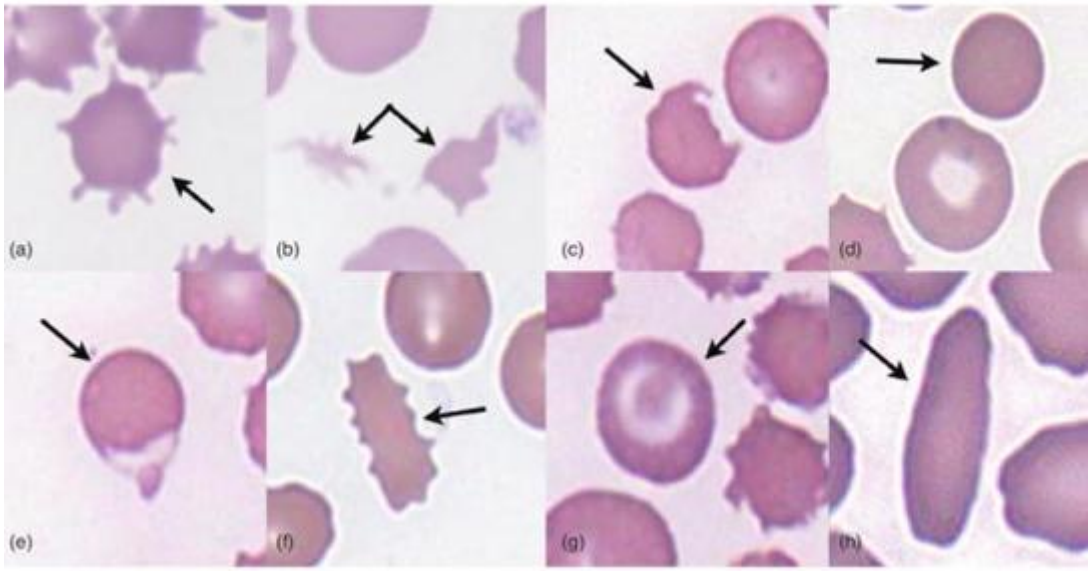


Figure 175: a) Acanthocyte b) Schistocyte c) Keratocyte d) Spherocyte e) Eccentrocyte f) Ovaloechinocyte g) Torocyte h) Ovalocyte as they appear in a blood smear under the microscope (Rosenfeld et al. 2011)

Echinocytes, are RBCs which exhibit many spiky protrusions on their surface and can be subcategorised into several sub-types including crenated erythrocytes and acanthocytes (Rosenfeld et al. 2011). Crenated cells have many uniformly distributed tent-shaped spikes and can be an artefact of slow drying blood smears or in dehydrated animals (Rosenfeld et al. 2011).

Figure 176 shows erythrocytes crenated by shearing after 150mins at 2000dynes/cm^2 (Sutera et al. 1972).

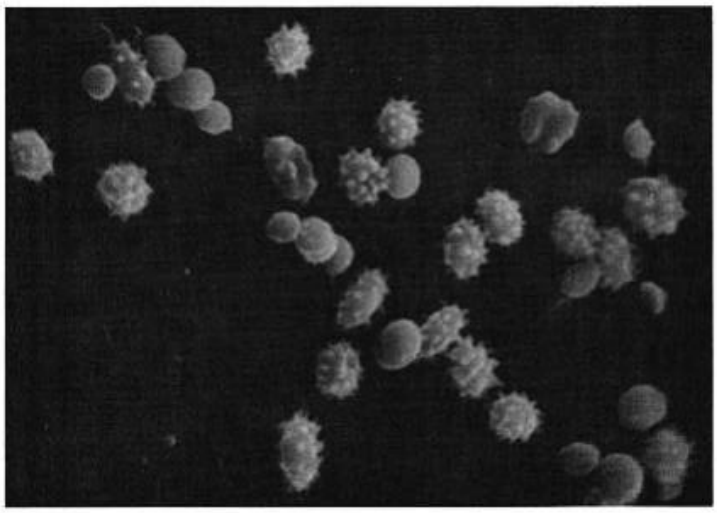


Figure 176: Erythrocytes crenated by shearing after 150mins at 2000dynes/cm^2 (Sutera et al. 1972)

Figure 177 shows the difference in appearance between erythrocytes crenated by shearing and erythrocytes crenated by ATP depletion when viewed under an SEM (Sutera et al. 1972).

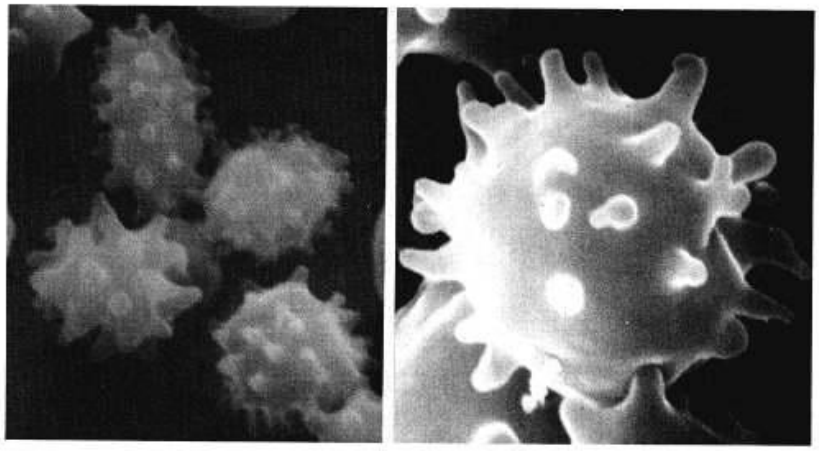


Figure 177: SEM images showing comparison between erythrocytes crenated by shearing (left, x7860 magnification) and erythrocytes crenated by ATP depletion (right, x8000 magnification) (Sutera et al. 1972)

Acanthocytes are not considered artefacts but true morphological changes, and differ from crenated cells in appearance as the thorn-like spicules projecting from their surface are uneven, irregularly placed, and have variable-sized knobs at the spicule tips (Ciesla 2011; Rosenfeld et al. 2011; Bain 2014). When acanthocytes are seen in large numbers they are associated with autoimmune haemolytic anaemia and renal and liver diseases, and when seen in low numbers are associated with iron deficiency and microangiopathy (the formation of fibrin clots in capillaries which damage RBCs) (Ciesla 2011; Rosenfeld et al. 2011). Echinocytosis occurs in hypothermic, heparinised CPB patients and has been attributed to a rise in free fatty acid concentration which has been reported to rise after heparinisation (Riemersma et al. 1982; Bain 2014). Additionally, echinocytosis and crenation often occur as a storage artefact in laboratories that make smears from EDTA-anticoagulated blood rather than fresh blood when there is a delay in making the blood smear, most likely as a result of a fall in ATP (Bain 2014).

Spherocytes are small erythrocytes which have near-normal volume but elevated haemoglobin content and when viewed under the microscope lack the central pallor seen in biconcave shaped erythrocytes and thus appear dark (Ciesla 2011; Rosenfeld et al. 2011; Bain 2014). Spherocytes are associated with several types of erythrocyte damage but particularly haemolytic anaemia (Rosenfeld et al. 2011). Spherocytes form when a macrophage removes a portion of the erythrocyte and it can no longer maintain its biconcave disc shape and instead takes a spherical form (Rosenfeld et al. 2011). If spherocytes are observed in conjunction with other erythrocyte changes (schistocytes, acanthocytes and keratocytes) then the mechanism of spherocyte formation is likely non-immune and may be the result of mechanical haemolytic anaemia, or

may relate to oxidative stress damage to the cell membrane with the formation of haemoglobin crystals in the membrane (Heinz bodies) (Rosenfeld et al. 2011). Similarly, Eccentrocytes are RBCs in which a part of the membrane has fused causing the haemoglobin to be pushed to one side, damage which is caused by oxidative stress (Caldin et al. 2005; Rosenfeld et al. 2011).

Oxidative stress is *"a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, which may lead to tissue injury"* (Betteridge 2000). Several compounds have the capacity to induce oxidative stress in erythrocytes which can lead to haemolysis (Edwards et al. 1996). Patients undergoing cardiac surgery, haemodialysis or extracorporeal membrane oxygenation exhibit an increased oxidative stress response which is related to poorer clinical outcomes and increased mortality (McDonald et al. 2014). Blood contact with the artificial surfaces of extracorporeal devices, the inflammatory response, hyperoxia and preoperative pathophysiological aspects are all contributing factors to the oxidative stress response (McDonald et al. 2014).

Schistocytes are irregularly sized and shaped RBC fragments and consequently are an indication of RBC fragmentation, which, if seen in experiments with the proposed device could be an indication of mechanical induced haemolysis but may also arise from microangiopathy (Rosenfeld et al. 2011; Bain 2014). Schistocytes are often seen along with spherocytes, acanthocytes and keratocytes, particularly when they are the result of mechanical damage (Rosenfeld et al. 2011; Bain 2014). In healthy adult patients 0.2% of RBCs may be schistocytes but in neonate this figure may be as high as 1.9% or even 5.5% in premature neonates (Bain 2014).

Keratocytes are erythrocytes which exhibit small blisters on their surface which subsequently rupture to form pairs of 'horn-like' protrusions, usually two but sometimes four or six, and which are associated with liver disease and iron deficiency but also mechanical damage including microangiopathy (Rosenfeld et al. 2011; Bain 2014).

Ovaloechinocytes (or burr-cells) are elongated cells which exhibit protrusions similar to those seen in crenated cells (Rosenfeld et al. 2011). Burr cells are associated liver and renal diseases but may also be seen in forcibly dried blood smears (Ciesla 2011).

Torocytes, also known as target cells, appear as bull's eye-shaped cells which form because of an excess of cell membrane either due to a decrease in their volume owing to haemoglobin loss or an increase in RBC surface membrane size often attributable to increased cholesterol (Ciesla 2011; Bain 2014). Torocytes are associated with iron deficiency and liver disease but may also appear as artefacts arising from dirty slides (Ciesla 2011; Bain 2014). Dacrocytes, also known as

teardrop cells, occur with conditions related to the bone marrow or in defective erythrocyte formation but also in some haemolytic anaemias (Bain 2014). The teardrop shape of these cells can be seen in Figure 178.

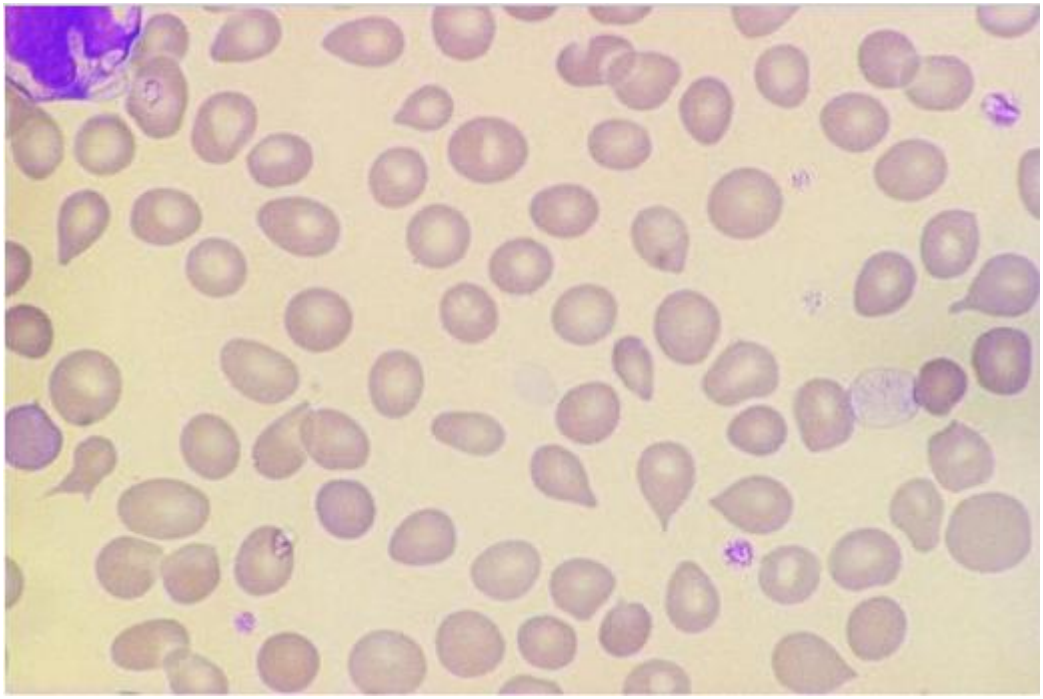


Figure 178: Dacrocytes (teardrop cells) as they appear in a blood smear under the microscope (Bain 2014)

Ovalocytes and elliptocytes are two similar poikilocytes with elongated morphology. However elliptocytes are more elongated with their long axis more than twice the length of their short axis whereas ovalocytes are more egg shaped with their long axis less than twice the length of their short axis (Ciesla 2011; Bain 2014). Ovalocytes and elliptocytes are generally the result of a hereditary condition (Ciesla 2011; Bain 2014).

4.1.2 Erythrocyte observation methodology

In order to compare the morphology of the erythrocytes before and after haemoconcentration blood smears were prepared on clean glass slides (*Sigma Aldrich*) by the 'wedge method' (Wintrobe et al. 2009). A drop of blood was placed in the middle of the slide approximately 15mm from one end and then a second 'spreader slide' is touched to the first slide in front of the blood drop at an angle of approximately 45°. The 'spreader slide' is moved backwards until it comes in contact with the blood drop at which point the drop spreads along the slide edge and the 'spreader slide' is moved forward quickly and smoothly to create a smear approximately 4cm long. No staining was used. a

Care must be taken when producing the smears to avoid artefacts which can arise from dirty slides, damaged slide edges, insufficient contact between the two slides, incorrect angle of contact between the two slides, or a spreading action which is too fast or too slow (Bain 2014). Figure 179 shows a typical smear used for viewing cell morphology.

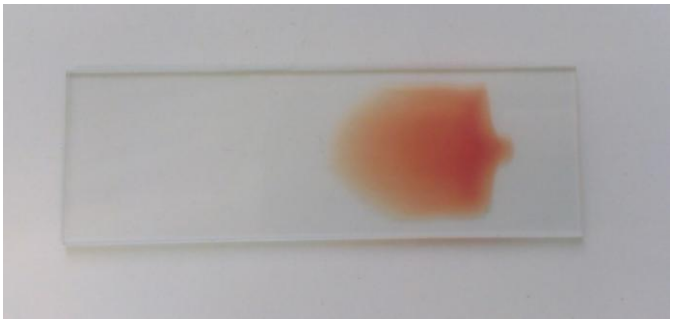


Figure 179: Typical blood smear used for viewing cell morphology

Smears were observed using a *Motic BA210 light microscope (Motic Hong Kong Ltd, Hong Kong)* by placing a coverslip over the area of the smear to be viewed and using oil immersion to view the samples at x100 magnification. Figure 180 shows images from a set of blood smears using the light microscope at x100 magnification of a sample from 400ml of 44.0% Hct whole undiluted bovine blood before haemoconcentration, a sample from 800ml of 22.0% Hct bovine blood diluted with saline before haemoconcentration, and a sample from 380ml of 43.0% Hct bovine blood after 70 mins of haemoconcentration.

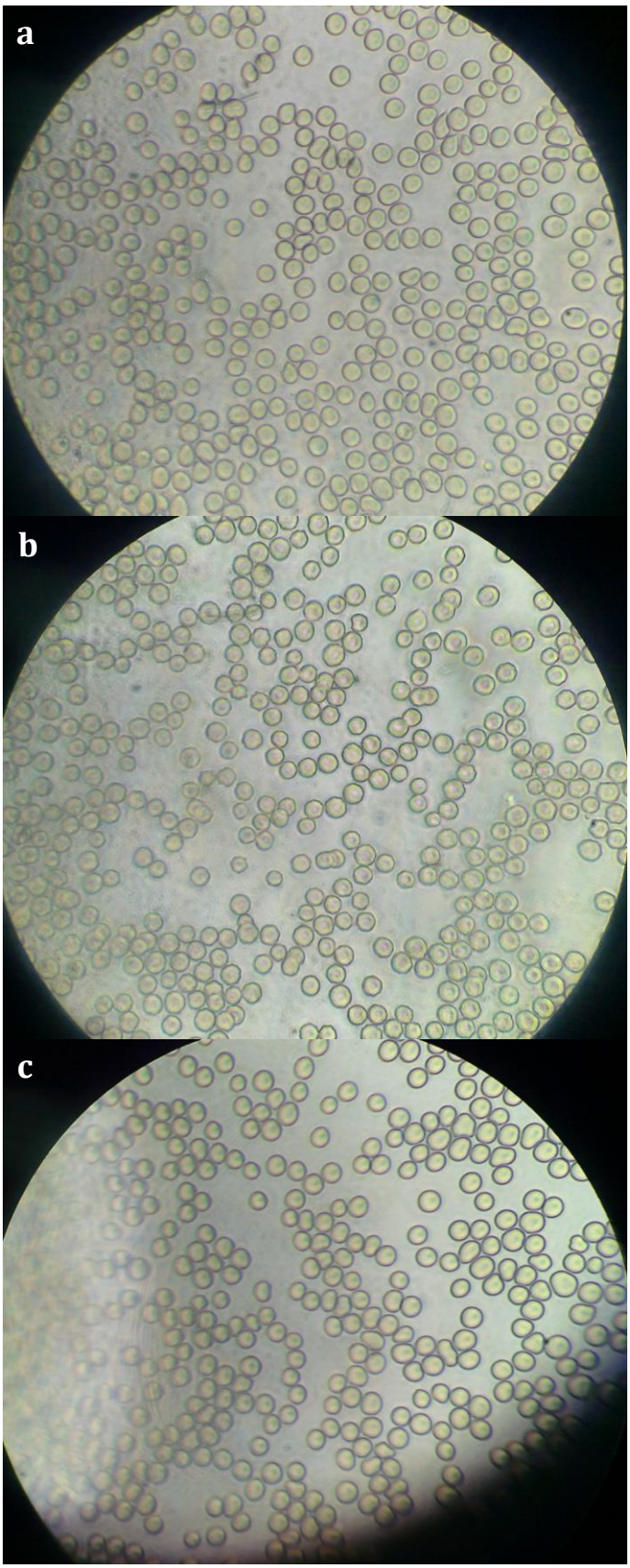


Figure 180: Optical microscope image at x100 magnification of a blood smears from samples from: a) 400ml of 44.0% whole undiluted bovine blood before haemoconcentration b) 800ml of 22.0% bovine blood diluted with saline before haemoconcentration c) 380ml of 43.0% Hct bovine blood after 70mins of haemoconcentration

It can be seen from Figure 180 that there appears to be similar numbers of poikilocytes in the whole undiluted pre-haemoconcentration blood smear (a) and the post-haemoconcentration blood smear (c). There appear to be some dacrocytes and perhaps ovalocytes in the pre-haemoconcentration sample (a) but fewer in the post-concentration sample (c). There do not appear to be echinocytes or fragmented cells such as spherocytes, schistocytes or keratocytes. In the diluted sample (b) there are a small number of echinocytes present but appear to be crenated cells rather than acanthocytes. Based on these initial observations it appears that the proposed device is not causing damage to the erythrocytes although further investigation is needed and will be discussed later in this chapter (*4.3 Complete Blood Counts*).

Similarly, the Figure 181 shows images from a second set of blood smears using the light microscope at x100 magnification of a sample from 400ml of 44.0% Hct whole undiluted bovine blood before haemoconcentration, a sample from 800ml of 22.0% Hct bovine blood diluted with saline before haemoconcentration, and a sample from 355ml of 46.0% Hct bovine blood after 80mins of haemoconcentration. The blood is from the same batch but used for testing the proposed device one day later.

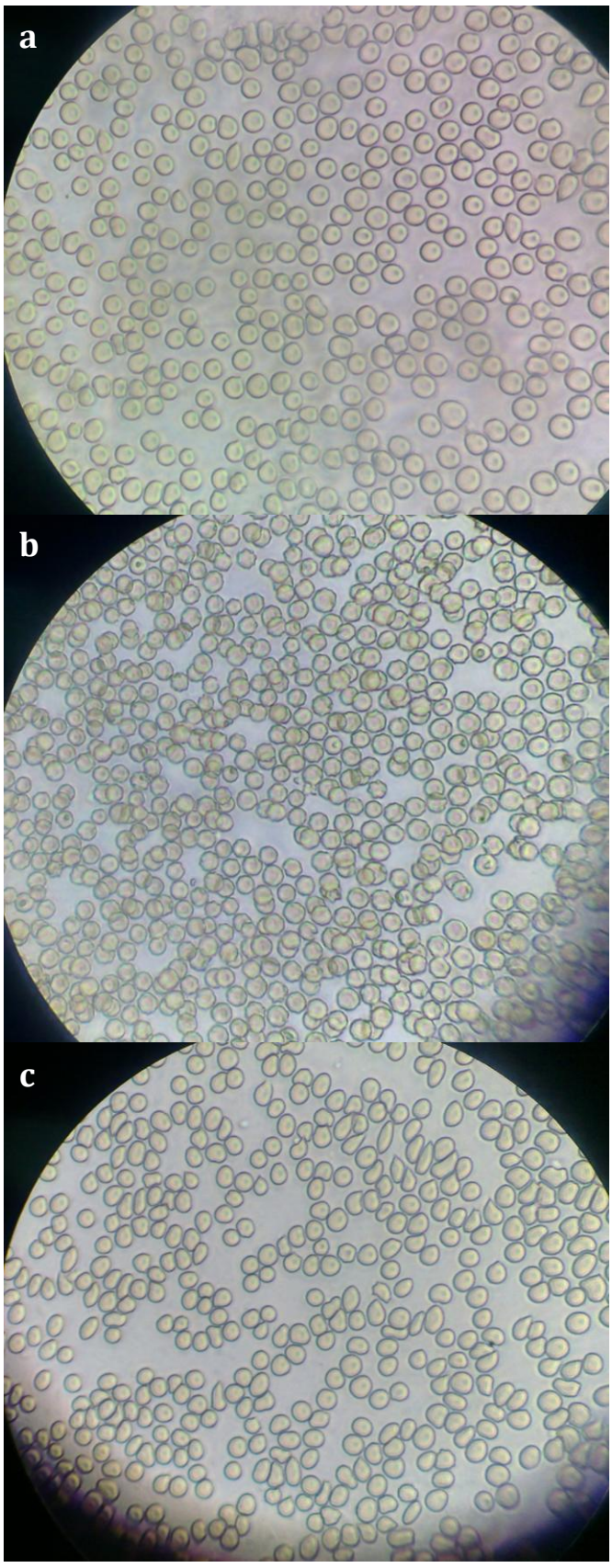


Figure 181: Optical microscope image at x100 magnification of blood smears from samples from from: a) 400ml of 44.0% Hct whole undiluted blood before haemoconcentration b) 800ml of 22.0% Hct bovine blood diluted with saline before haemoconcentration c) 355ml of 46.0% Hct bovine blood after 80mins of haemoconcentration

In this instance from Figure 181: Optical microscope image at x100 magnification of blood smears from samples from from: a) 400ml of 44.0% Hct whole undiluted blood before haemoconcentration b) 800ml of 22.0% Hct bovine blood diluted with saline before haemoconcentration c) 355ml of 46.0% Hct bovine blood after 80mins of haemoconcentration there appears to be more poikilocytes in both the pre-haemoconcentration (a) smear and the post-concentration smear (c). The fraction of poikilocytes appears to be higher in the post-haemoconcentration sample (c) with more dacrocytes and ovalocytes visible, but again there is no evidence of echinocytes or fragmented cells such as spherocytes, schistocytes or keratocytes. Again the diluted sample shows some crenated cells (b). These observations seem to suggest there may be some degree of cell damage attributable to the proposed device however, the dacrocytes, and ovalocytes observed are generally not associated with mechanically induced haemolysis. Moreover, the fraction of poikilocytes is not high, but again, further investigation of cell damage by other measures is required and will be discussed later in this chapter (*4.3 Complete Blood Counts*).

4.2 Manual Platelet Counts

Counting platelets in the blood before and after haemoconcentration with the proposed device will give an indication what fraction of platelets are lost in the procedure either by adhesion to the artificial surfaces (as described in *3.2.3.5. Protein behaviour at a surface*), shear induced aggregation (as described in *3.3.3. Effect of flow on blood*) or lost in the filtrate. Low platelet counts (thrombocytopenia) will have an adverse affect on coagulation and increase the risk of bleeding (MacDonald 2005). In humans a normal platelet count is between approximately 150,000/mm^3 and 400,000/mm^3, with counts below 100,000/mm^3 considered thrombocytopenic (Wood et al. 2003; MacDonald 2005; McConnell 2007). The blood being used to test the device is bovine and thus a platelet count of between 100,000/mm^3 and 800,000/mm^3 is considered normal (Merck et al. 2010).

Initially, platelet counts were done manually with a haemocytometer. A haemocytometer is a precision-made glass slide with two counting areas used for counting blood cells (Estridge et al. 2000). The counting areas of the haemocytometer are two polished raised platforms surrounded by a H-shaped 'moat' so that when a coverslip is positioned on top of the haemocytometer, two counting chambers 0.1mm deep are created as shown in Figure 182 and Figure 183 (Estridge et al. 2000).

Figure 182: Labelled diagram of a haemocytometer with coverslip (Estridge et al. 2000)

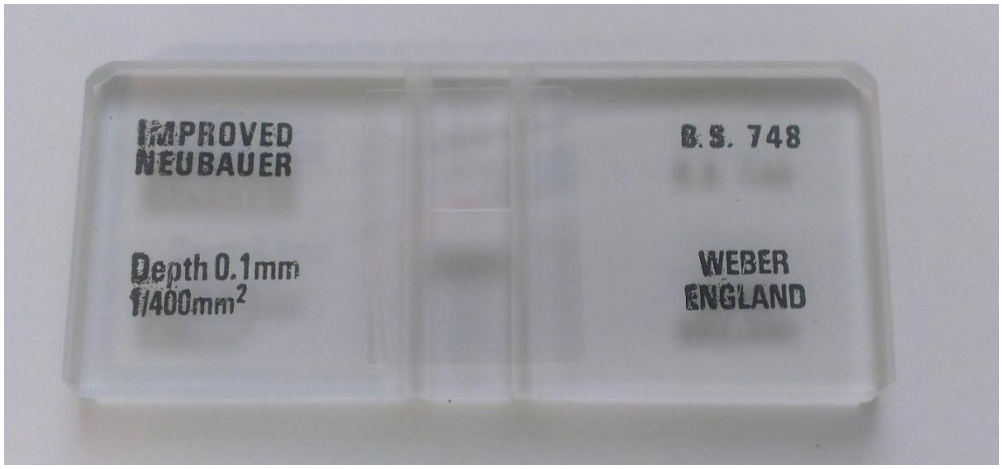


Figure 183: Haemocytometer with coverslip before adding sample

To aid counting, the two counting areas have lines precisely etched on their surface (as shown in Figure 184) which allow the observer to count cell populations within known dimensions, and thus known volumes (Estridge et al. 2000).

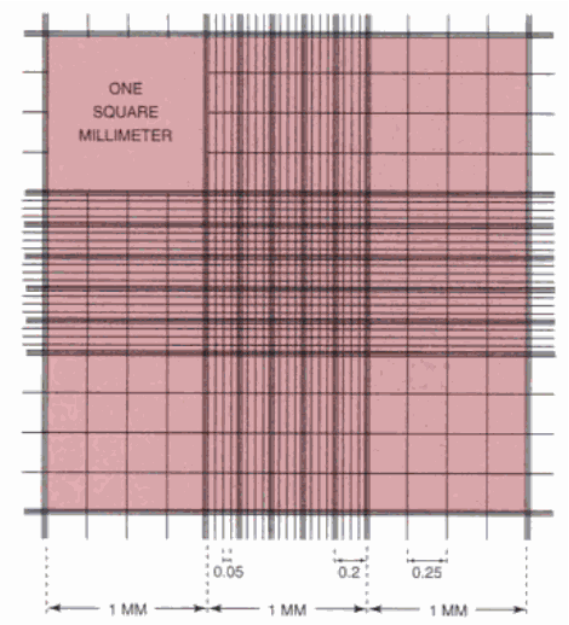


Figure 184: Precisely etched lines in the counting area of the haemocytometer (Estridge et al. 2000)

In order to count platelets 50µl of whole blood was diluted in 950µl of ammonium oxylate and left to stand for 15mins to lyse the erythrocytes which results in a more accurate count (Bain 2014). Next a micropipette was used to carefully load the solution into each counting chamber by touching the tip to the point of contact between the coverslip and the counting platform and allowing the chamber to fill by capillary action (Estridge et al. 2000). The haemocytometer was then covered by a plastic container and allowed to stand for a further 15mins to allow the cells to settle (Estridge et al. 2000). The haemocytometer slide was then place on a *Leitz Diavert optical microscope (Leica Microsystems, Wetzlar, Germany)* and viewed at x40 magnification so that the number of platelets could be counted in each of the four corners square of the fine grid as well as the centre square as shown in Figure 185.

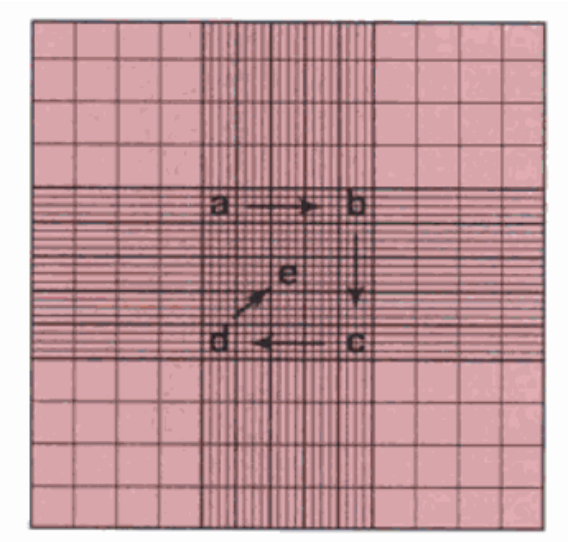


Figure 185: The four corner squares and centre square of the fine grid used for counting platelets (Estridge et al. 2000)

In order to prevent 'double counting' of platelets on the boundary lines between squares, platelets on the bottom and left edges of each square are counted, whilst platelets on the top and right boundary lines are ignored. The sum of the counts in these five squares is equal to the number of thousand platelets per mm^3 (or per µl) of the original blood sample. This is because the total volume of these 5 'squares' is $0.02mm^3$ (*5 x (0.2mm x 0.2mm x 0.1mm)*), or $1/50^{th}$ of a mm^3, and the original blood sample was diluted by a factor of 20 (*50µl blood in 950µl ammonium oxylate*), and thus the platelet count in the haemocytometer is 1000 *(50 x 20)* times smaller than the true value in the original blood sample.

This counting method was performed for both counting chambers on the haemocytometer and the average of the two counts was taken. Platelets appear as small distinct circular or oval shaped elements which are easily identifiable due to their refractivity (Bain 2014). Figure 186 shows images of the haemocytometer counting grid for typical manual platelet counts of bovine blood; a) undiluted before haemoconcentration, b) diluted before haemoconcentration, and c) after haemoconcentration with the proposed device. The platelet counts for three images shown are a) $8,000/mm^3$, b) $3,000/mm^3$, and c) $7,000/mm^3$.

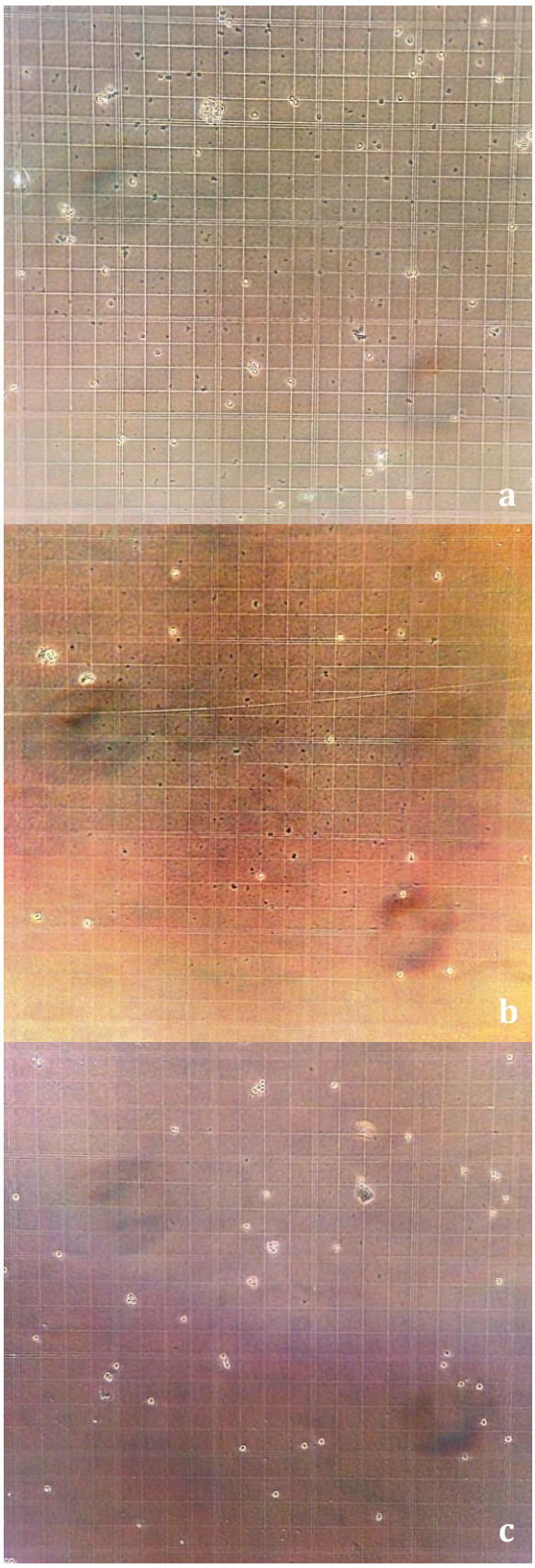


Figure 186: Haemocytometer counting grid for the manual platelet count of bovine blood: a) undiluted before haemoconcentration. The platelet count is 8,000/mm³ b) diluted before haemoconcentration. The platelet count is 3,000/mm³ c) after haemoconcentration with the proposed device. The platelet count is 7,000/mm³

Figure 187 shows the mean platelet count over a series of nine experiments exhibiting successful haemoconcentration of 800ml bovine blood with the proposed device. In each experiment, three samples were taken; two before haemoconcentration, an undiluted sample (labelled 'whole') and a diluted sample (labelled 'diluted'), and one sample after haemoconcentration with the proposed device (labelled 'post-MUF'). The mean platelet count over the nine experiments was calculated after the 'diluted' and 'post-MUF' readings were corrected for the change in Hct from the original 'whole' sample in order to factor out reduced platelet counts due to dilution, and after the whole series was 'standardised' by correcting for Hct a second time to factor out differences in platelet count due to variation in Hct of the original 'whole' sample between the nine experiments.

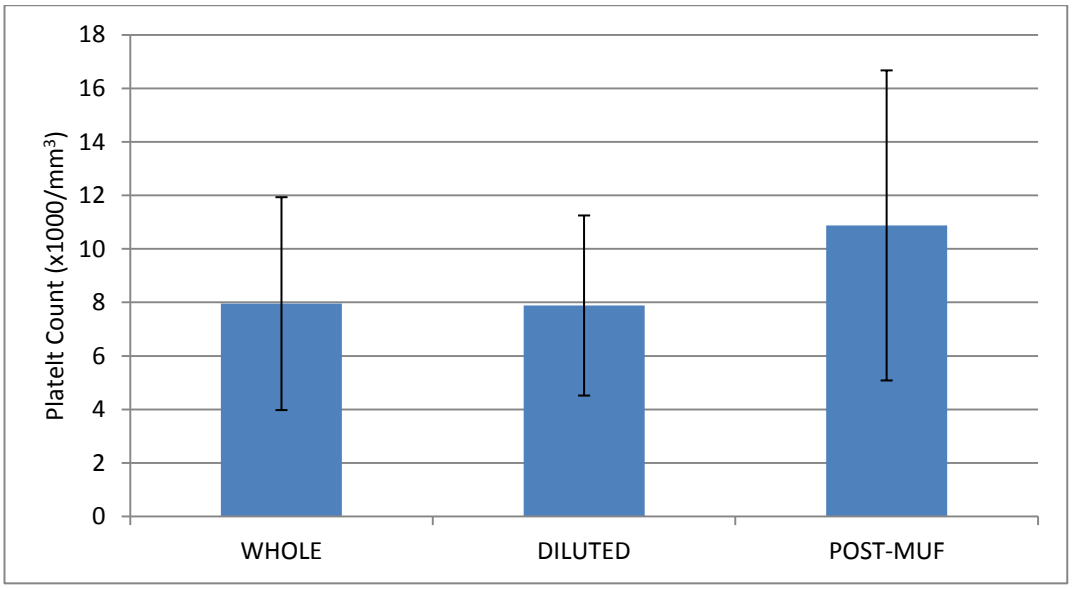


Figure 187: Mean platelet count for a series of nine experiments (n=9, standard deviation error bars) exhibiting successful haemoconcentration of 800ml bovine blood with the proposed device at three intervals; undiluted before haemoconcentration ('whole'), diluted before haemoconcentration ('diluted'), and after haemoconcentration with the proposed device ('post-MUF')

Figure 188 shows the manual platelet counts from the same series of nine experiments exhibiting successful haemoconcentration of 800ml bovine blood with the proposed device; corrected for haematocrit in order to factor out changes in platelet count due to dilution. Again, three samples per experiment were taken; undiluted before haemoconcentration (labelled 'whole'), diluted before haemoconcentration (labelled 'diluted'), and after haemoconcentration with the proposed device (labelled 'post-MUF').

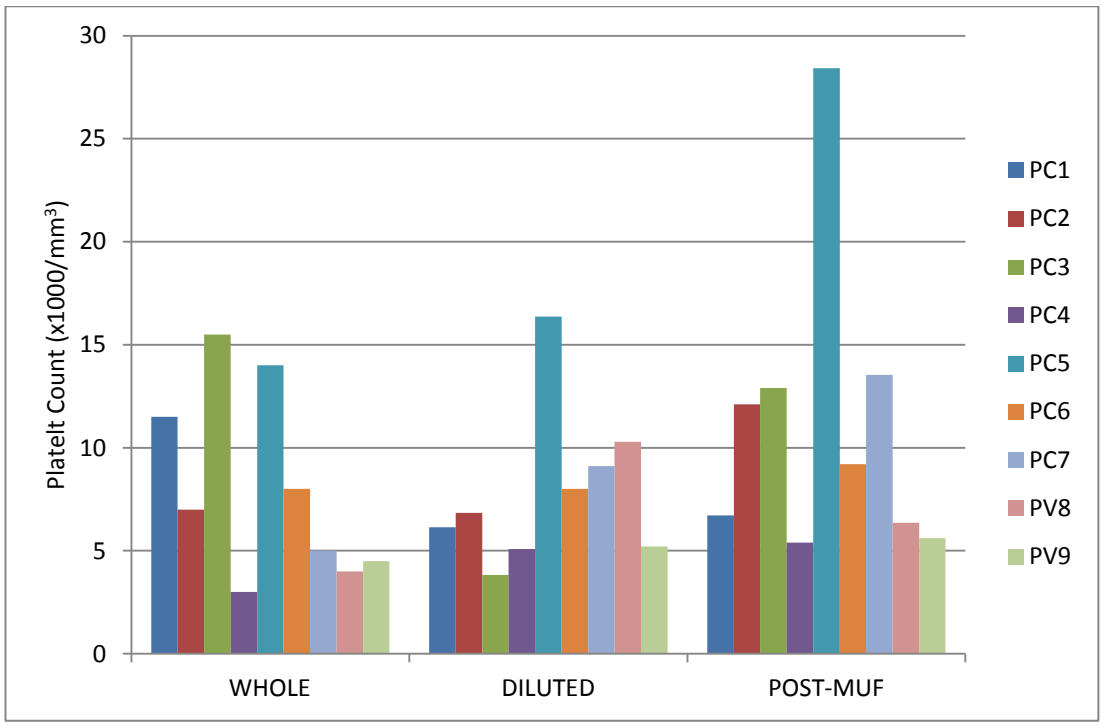


Figure 188: Single observation manual platelet counts from a series of nine experiments exhibiting successful haemoconcentration of 800ml bovine blood with the proposed device at three intervals; undiluted before haemoconcentration ('whole'), diluted before haemoconcentration ('diluted'), and after haemoconcentration with the proposed device ('post-MUF'). Data at the three intervals has been corrected for haemodilution

As can be seen from Figure 187 and Figure 188 the manual platelet counts are surprisingly low, far below the aforementioned normal range of between 100,000/mm^3 and 800,000/mm^3 for bovine blood (Merck et al. 2010). Additionally the standard deviation from the mean in Figure 187 is quite large, suggesting a large amount of variation or error in the readings. These factors suggest that there is either an issue with the blood itself, or that the manual platelet count technique is giving inaccurate results. Manual platelet counts are known to be generally imprecise, particularly when the true count is low, and this, in conjunction with the laborious nature of the manual count procedure, means that manual counts are only used if there is a particular clinical requirement and they are the only technique available (Bain 2014). Subsequently, therefore, the platelet counts produced by the manual technique were compared against the platelet counts produced by the far more accurate flow cytometry technique (flow cytometry will be discussed in more detail in the next section *4.3.1 Methodology*) in order to ascertain the accuracy of the manual platelet count technique. Figure 189 shows the comparison between the manual platelet count and the flow cytometry platelet count.

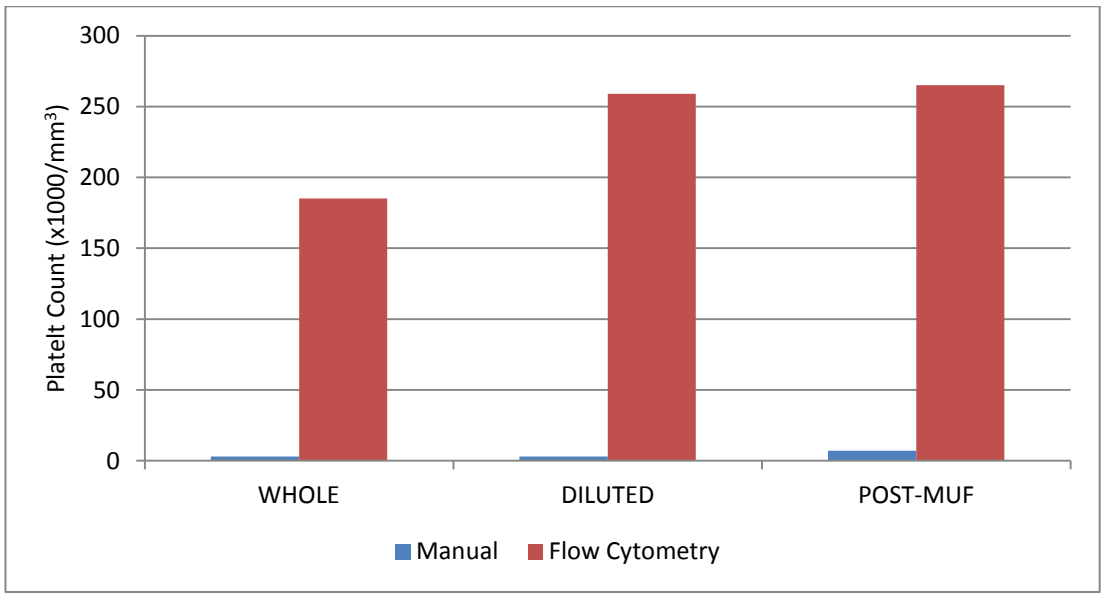


Figure 189: Comparison between the manual platelet count and the flow cytometry platelet count (n=1). Data at the three intervals has been corrected for haemodilution as before

As can be seen, there is a vast difference between the platelet counts produced by these two techniques and, taking into account the known issues with accuracy of manual counts, it is assumed that the manual counts performed thus far have not been reliable and therefore in order to properly ascertain the impact the proposed device has on platelet levels, analysis with a more accurate technique is needed. It should be noted that manual platelet counting (and also blood smear examination) is technique in which the results and outcomes are strongly influenced by the experience the examiner has, and that with repeated attempts and development of skills accurate measures can be achieved which would more closely resemble the findings of automated processes.

4.3 Complete Blood Counts

4.3.1 Methodology

The manual techniques for assessing blood quality have given some insight into the impact of the proposed device but a more accurate and detailed analysis is needed to properly ascertain the impact of the proposed device on blood quality.

Consequently, an experiment series was devised in which the impact directly attributable to the proposed device on both the blood constituents and also selected inflammatory mediator levels could be assessed. In these experiments there were four groups; a static control group, a dynamic control group, a low inflammation group, and a high inflammation group. In the static

control group blood was allowed to sit undisturbed in a polypropylene beaker for 60mins (the standardised MUF time for this experiment series). The static control group would demonstrate the changes in blood constituents and inflammatory mediator levels which occur as a result of the time elapsed. In the dynamic control group blood was circulated through the MUF circuit including the proposed device for 60mins, however, no superabsorber was used and in place of the polycarbonate membrane was a single cellulose acetate sheet. The dynamic control would demonstrate the changes in blood constituents and inflammatory mediator levels which occur as a result of the circulation of the blood and the time elapsed. Additionally, comparisons with a dynamic control is a requirement of blood damage analysis CPB arterial line blood filters as set out by the FDA 510(k) guidance (FDA 2000a). In the low inflammation group blood was haemoconcentrated using the proposed device and the technique described in *3.7. Value of design improvements* (the detailed method is given later in this section) for a period of 60mins. The low inflammation group would demonstrate the changes in blood constituents and inflammatory mediator levels which occur as a result of haemoconcentration with proposed device. In the high inflammation group blood was again haemoconcentrated using the proposed device and the procedure was identical to that of the low inflammation group, but elevated levels of inflammatory mediators were present in the blood. The high inflammation group would again demonstrate the changes in blood constituents and inflammatory mediator levels which occur as a result of haemoconcentration with proposed device, but with particular regards to the potential ability of the proposed device to remove inflammatory mediators in patients with elevated levels.

Each group comprised three experiments, two with blood sourced <6 hours earlier and one with blood sourced the previous day. This was necessary for logistical reasons but it was hoped that it may also provide an insight into the impact of blood age on blood quality in experimental situations such as these and could perhaps inform later studies as to the change in suitability of blood for experimentation over time. As mentioned previously (*3.1.5. Blood source and pre-treatment*), the blood was collected from a bovine source on site at a local abattoir.

In each experiment the bovine blood was 'spiked' with a predetermined amount of both TNF-α and IL-6. These cytokines are both full length recombinant bovine proteins, supplied by *abcam Plc (Cambridge, UK)* and were reconstituted with sterile phosphate-buffered saline (PBS) containing at least 0.1% bovine serum albumin (BSA) and aliquoted and frozen for storage as instructed by the supplier. In the high inflammation group the 'spiked' amount was 1000pg/ml of both TNF-α and IL-6 to represent an elevated level, whereas, in the static control, dynamic control and low inflammation groups the 'spiked' amount was 100pg/ml, representing more normal level (Sekiyama et al. 1994). The reasoning behind studying these two cytokines in particular is discussed in the next section (*4.4.1 Procedure*). The working volume for this series

of experiments was 800ml, 400ml of bovine blood diluted with 400ml of normal saline (0.9% NaCl) and thus the 'spiked' amounts of TNF-α and IL-6 were adjusted accordingly.

The first step of each experiment was to carefully measure 400ml of the bovine blood and record the Hct. To reduce the error associated with the Hct readings, every time Hct needed to be measured two samples were taken and the mean reading was recorded, the two readings never differed by more than 1%. Next the blood was 'spiked' with TNF-α and IL-6 as described above using a micropipette. The blood was gently stirred by hand after which two samples were taken; the first, a 2ml sample, was placed in an EDTA vacutainer to be used for analysis of the blood constituents, and the second, a 1ml sample, was placed in a microcentrifuge tube and centrifuged for 5mins at 5000rpm after which the separated plasma was drawn off and frozen for later use in ELISAs. In tandem with the EDTA sample, a blood smear was also taken for analysis of the blood constituents. After this, the blood was diluted with 400ml of normal saline and gently stirred by hand before again taking an Hct reading, an EDTA sample and blood smear, and a plasma sample.

Once the above steps were completed the testing procedures could commence. As described previously in this section, the static control group blood was left undisturbed in a polypropylene beaker for 60mins, the dynamic control group blood was circulated through the MUF circuit for 60mins with an acetate sheet in place of the for polycarbonate membrane in the proposed device, and in both the low inflammation and high inflammation groups the blood was haemoconcentrated using the proposed device for 60mins (detailed procedure is described later in this section). In each experiment Hct samples were recorded every ten minutes between t=0 and t=60 inclusive, plasma samples were taken at t=0, t=15, t=30 and t=60, and EDTA samples and smears were taken at t=0 and t=60.

The proposed device testing prototype had an exchange surface area of 155 x 200mm with a 2mm channel depth and a greatly reduced internal priming volume of approximately 76.3ml. The polycarbonate membrane was attached to the rig using the cellulose acetate 'framing' method described in *3.1.2 Proposed device test rig* and was allowed to be slack in its frame to encourage wave motion of the membrane (described in *3.5. Agitation and Wave motion*).

The MUF procedure began by priming the membrane a superabsorber with 200ml saline which was pumped into the flow channel and allowed to absorb which happened within approximately 2mins. After this, the blood was pumped through the MUF circuit at a flow rate of 150ml/min using a Stockert roller pump *(Stockert, Munich, Germany)*. The proposed device testing prototype was agitated at 100rpm using a Stuart Scientific SO5 mini orbital shaker *(Bibby Scientific Ltd, Stone, UK)*. Surface bubble mixing was utilised to control membrane fouling and a

retaining film was in place above the superabsorber to maintain contact between it and the control membrane. After 60 minutes the procedure was halted and the concentrated blood was drained from the MUF circuit ready for the aforementioned samples to be taken.

As mentioned the plasma samples were collected and frozen for later use in ELISAs, which will be discussed in the next section (*4.4.1 Procedure*). The EDTA samples and smears were taken immediately for full haematology analysis and smear examination by *Veterinary Diagnostic Services* at the *School of Veterinary Medicine* at the *University of Glasgow* and reports on the full haematology analysis and smear examinations were provided within approximately 24-48 hours.

Each report included the results of 18 haematological parameters: red blood cell count (RBC), total haemoglobin concentration (Hb), cellular haemoglobin concentration (CHb), haematocrit (Hct),mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cell count (WBC), Neutrophil count, Lymphocyte count, Monocyte count, Eosinophil count, Basophil count, platelet count (PLT) mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW).

Many of these parameters are easy to understand. For example RBC, WBC, PLT, and the WBC differential counts (Neutrophil, Lymphocyte, Monocyte, Eosinophil, and Basophil) are simply a measure of the various cell populations expressed as a millions or trillions per litre. Hb and CHb refer to two different method of measure Hb levels (explained in more detail later in this section) and are expressed as a concentration in g/dl. Hct has been discussed previously (*1.2. Conventional Ultrafiltration*) and is a measure of the proportion of a volume of blood that is RBCs, and similarly, PCT is a measure of the proportion of blood that is platelets, both measured as a percentage. Other parameters are calculated from these measured parameters, for example, MCV is a measure of the mean RBC volume in the sample, it is calculated by MCV = *1000 x (Hct/RBC)* when RBC is expressed in trillions per litre, MCV is typically given in fL (Wintrobe et al. 2009). MCH is a measure of the mean mass of Hb in the RBCs and is calculated by *MCH = (10 x Hb) / RBC* and expressed in pg (Wintrobe et al. 2009). Similarly, MCHC is a measure of the mean concentration of Hb in the RBCs and is calculated by *MCHC = (Hb x 100) / Hct* and expressed in g/dl (Wintrobe et al. 2009). PDW is a representation of the variation in RBC size, it is calculated by *RDW = (One Standard deviation of red cell volume ÷ MCV) x 100* and is reported as a percentage (Beckman Coulter 2007). The way in which the initial parameters are measured is described in the following paragraph.

Full haematology analysis was performed using a *Siemens Advia 120 Hematology System (Siemens AG, Berlin, Germany)*. The Advia 120 is a fully automated haematology analyser which performs a complete blood count (CBC), five-part WBC differential (Neutrophil, Lymphocyte, Monocyte, Eosinophil, Basophil), 2-dimensional platelet analysis, and reticulocyte analysis using flow cytometry by a combination of laser light scatter and cytochemical staining (Ameri et al. 2011).

Flow cytometry is a technique which simultaneously measures various cell properties (cytometry) and the cell move in single file (flow) in a fluid column and past a beam of laser light, which allows both qualitative and quantitative analysis of the cells (Turgeon 2005; Wintrobe et al. 2009). As each cell passed through the laser beam it scatters the light by refraction and reflection which is detected by photodetectors and converted to electrical pulses at two points; along the axis of the beam known as forward scatter, and at 90° to the beam axis known as side scatter (hence the 'two-dimensional platelet analysis' mentioned above (Kunicka et al. 2000)) (Turgeon 2005; Wintrobe et al. 2009). The number of pulses generated is proportional to the number of cells passing though the laser beam whilst the degree of light scatter is related to the intrinsic properties of the cells such as their size, shape and granularity (Turgeon 2005; Wintrobe et al. 2009). The Forward scatter is known to be proportional to the cell size and can be useful in discriminating between complete cells and cell debris, and between live and dead cells, whereas side scatter is related to the cell granularity and, in conjunction with forward scatter, can be used to distinguish between lymphocytes, monocytes and granulocytes (neutrophils, eosinophils, and basophils) (Wintrobe et al. 2009). Figure 190 depicts the typical construction of a laser flow cytometer.

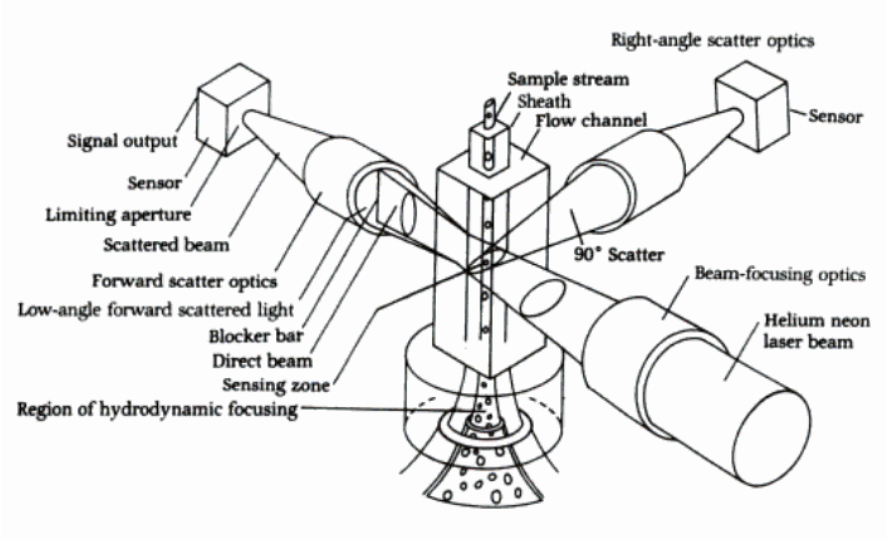


Figure 190: Typical construction of a laser flow cytometer (Turgeon 2005)

In the Advia 120, Leukocytes, erythrocytes and platelets are measured optically and in different channels (Prins et al. 2009; Meintker et al. 2011). RBC analysis is achieved using the laser flow cytometry procedure described above but first the erythrocytes are 'isovolumetrically sphered' (their shape is changed to a sphere) and fixed using a reagent such as a sodium dodecyl sulphate (SDS) and bovine serum albumin (BSA) mix which removes shape variation and improves accuracy of measurements (Kim et al. 1983; Kakkar et al. 2009). By this method the RBC count, Hct and MCV parameters can be found.

Platelets are also counted by a similar two-dimensional laser scatter flow cytometry method and are distinguished from erythrocytes based on their size and refractive index (Prins et al. 2009). By this method the PLT, MPV, PCT and PDW parameters can be measured.

WBC differential is analysed in both a 'peroxidase channel' and a 'basophil/lobulatiry channel' (Kakkar et al. 2009; Prins et al. 2009; Meintker et al. 2011). In the peroxidase channel a reagent lyses the RBCs and fixes and stains the WBCs (Turgeon 2005; Kakkar et al. 2009). The staining occurs in the primary granules of leukocytes containing peroxidase which are numerous in eosinophils and neutrophils, less numerous in moncytes, and not present in basophils, lymphocytes, blasts, or large unstained cells (LUCs) which include activated lymphocytes, myeloid and lymphatic blasts, and plasma cells (Turgeon 2005; Kakkar et al. 2009; Meintker et al. 2011). Cells are then characterised by their size and peroxidase activity which is determined by the scatter and absorbance of tungsten light respectively by flow cytometry (Turgeon 2005; Meintker et al. 2011). In the basophil channel the conformation of the nucleus of the WBCs is measured by exposing the cells to a surfactant at low pH which destroys the membranes and cytoplasm of neutrophils, eosinophils, lymphocytes, and monocytes, leaving the bare nuclei and the intact basophils (Turgeon 2005). Laser flow cytometry then distinguished the leukocytes by the nucleus shape and counts the basophils (Turgeon 2005). The results obtained from both the peroxidase and basophil/lobularity channels are used in conjunction to determine; WBC count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, and basophil count (Turgeon 2005; Kakkar et al. 2009; Prins et al. 2009; Meintker et al. 2011). Additionally, *Veterinary Diagnostic Services* also manually assessed the WBC differential count using a Romanowsky stain to confirm the results provided by the Advia 120. A Romanowsky stain imparts specific colour to various blood components of a blood smear which is then assessed under a microscope (Turgeon 2005).

The Advia 120 measures haemoglobin by two methods; one using two-dimensional flow cytometry on isovolumetrically sphered erythrocytes and reticulocytes (referred to as CHb in this thesis), and one in which cells are lysed and cell-free haemoglobin is measured by a modified cyanmethemoglobin colorimetric method (referred to as Hb in this thesis) (Bauer et al.

2008; Prins et al. 2009). In the flow cytometry method, cellular Hb is measured knowing that high angle laser scatter is related to Hb concentration within the cells (Bauer et al. 2008; Prins et al. 2009). The cellular Hb mass is measured by multiplying the erythrocyte or reticulocyte volume by the Hb concentration on a cell-by-cell basis, and the reported cellular Hb is a mean of these values (Bauer et al. 2008). The colorimetric method is based on oxidation of the haem iron in the Hb from the ferrous to the ferric state and is subsequently combined with cyanide to form cyanmethemoglobin which has an absorbance of 540nm light which is directly proportional to Hb concentration (Bauer et al. 2008; Rodak et al. 2013). In this method the Hb content of a haemolysed sample is measured and then the haematocrit is used to calculate a mean concentration of Hb within those cells (Bauer et al. 2008). Hb measured by this method (MCHC) can be falsely elevated by haemolysis whereas Hb measured by the cellular flow cytometry method (sometime termed corpuscular HGB concentration mean or CHMC) will not (Bauer et al. 2008). The difference between the MCHC and the CHCM (or between Hb and CHb) can therefore accurately quantify the cell-free haemoglobin and thus degree of haemolysis in a sample (Bauer et al. 2008).

The figures in the following sections show the mean measurements for the various aforementioned blood constituent parameters. Reference range values from the Merck Veterinary Manual (Merck et al. 2010) are shown as maximum and minimum where appropriate. Significant differences between time intervals within the same group were calculated by a paired t-test with $p<0.05$ considered significant.

4.3.2 Haemoconcentration

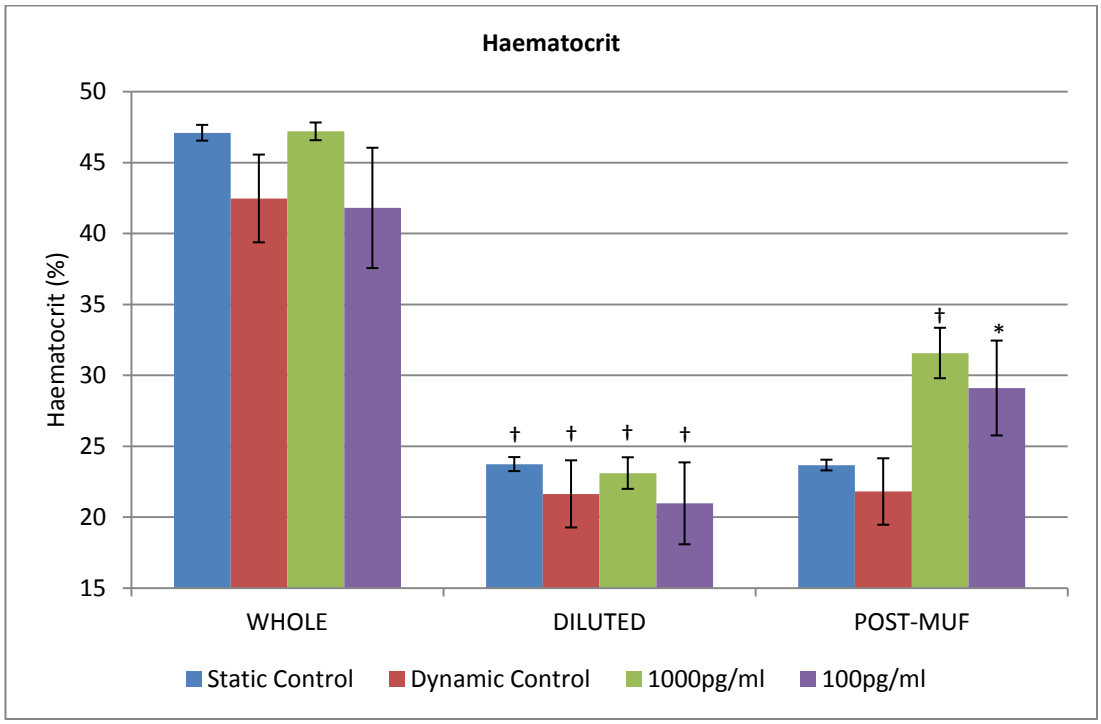


*Figure 191: Hct for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

From Figure 191 it can be seen that, as expected, there is a significant decrease in Hct after dilution in each group. In the static control and dynamic control groups where there was no attempt at haemoconcentration there is no significant change in Hct after the 60min period. In the high inflammation and low inflammation groups that received MUF with the proposed device there was a significant rise in Hct after the 60min period. This further demonstrates the haemoconcentrating ability of the proposed device.

4.3.3 Erythrocyte levels and condition

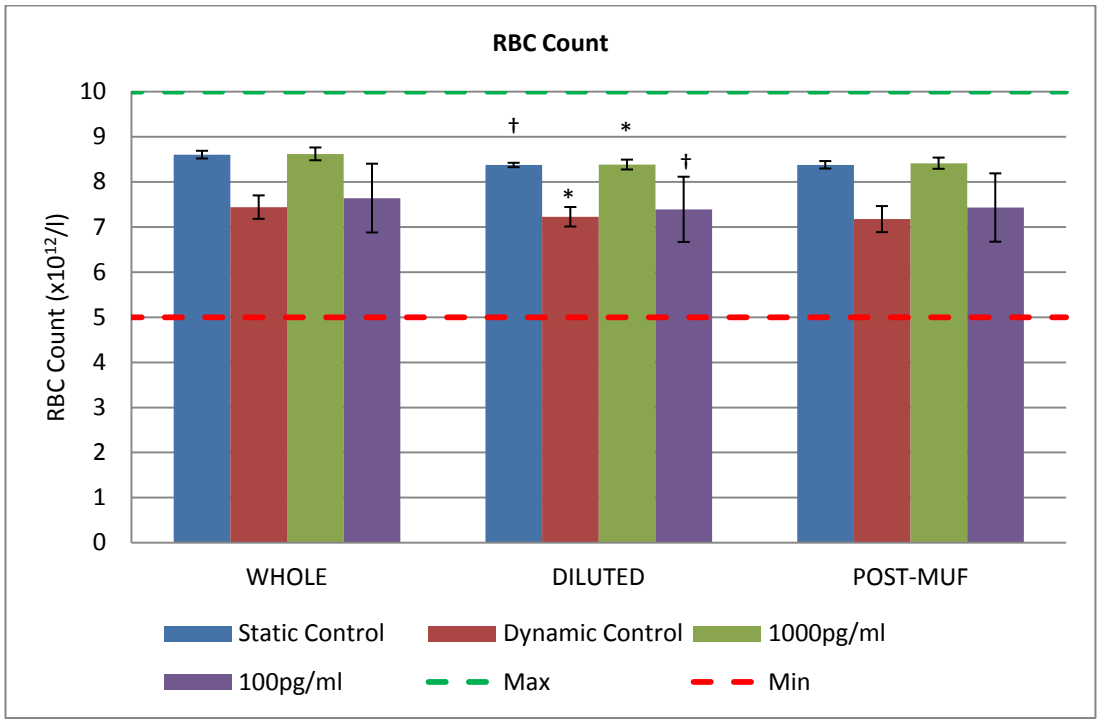


*Figure 192: RBC count (x10¹²/L) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. Counts have been corrected for differences in Hct to eliminate changes due to dilution of blood alone. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

It can be seen from Figure 192 that there is a significant decrease in RBC count after dilution in each group but no significant difference in RBC count after 60mins MUF or no intervention in any group. The decrease in RBC count after dilution may be due to haemolysis or aggregation of RBCs arising from the mixing procedure (after adding the saline for dilution the blood-saline mixture was stirred by hand) or from the saline itself e.g. an adverse tonicity. Although there is a significant decrease in RBC count after dilution, all the values are within the reference range. More importantly however, these results show that the MUF device does not lead to any loss of RBCs.

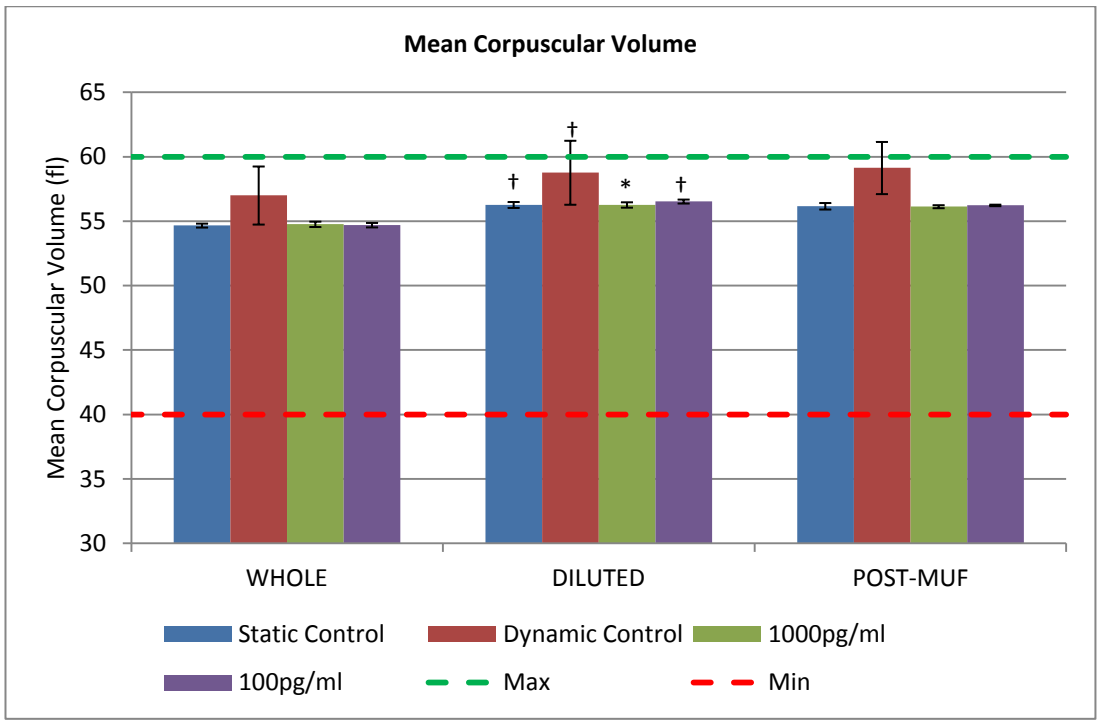


*Figure 193: MCV (fL) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

It can be seen from Figure 193 that there was a significant rise in MCV in each group after dilution but no significant difference after 60mins MUF or no intervention in any group. The increase in MCV after dilution could be due to erythrocyte swelling if the saline solution used for dilution was hypotonic, which, if coupled with lysis of RBC through bursting could also explain the decrease in RBC count seen previously in this section. Again, however, the most important observation with regards to assessing the device impact on blood quality is that the MUF device does not lead to any rise or fall in MCV and thus there is no indication that the functioning of the RBCs will be adversely affected by its use.

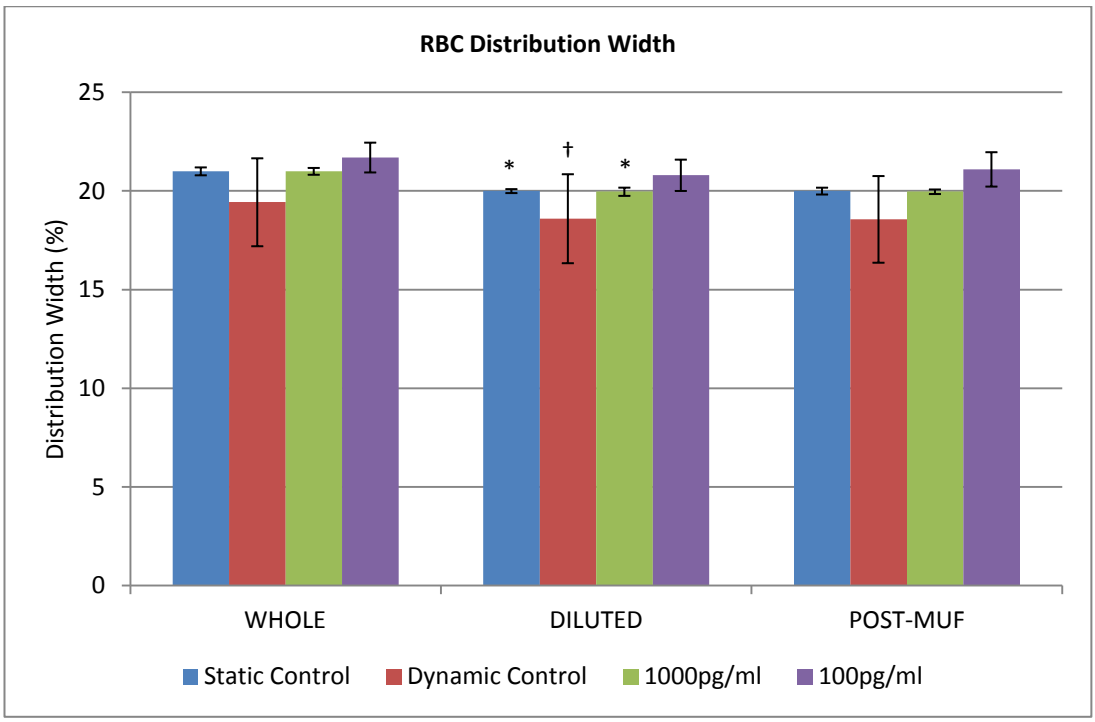


*Figure 194: RDW (%) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

Figure 194 shows that in the static control, dynamic control and high inflammation groups there was a significant decrease in RDW after dilution but again no significant difference after 60mins MUF or no intervention in any group. A reduction in RDW after dilution fits with the previous observations of a decrease in RBC count and an increase in MCV after dilution and particularly with the suggestion of RBC swelling which would reduce the variation in RBC sizes and hence reduce the RDW. Small immature RBCs could also be more susceptible to damage during the dilution procedure. Most importantly, however, as with MCV, this result shows that the MUF device does not lead to changes in RDW and thus there is still no indication that the functioning of the RBCs will be adversely affected its use.

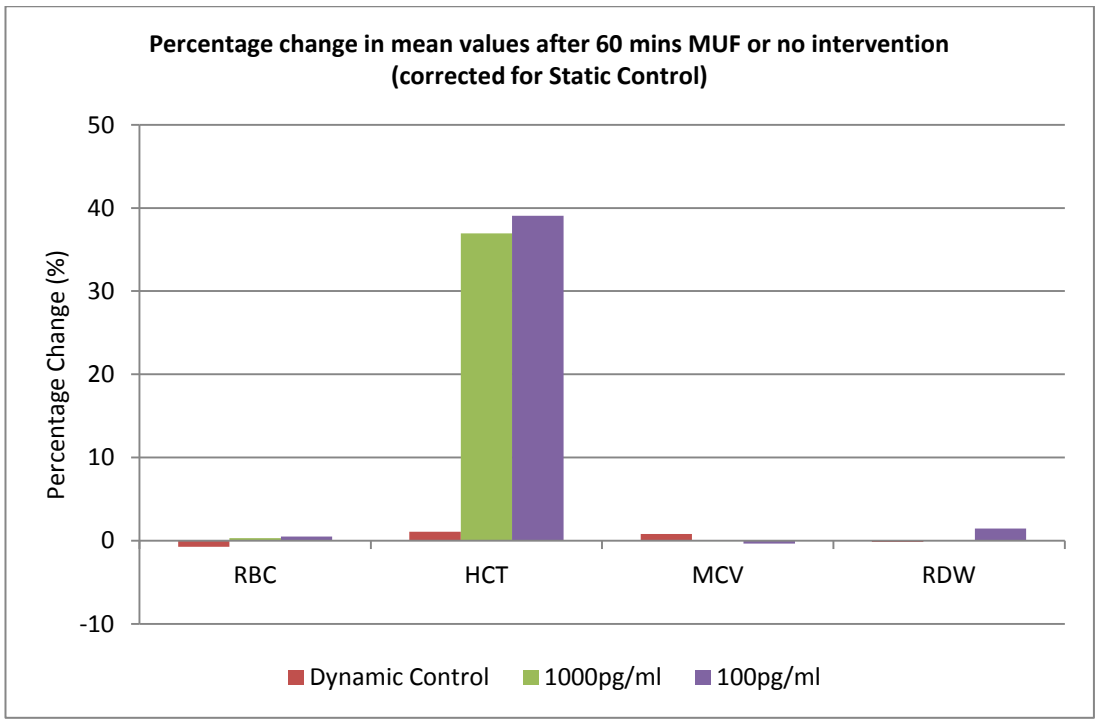


Figure 195: Percentage change in mean value (n=3) of the various RBC parameters after 60mins of MUF or no intervention in the dynamic control, high inflammation and low inflammation groups after the means have been corrected for the change observed in the static control.

Figure 195 shows the percentage change in mean value of the various RBC parameters after 60mins of MUF or no intervention in the dynamic control, high inflammation and low inflammation groups after the means have been corrected for the change observed in the static control. By correcting for the static control, the change shown in Figure 195 is the change which can be attributed to flow through the device, and in the case of the high and low inflammation groups, flow and filtration. Again, this highlights the haemoconcentrating ability of the device whilst affecting very small changes in RBC parameters.

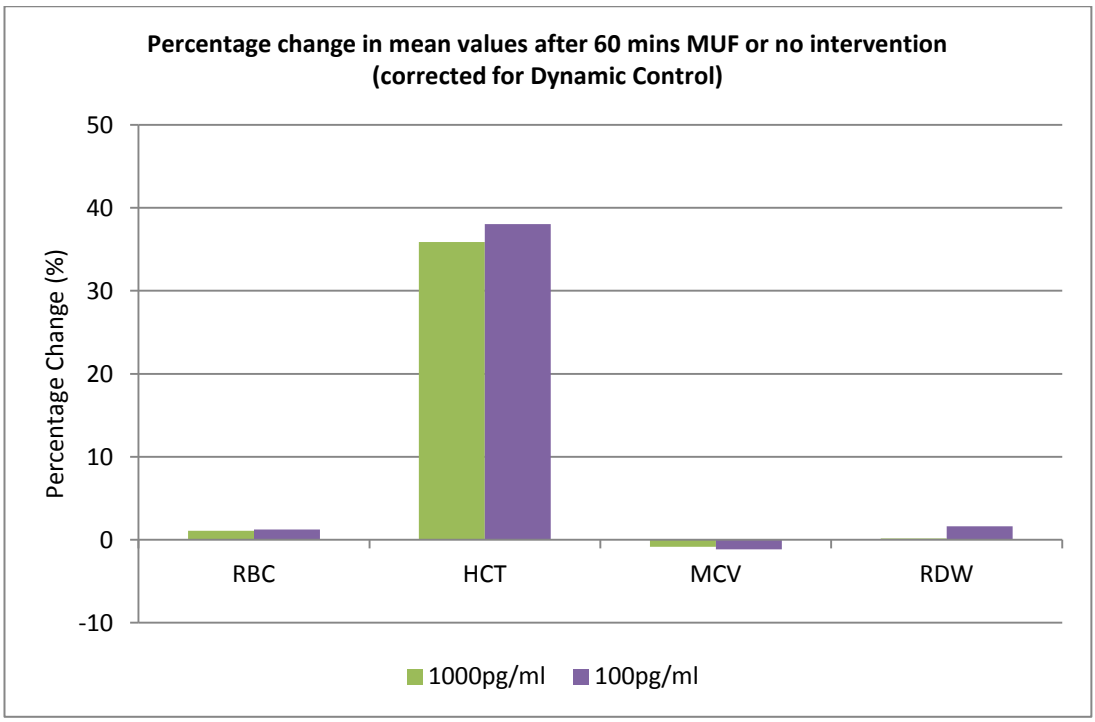


Figure 196: Change in mean value (n=3) of the various RBC parameters after 60mins of MUF or no intervention in the high inflammation and low inflammation groups after the means have been corrected for the change observed in the dynamic control.

Figure 196 shows the percentage change in mean value of the various RBC parameters after 60mins of MUF or no intervention in the high inflammation and low inflammation groups after the means have been corrected for the change observed in the dynamic control. By correcting for the dynamic control, the change shown in Figure 196 is the change which can be attributed to filtration alone. Yet again, the change in Hct is clear whereas there are only very small changes observed in the other RBC parameters due to filtration. Note that this change is a percentage change relative to the immediate pre-MUF levels and not a Hct percent change (fraction of blood volume that is cells).

4.3.4 Leukocyte levels and differential

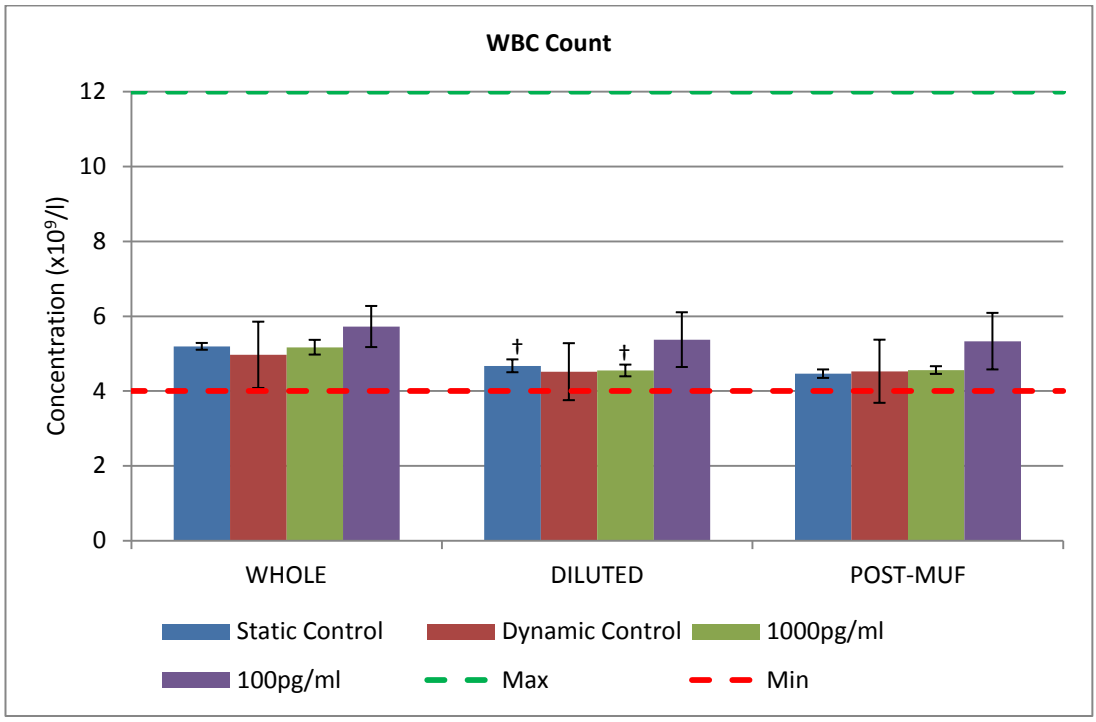


*Figure 197: WBC count (x10⁹/L) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. Counts have been corrected for differences in Hct to eliminate changes due to dilution of blood alone. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

Figure 197 shows that there was a significant decrease in WBC count after dilution in both the static control and high inflammation groups but WBC levels remain within the normal range. There is no significant change in WBC count after 60mins MUF or no intervention in any group and thus the proposed device does not have a deleterious effect of WBC populations. Overall, the WBC counts are at the low end of the normal expected range which may indicate an issue with blood quality at the source which is discussed later in this chapter (*4.5 Impact of Blood Age and Source*).

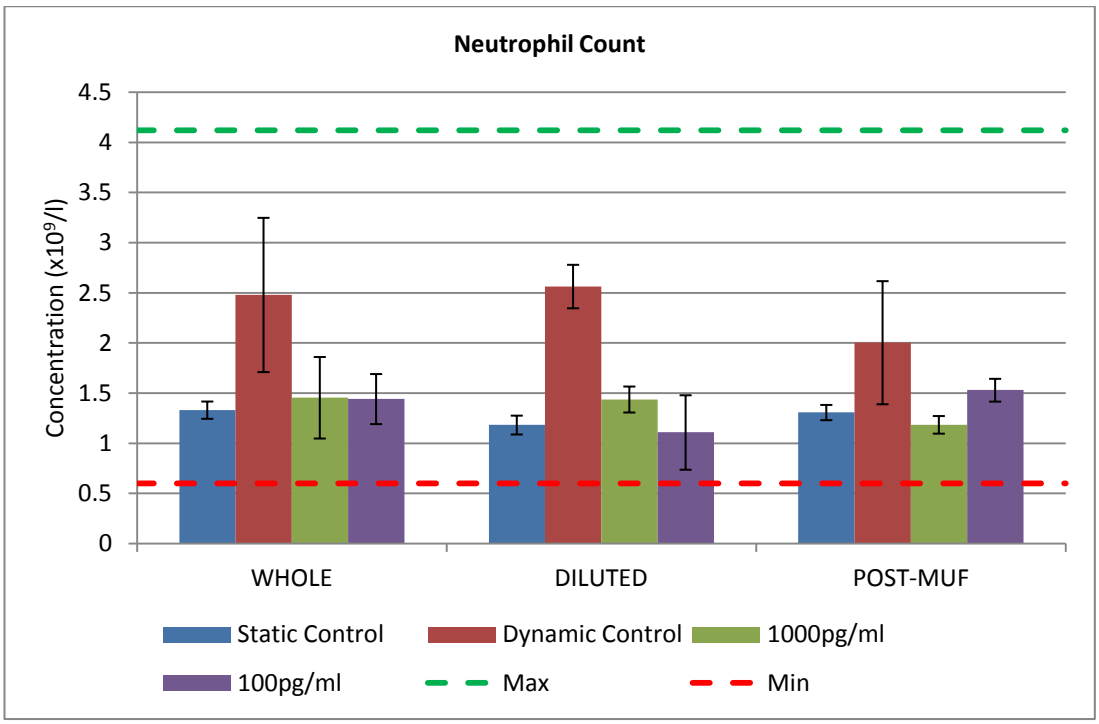


*Figure 198: Neutrophil count (x10⁹/L) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. Counts have been corrected for differences in Hct to eliminate changes due to dilution of blood alone. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

Neutrophils account for 40-75% of circulating leukocytes and are involved in the early stages of the inflammatory response (Colville et al. 2015). Neutrophils travel in the blood to the site of inflammation and are attracted by chemotaxis to an infection site where they will phagocytise microorganisms in the tissue such as bacteria and other microscopic debris (Colville et al. 2015). Figure 198 shows that there was no significant difference in neutrophil levels at any time in any group and thus the proposed device is not associated with any change in neutrophils. The counts are within the normal reference range.

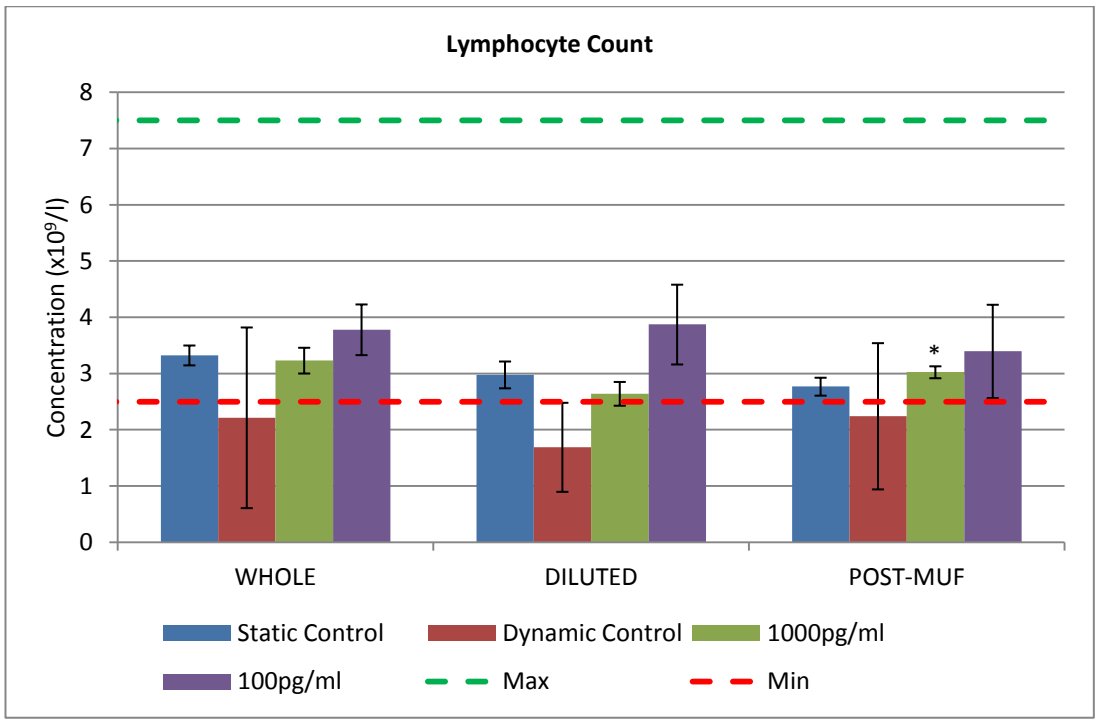


*Figure 199: Lymphocyte count (x10⁹/L) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. Counts have been corrected for differences in Hct to eliminate changes due to dilution of blood alone. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

There are four types of lymphocyte: T-cells which are responsible for cell-mediated immunity and activating B-cells, B-cells which are ultimately responsible for the antibody-mediated humoral immunity, Plasma cells which are derived from B-cells in response to an antigenic stimulus and produce, store and release antibodies, and 'Natural Killer' (NK) cells which are granular lymphocytes that bind to virus-infected/stressed/tumour cells and induce apoptosis (Colville et al. 2015). Most of the lymphocytes in the peripheral blood are T-cells, though NK-cells are also found there whereas B-cells and plasma cells are rarely present (Colville et al. 2015). Figure 199 shows that, with the exception of a significant rise in the high inflammation group after MUF, there was no significant difference in neutrophil levels at any time in any group. Since the significant rise in lymphocytes after MUF in the high inflammation group was not replicated in the low inflammation group the cause may be related to the cytokine levels although it is unclear by what mechanism this could occur in vitro and further investigation may be needed. Importantly however, the proposed device is not associated with any significant reduction in lymphocyte populations. Although the rise in lymphocyte count in the high inflammation group is significant, the numbers remain within the reference range. Again, overall the Lymphocyte counts are at the low end of the normal expected range which may indicate an issue with blood quality at the source which is discussed later in this chapter (*4.5 Impact of Blood Age and Source*).

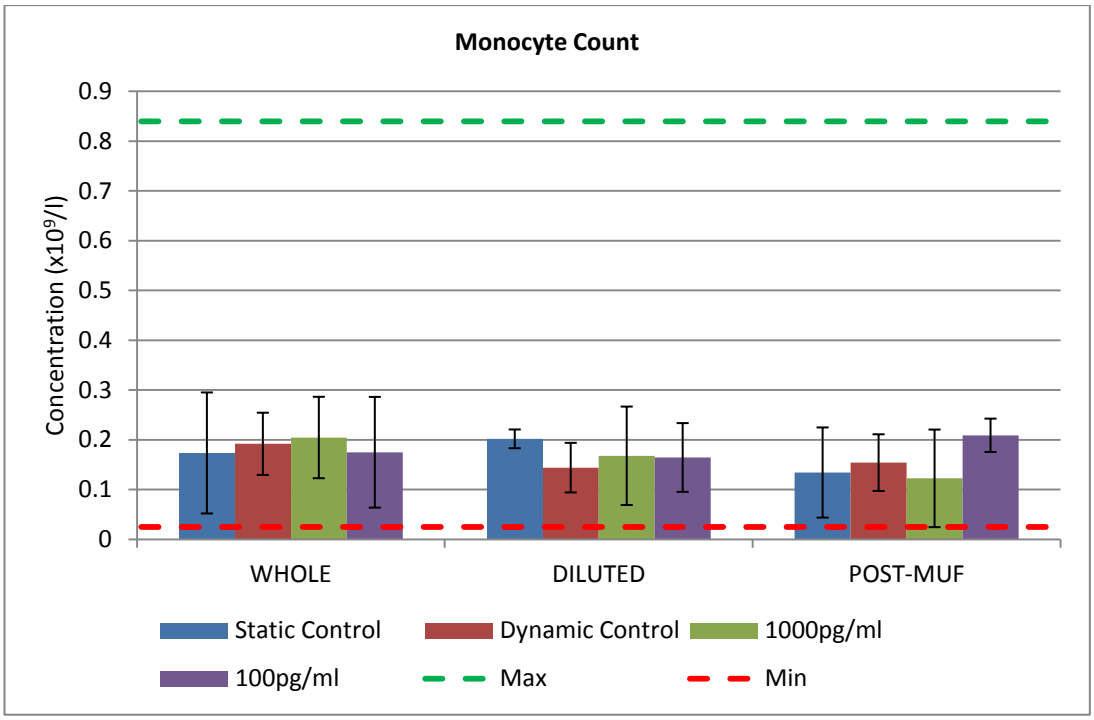


*Figure 200: Monocyte count (x10⁹/L) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. Counts have been corrected for differences in Hct to eliminate changes due to dilution of blood alone. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

Monocytes comprise 5-6% of circulating leukocytes and participate in the inflammatory response by phagocytosing cell debris and presenting antigens on their cell membranes for lymphocytes to destroy (Colville et al. 2015). Figure 200 shows that there was no significant difference in monocyte levels at any time in any group and thus the proposed device is not associated with any change in monocytes. The counts are within reference range in each group from baseline, although there was a lot of variation.

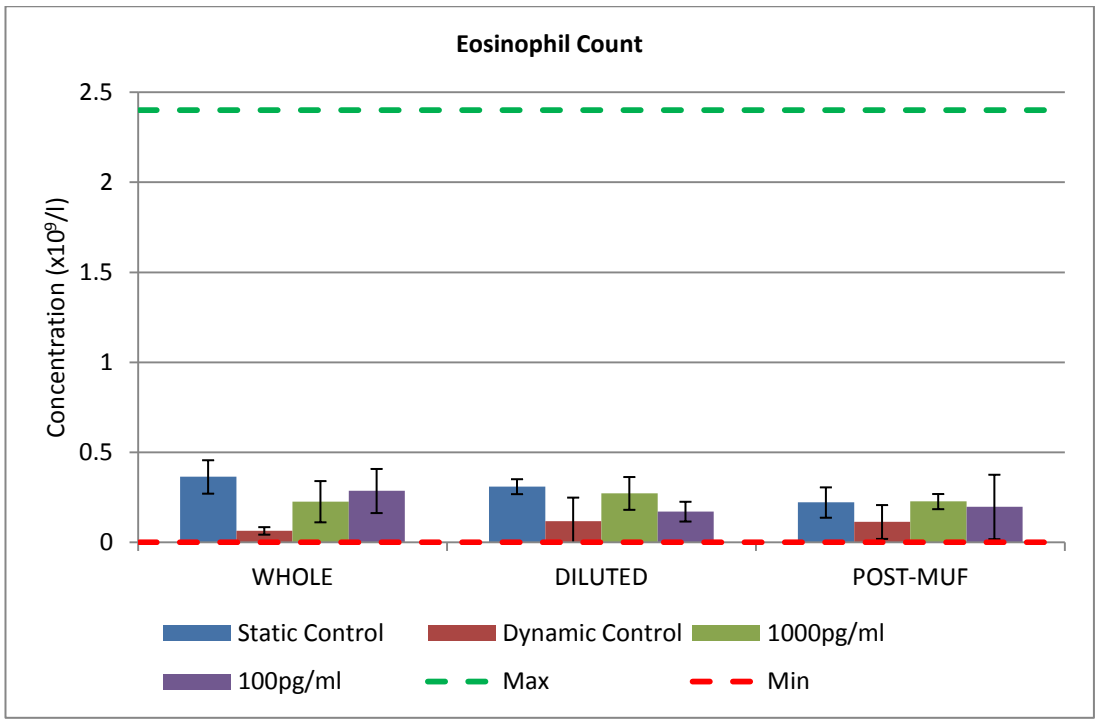


*Figure 201: Eosinophil count (x10^9/L) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. Counts have been corrected for differences in Hct to eliminate changes due to dilution of blood alone. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

Eosinophils constitute 1-6% of circulating leukocytes and are mainly involved in the inflammatory response through inhibiting local allergic and anaphylactic reactions, though they also ingest substances involved in the humoral immune response and have some phagocytic and bactericidal functions (Colville et al. 2015). Figure 201 shows that there was no significant difference in eosinophil levels at any time in any group and thus the proposed device is not associated with any change in eosinophils. The counts are within the normal reference range and although they may appear toward the low end, it should be noted that eosinophil counts are generally lowest in the morning which was the time at which the blood specimens were sourced and moreover, the normal reference range includes any values above zero, therefore so the values seen are not concerning (Fischbach et al. 2009).

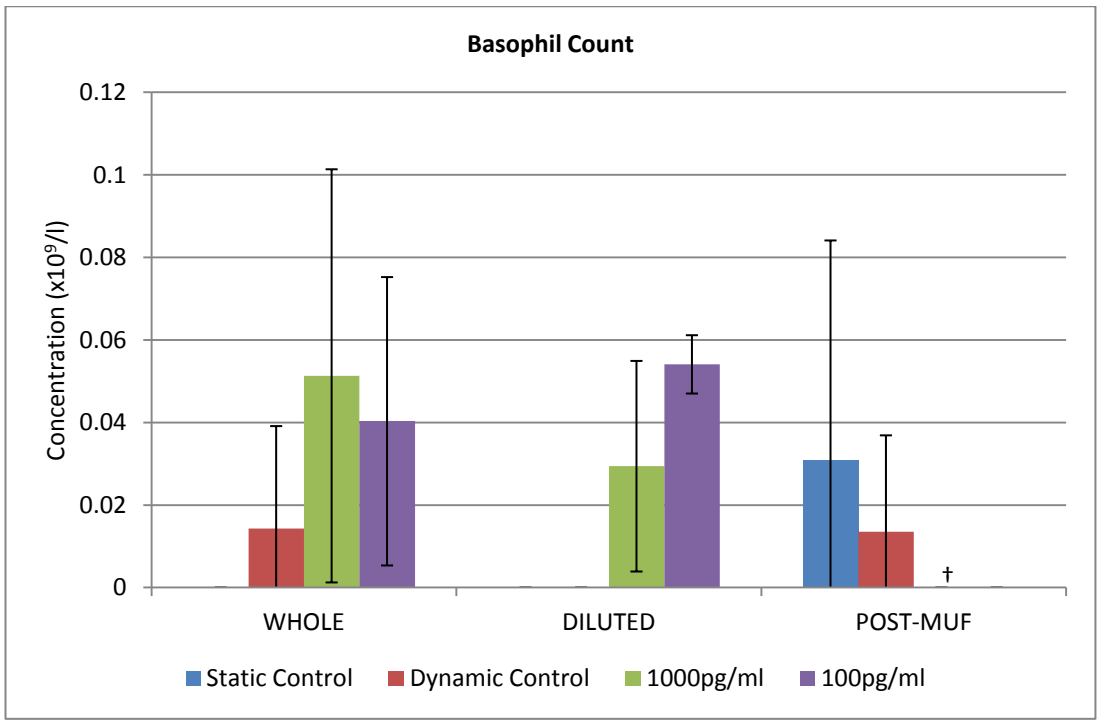


*Figure 202: Basophil count (x10⁹/L) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. Counts have been corrected for differences in Hct to eliminate changes due to dilution of blood alone. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

Basophils are the least common leukocyte and make up only 1% of circulating leukocytes (Colville et al. 2015). Basophil function is not well understood, their granules contain heparin and histamine (which helps initiate inflammation and allergic reactions) but they are the least phagocytic of the granulocytes (Colville et al. 2015). Figure 202 shows that, with the exception of a significant rise in the low inflammation group after MUF, there was no significant difference in neutrophil levels at any time in any group. The basophil concentrations were all particularly low and variable, for example, in the static control group there were no basophils detected in the initial whole blood or the diluted blood before MUF but after 60mins of no intervention basophils were detected. This, coupled with the previous observations for other WBC types and the overall WBC count, implies that there is no strong evidence to suggest that the proposed device has a harmful impact upon basophil levels. No reference range is provided for basophil levels. Error bars appear large in Figure 202 compared to other leukocyte type counts, however the concentration is an order of magnitude lower and so variability of count appears to be similar.

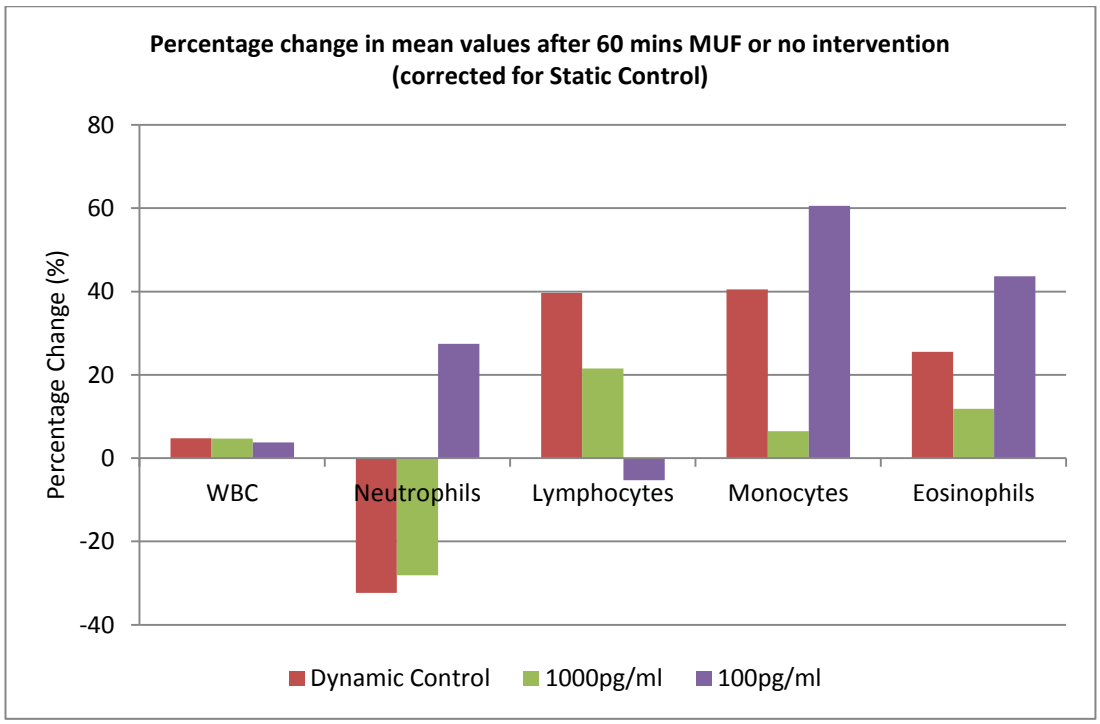


Figure 203: Percentage change in mean value of the WBC and differential counts after 60mins of MUF or no intervention in the dynamic control, high inflammation and low inflammation groups after the means have been corrected for the change observed in the static control (n=3).

Figure 203 shows the percentage change in mean value of the WBC and differential counts after 60mins of MUF or no intervention in the dynamic control, high inflammation and low inflammation groups after the means have been corrected for the change observed in the static control. By correcting for the static control, the change shown in Figure 203 is the change which can be attributed to flow through the device, and in the case of the high and low inflammation groups, flow and filtration. It can be seen that changes do occur in the various WBC type populations as a result of flow but that they are not consistent across the three groups. The dynamic control, which represents the change attributable to flow alone (i.e. not in conjunction with filtration), shows a drop in neutrophils coupled with a rise in lymphocytes, monocytes and eosinophils. Despite these percentage changes, we have seen previously in this section that there were no significant differences in the absolute measured values of these parameters. Importantly, the overall WBC count does not undergo a large change as a result of flow through the device.

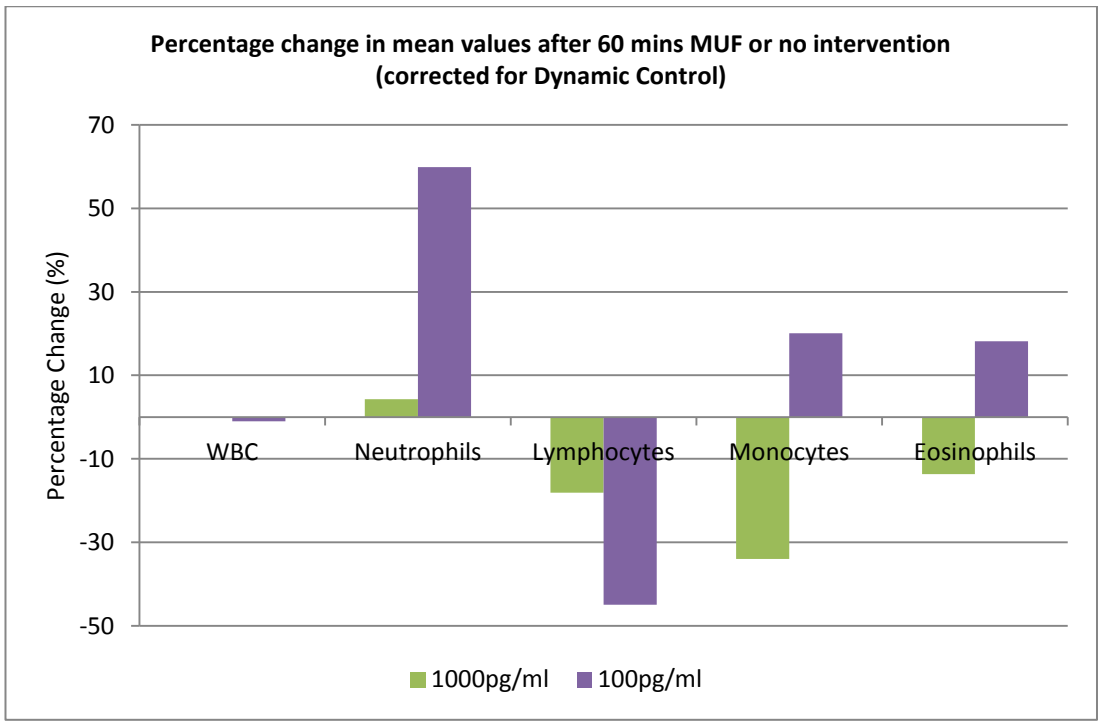


Figure 204: Percentage change in mean value (n=3) of the WBC and differential counts after 60mins of MUF or no intervention in the high inflammation and low inflammation groups after the means have been corrected for the change observed in the dynamic control.

Figure 204 shows the percentage change in mean value of the WBC and differential counts after 60mins of MUF or no intervention in the high inflammation and low inflammation groups after the means have been corrected for the change observed in the dynamic control. By correcting for the dynamic control, the change shown in Figure 204 is the change which can be attributed to filtration alone. Again, although there appear to be large changes attributable to filtration in the differential count, they are not consistent between the two groups which only differed in terms of the TNF-α and IL-6 concentrations. Importantly, the overall WBC count undergoes only a very small change attributable to filtration in the low inflammation group and no change attributable to filtration in the high inflammation.

4.3.5 Platelet levels and condition

During early testing of the proposed device, an assessment of platelet preservation with the MUF device using activated clotting time (ACT) as an indicator was attempted. ACT was measured by placing a very small sample (one or two drops) of blood on a Hemochron Jr. ACT plus test slide *(Elitech UK Ltd, Herefordshire, UK)* and placing it in the Hemochron Jr. signature plus reader (*Elitech UK Ltd, Herefordshire, UK)*. The Hemochron Jr. signature plus reader automatically produces a clotting time reading measured in seconds. However, when measuring ACT it was common for the clotting time to be reported as 'out of range' by the Hemochron Jr. signature plus reader, that is, the clotting time was longer than 1000secs. This long clotting time was most likely due to excessive heparinisation of the blood which, as described previously (*3.1.5. Blood source and pre-treatment*), was 10kIUs of heparin sodium from porcine mucosa (*Sigma Aldrich*) in each 5 litre collection container. Reduced heparinisation was experimented with but this often led to rapid clotting of the blood and thus it was decided that platelet analysis would be better achieved through a complete blood count.

In the data represented in the figures in this section, the results for the static control group and the high inflammation group are for a single experiment rather than a mean of three experiments as was the case for the dynamic control group and the low inflammation group. This is due to the fact that for two experiments in both the static control group and high inflammation group platelets were aggregated and could not be properly assessed. As a result, there are no error bars present as it was not possible to calculate the standard deviation and it was not possible to calculate measures of significance for these groups. Additionally, it was not possible to reliably correct for the static control or to correct the high inflammation group for the dynamic control and thus these measures have been omitted. However, it should be noted that the complete blood count reports stated clearly that although the platelets were aggregated the number appeared to be within the reference ranges. Additionally, since the aggregation appeared in both the static control and the high inflammation groups in equal frequency but not in the low inflammation group, the aggregation cannot be reasonably attributed to the proposed device.

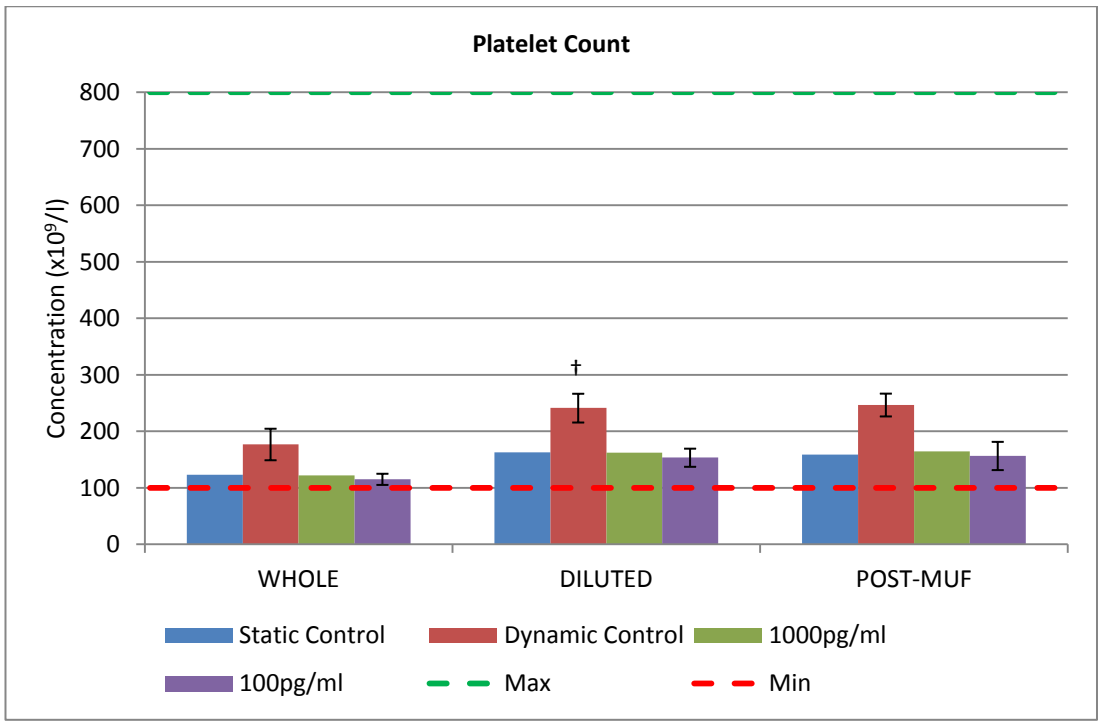


*Figure 205: Platelet count (x10^9/L) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. Counts have been corrected for differences in Hct to eliminate changes due to dilution of blood alone. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval. Static control and 1000pg/ml n=1, Dynamic control and 100pg/ml (n=3).*

It can be seen from Figure 205 that there is a significant increase in platelet count for the flow control group after dilution but no significant difference at any other instance. This suggests that platelet populations are preserved with use of the proposed device. Platelet counts are at the low end of the normal reference range which again could imply an issue with the blood quality at the source which is discussed later in this chapter (*4.5 Impact of Blood Age and Source*).

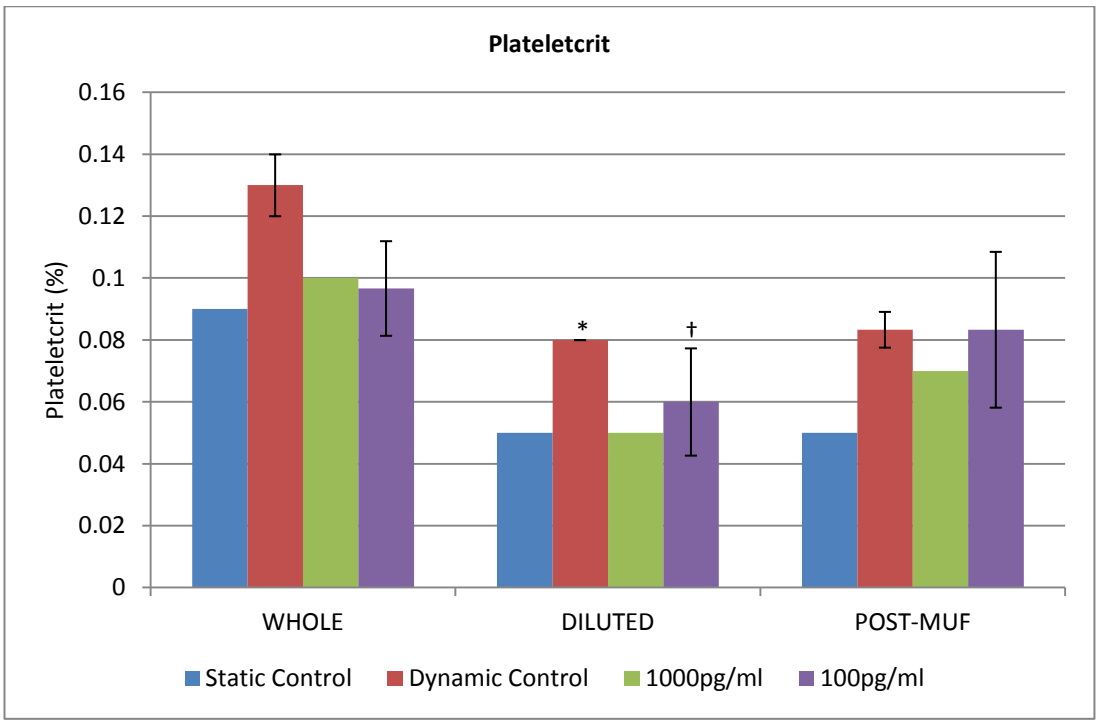


*Figure 206: Plateletcrit (%) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

As expected, the plateletcrit observations shown in Figure 206 are similar to those of the haematocrit, exhibiting a significant drop after dilution and no change in the control groups but a rise in the filtration groups. Although the rise in plateletcrit after 60mins of MUF was not significant this may have been due to the values measured being particularly low and perhaps close to the limits of resolution and sensitivity of the CBC making it difficult to observed changes.

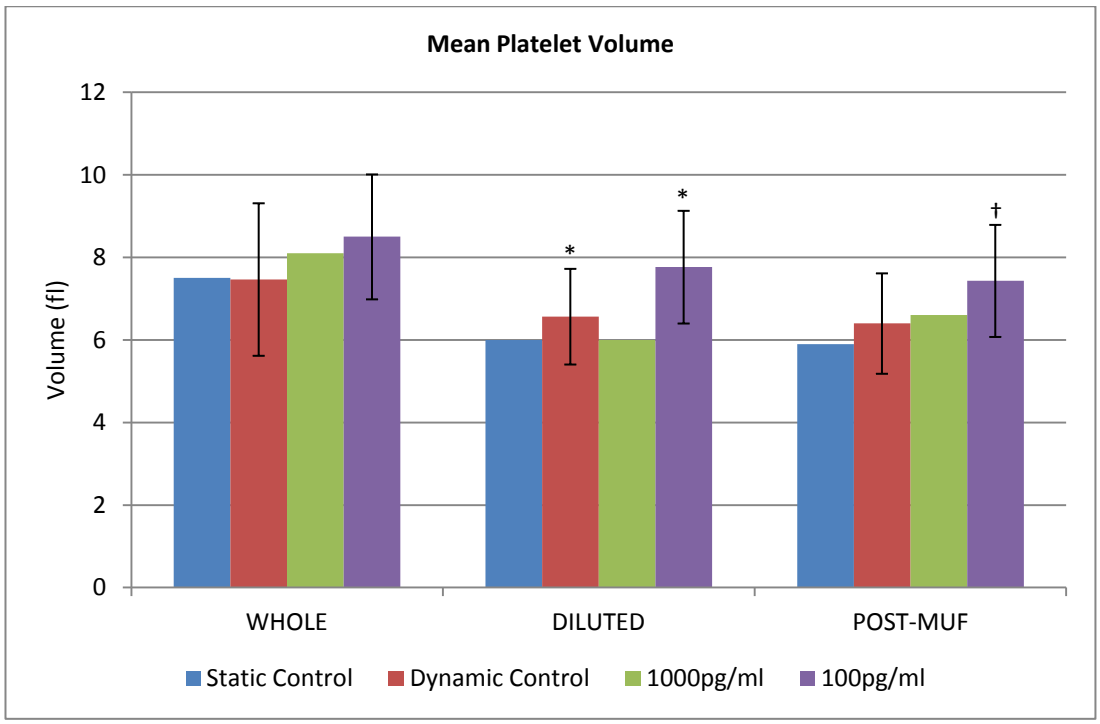


*Figure 207: MPV (fl) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

It can be seen from Figure 207 that there was a significant decrease in MPV in both the dynamic control and low inflammation groups after dilution. Subsequently, there was a further significant decrease in MPV in the low inflammation group. Conversely, in the high inflammation group MPV was seen to increase, however this was a result form a single experiment as explained previously in this section. No reference ranges are provided for MPV but the William R. Pritchard Veterinary Medical Teaching Hospital at The University of California Davis, report CBC reference ranges from their own laboratory which state a normal reference range for MPV as 4.5-7.6fl (UCDavis 2001). The values shown in Figure 207 are at the upper limit of this reference range and in fact the significant decreases seen bring the values towards a more normal level. A significant decrease in MPV is normally only associated with impaired thrombopoiesis (platelet generation) however this could not explain the decrease seen here (Lee 2013). When this data is considered in conjunction with PDW data in Figure 208 another possible explanation arises.

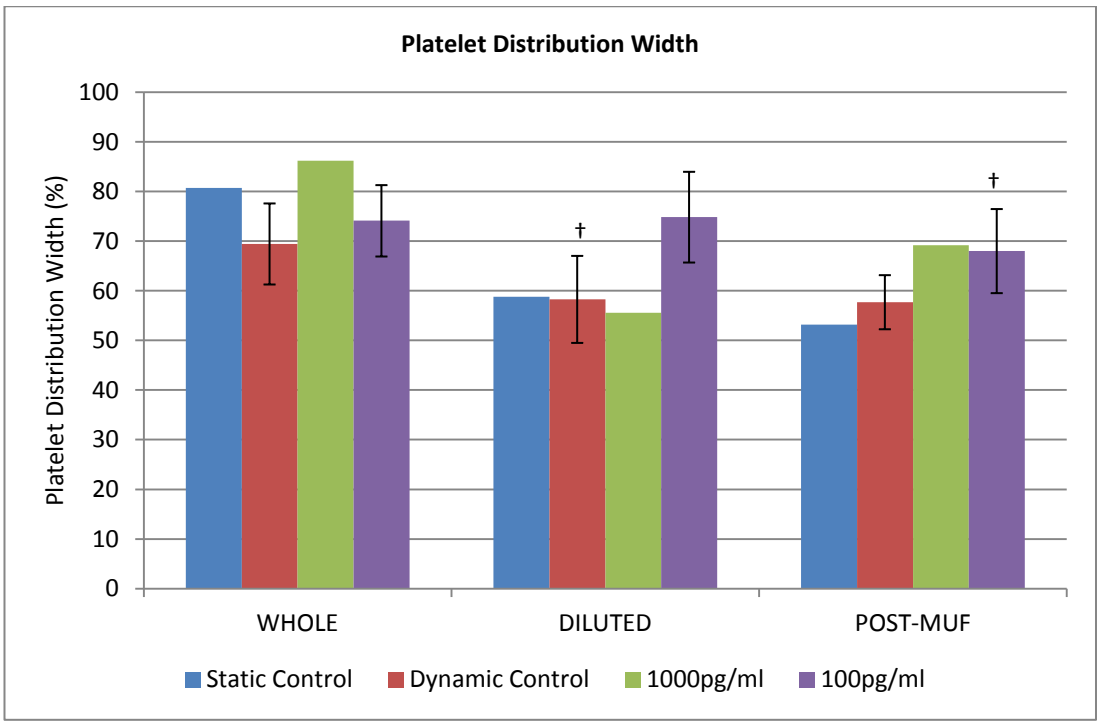


*Figure 208: PDW (%) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

It can be seen from Figure 208 that there was a significant decrease in PDW in the low inflammation group after MUF with the proposed device. This could be explained by the fact that the very smallest platelets or immature platelet may be lost in filtration through the pores of the control membrane thus reducing the range of platelet sizes observed and hence reducing the PDW, however it was seen previously in this section that MPV decreased significantly in the low inflammation suggesting a loss of larger platelets. Since larger platelets are more reactive and aggregable it is possible that larger platelets are being lost through small scale aggregation or platelet adhesion which, although not large enough to result in significant decreases in platelet count, may be sufficient to skew the mean and distribution of the measured platelet sizes (Michelson 2013). As with MPV no reference ranges are provided but the UCDavis reported reference ranges state a normal PDW as 56-80% which the majority of these value fall within even after the aforementioned significant decreases (UCDavis 2001).

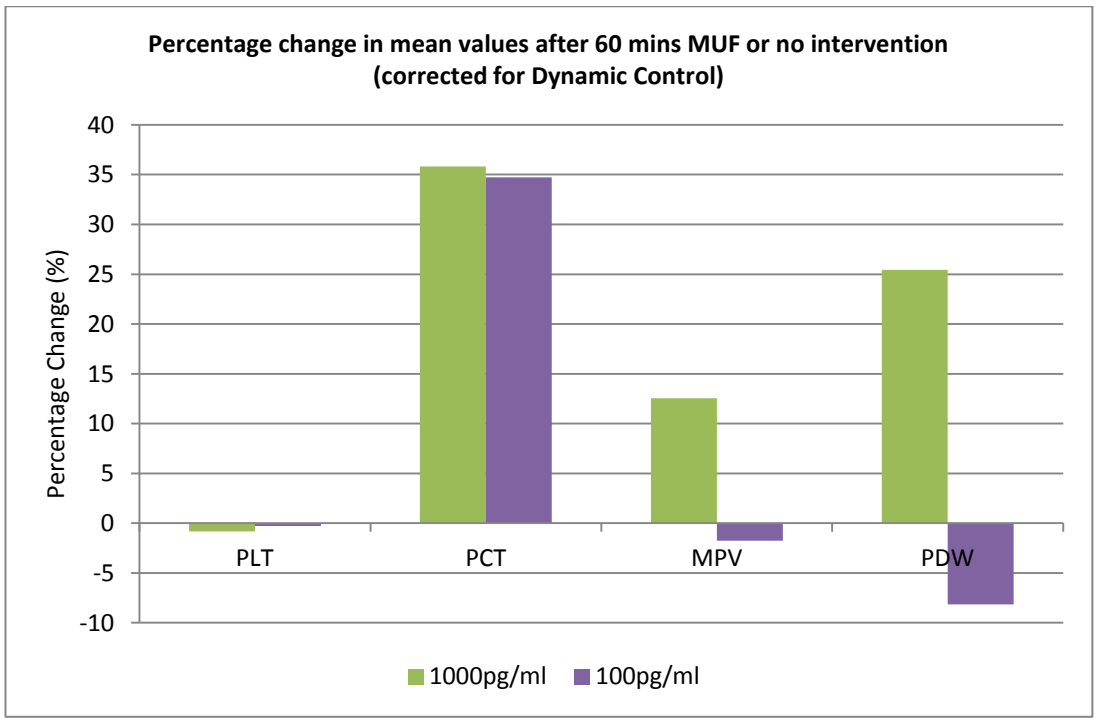


Figure 209: Percentage change in mean value (n=3) of the platelet parameters after 60mins of MUF or no intervention in the high inflammation and low inflammation groups after the means have been corrected for the change observed in the dynamic control.

Figure 209 shows the percentage change in mean value of the platelet parameters after 60mins of MUF or no intervention in the high inflammation and low inflammation groups after the means have been corrected for the change observed in the dynamic control. By correcting for the dynamic control, the change shown in Figure 209 is the change which can be attributed to filtration alone. As expected there is a large rise in plateletcrit attributable to filtration. Encouragingly, there was minimal change in platelet counts suggesting that platelets are preserved with the proposed MUF device.

Changes in MPV and PDW coupled with minimal changes in platelet count could be explained by low scale aggregation and activation of the more aggregable and reactive large platelets.

4.3.6 Haemolysis

Haemolysis and its sources have been discussed at length in the previous chapter (*3.3.3. Effect of flow on blood*). It is vitally important that haemolysis is greatly minimised and if possible avoided completely. Evaluation of haemolysis is important for assessing the device impact on blood quality as it is perhaps the best indicator of damage occurring to the blood which will have a strong negative impact on blood quality. Haemolysis is generally quantified by changes in levels of free haemoglobin in the blood which is taken to signify haemoglobin release from lysed

RBCs (Gregoretti 1996; Najarian et al. 1996b; Sowemimo-Coker 2002; Sakota et al. 2008). Human patients with normal renal function can clear up to 14mg of plasma free haemoglobin a day (Naito et al. 1994). Up to 130mg/dL of plasma free haemoglobin may be bound in haemoglobin-haptoglobin complexes in vivo and if plasma free haemoglobin is above this level haemoglobinuria and organ failure can occur (Naito et al. 1994).

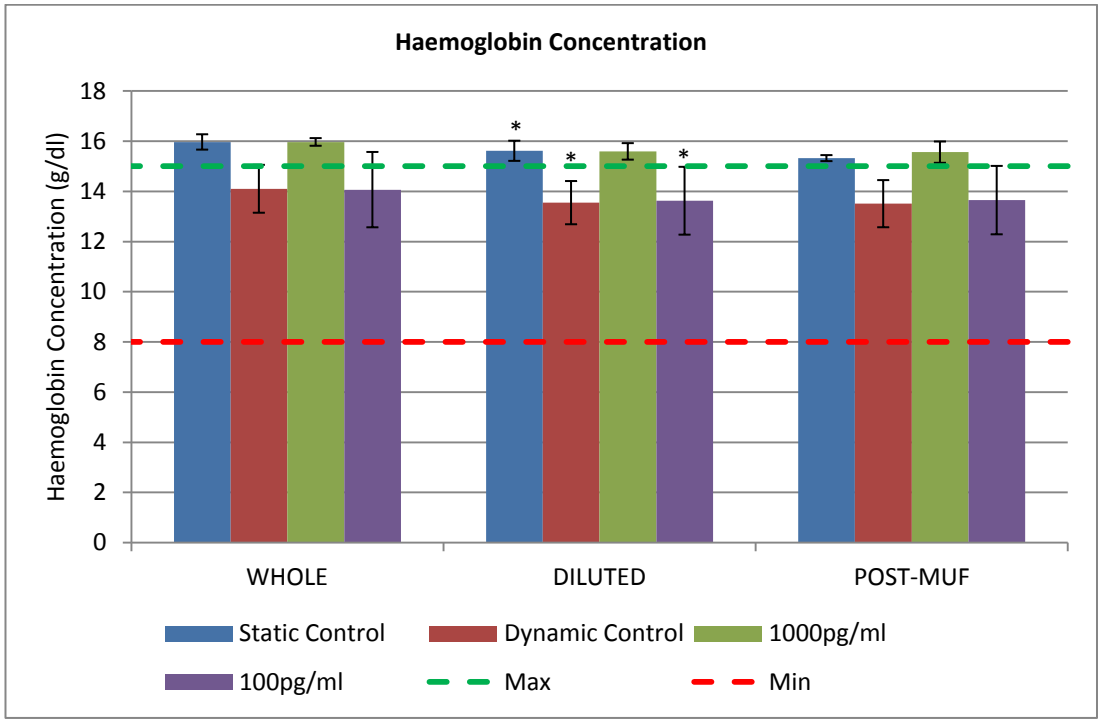


*Figure 210: Total haemoglobin concentration (Hb) in g/dl for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

As described previously in this section, Hb is the haemoglobin content as measured using the cell lyse cyanmethemoglobin colorimetric method. Since it is a lyse method, the measurement includes both haemoglobin inside RBCs and free plasma haemoglobin. It can be seen from Figure 210 that there is a significant decrease in Hb in the static control, dynamic control and low inflammation groups and a slight decrease in the high inflammation group. Since the lyse method includes plasma free haemoglobin, this change cannot be attributed to haemolysis. Importantly however, there is no significant change in the Hb level after 60mins of MUF or no intervention in any of the groups which suggests that there is no haemolysis induced by the proposed device. Hb is high and above the normal reference range in some cases and even from baseline which could suggest an issue with the blood quality from the source or haemolysis due to the collection procedure, however it should be noted that Hb measurements can be falsely elevated by high blood concentrations of heparin (Fischbach et al. 2009). Since each 5L batch of blood was heparinised with 10kIUs of heparin sodium from porcine mucosa it is possible that

this was the case. This may also explain the significant reduction in Hb observed in the static control, dynamic control and low inflammation groups (as well as the slight decrease observed in the high inflammation group) as the false elevating effect of the high blood heparin content would be diminished by dilution of the blood and the heparin within the blood.

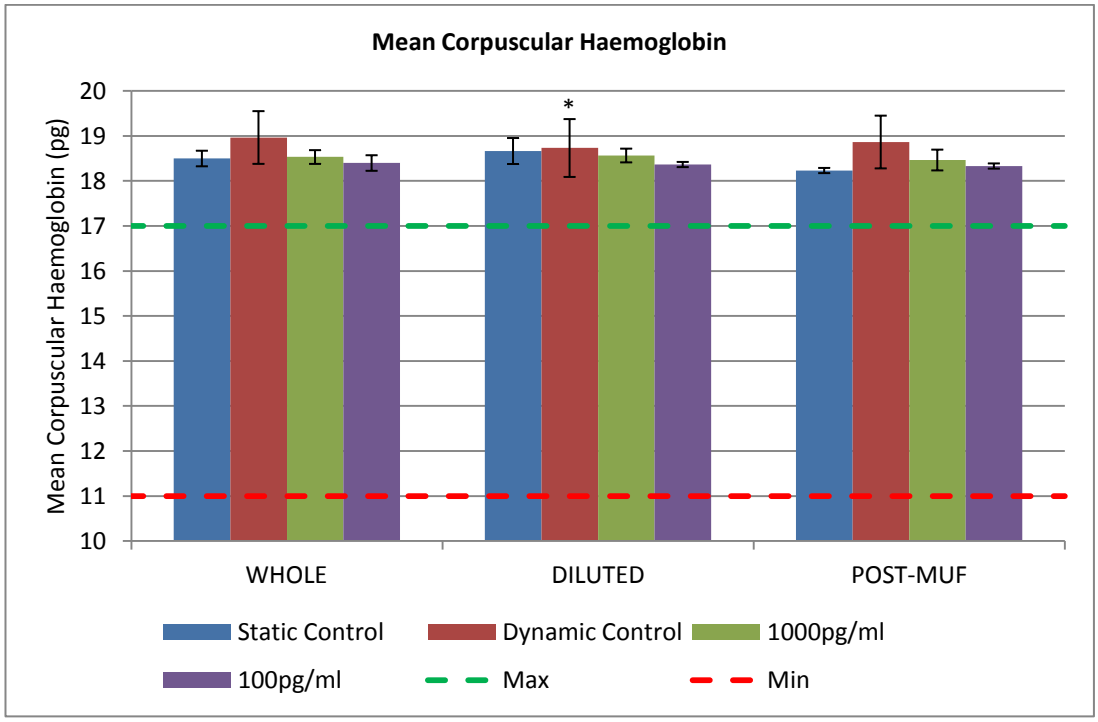


*Figure 211: MCH pg) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

As mentioned previously (*4.3.1 Methodology*) MCH is a measure of the mean mass of Hb in the RBCs and is calculated by *MCH = (10 x Hb) / RBC* and expressed in pg (Wintrobe et al. 2009). It can be seen from Figure 211 that there was a significant decrease in MCH after dilution in the dynamic control group alone, but no subsequent changes in any group after 60mins of MUF or no intervention. Decreased MCH can be a sign of subhaemolytic RBC trauma which could be occurring in the dynamic control group, but since no decrease occurred in the MUF groups it can again be said that there is no indication that the proposed device induced haemolysis (Sakota et al. 2008). Since MCH is calculated from Hb it too can be falsely elevated by high blood heparin concentrations which may explain why, as with the Hb levels, MCH levels are above the normal reference range.

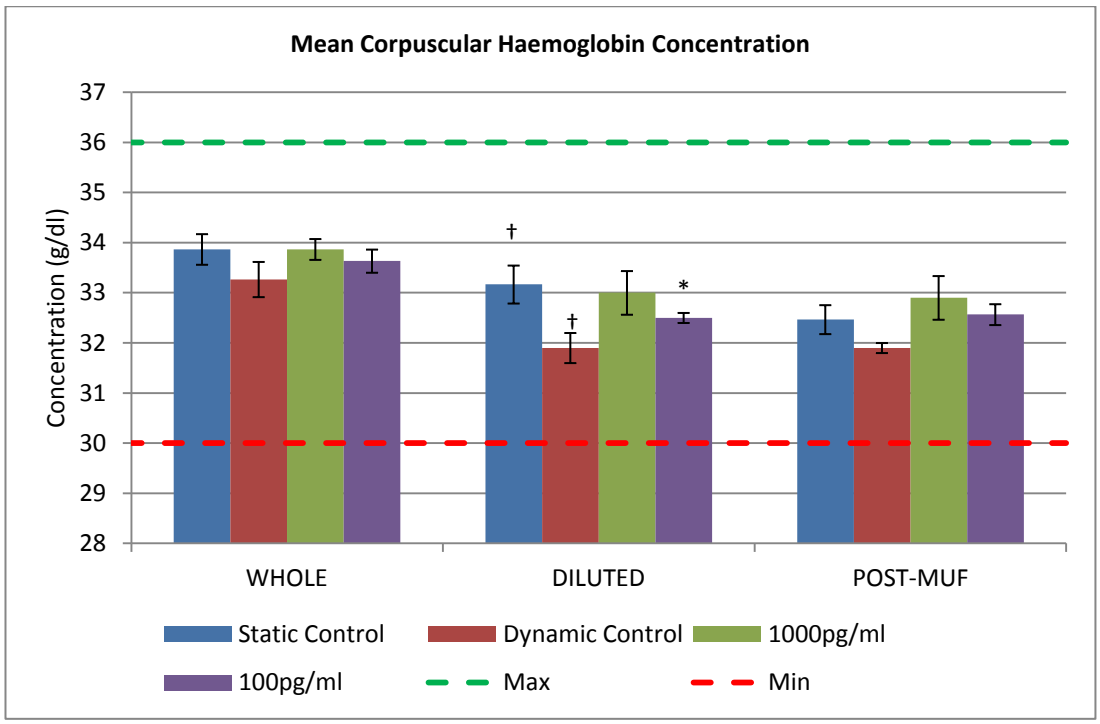


*Figure 212: MCHC (g/dl) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

MCHC is a measure of the mean concentration of Hb in the RBCs and is calculated by *MCHC = (Hb x 100) / Hct* and expressed in g/dl (Wintrobe et al. 2009). Consequently, MCHC can also be falsely elevated by high blood heparin concentrations although in this instance MCHC values are within the normal reference range. As shown in Figure 212 there is a significant decrease in MCHC after dilution in the static control, dynamic control and low inflammation groups which suggests there may be an issue with the dilution procedure which corroborates with changes observed in RDW and MCV after dilution. Again however, most importantly there was no significant change in MCHC in any group which means there is no indication of negative impact on the RBCs with use of the proposed device.

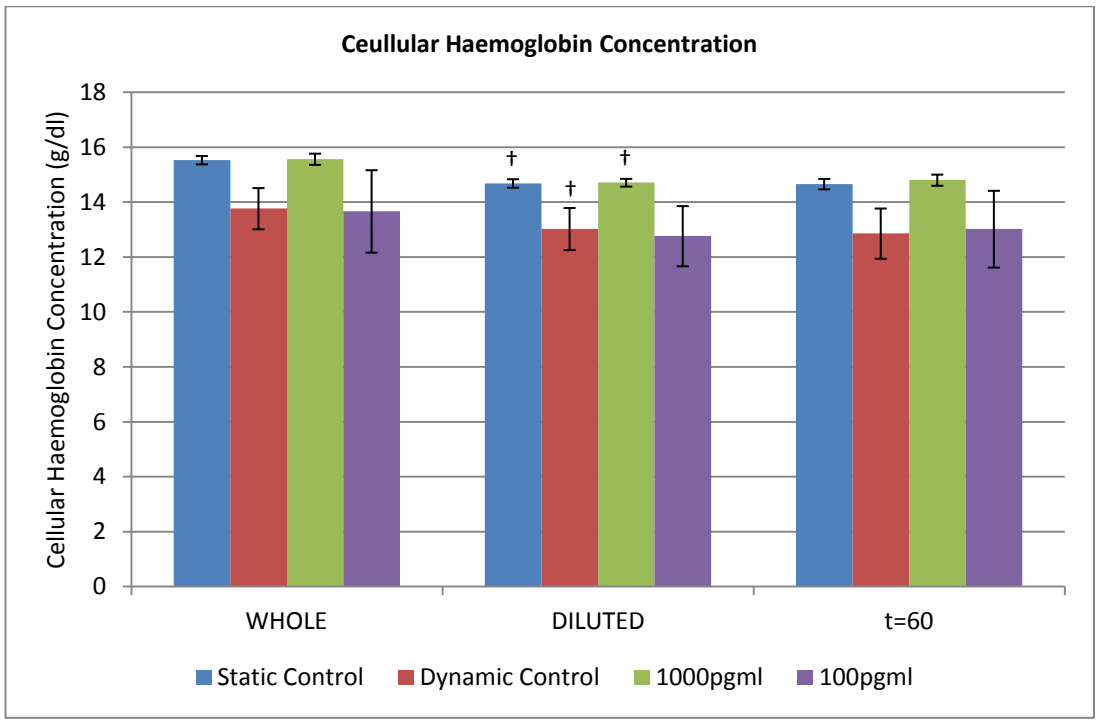


*Figure 213: Cellular haemoglobin concentration (CHb) in g/dl for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

As described previously in this section, CHb is the haemoglobin content as measured using two-dimensional flow cytometry on isovolumetrically sphered erythrocytes and reticulocytes. As can be seen from Figure 213 there was a significant decrease in CHb after dilution, again this suggests an issue with the dilution procedure, possibly the swelling of RBCs as indicated by the decrease in cellular haemoglobin and MCHC, and the significant increase in MCV seen previously. Importantly however there was no significant change in any group after 60mins of MUF or no intervention which indicates there was no change in the haemoglobin content of RBCs induced by the proposed device and thus no indication of any detrimental effect of the device.

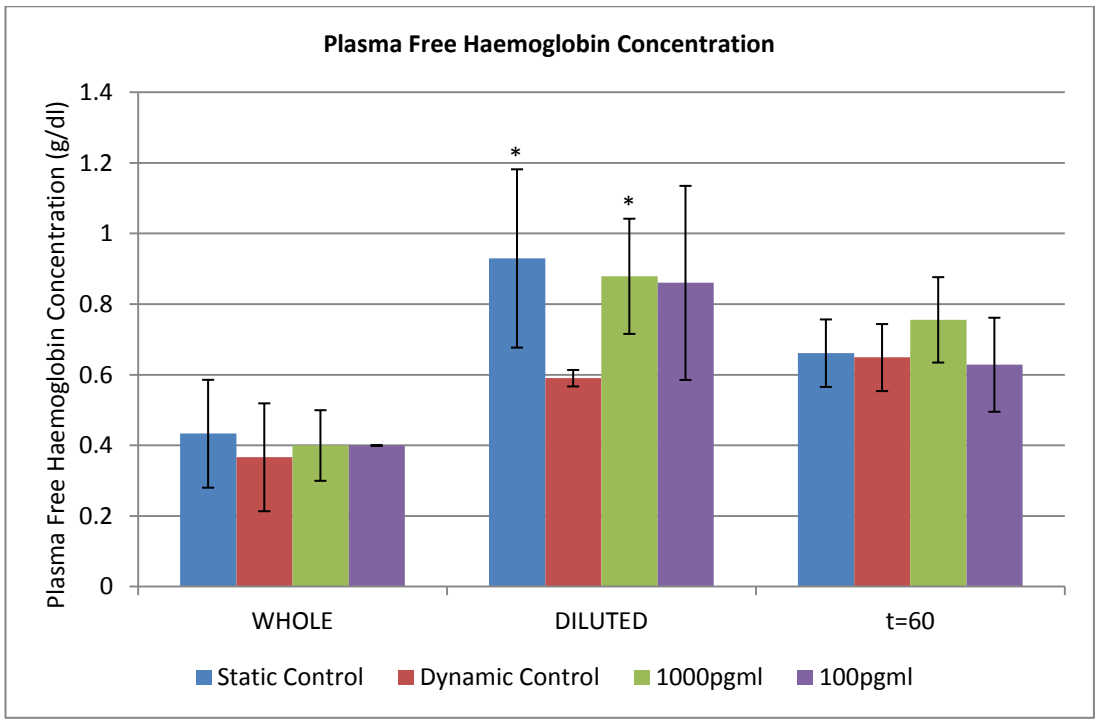


*Figure 214: Plasma Free Haemoglobin (g/dl) for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

Plasma Free Haemoglobin was calculated by subtracting the flow cytometry cellular haemoglobin measurement (CHb) which directly measures haemoglobin concentration in the RBCs from the lyse-method total haemoglobin measurement (Hb) which measures haemoglobin concentration in the fluid blood after cell destruction. The result represents the haemoglobin concentration in the blood excluding that which was present in the RBCs and thus the plasma free haemoglobin. As can be seen from Figure 214, there was a significant increase in plasma free haemoglobin after dilution in the static control and high inflammation groups which is an indication of some cell destruction as a result of the dilution procedure as mentioned previously and as indicated by the significant decrease in RBC counts seen previously. Yet again and most importantly however, there was no significant change in any group after 60mins of MUF or no intervention which is a good indication that there was no haemolysis induced by the proposed device.

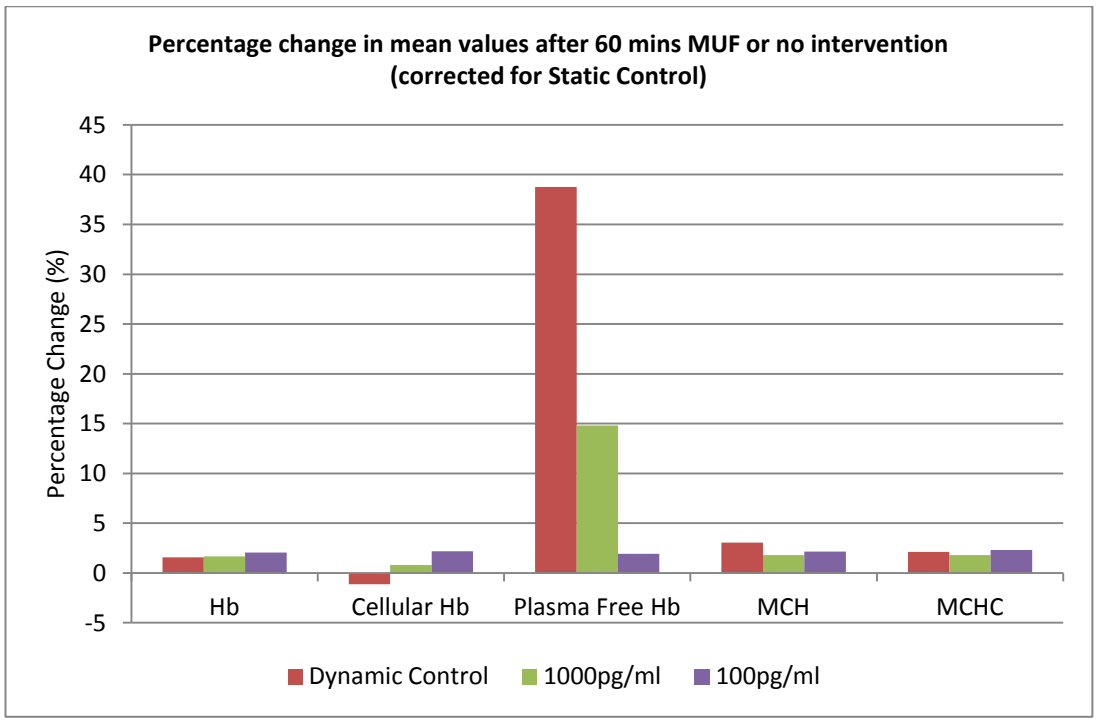


Figure 215: Percentage change in mean value of the various haemoglobin parameters after 60mins of MUF or no intervention in the dynamic control, high inflammation and low inflammation groups after the means have been corrected for the change observed in the static control (n=3).

Figure 215 shows the percentage change in mean value of the various haemoglobin parameters after 60mins of MUF or no intervention in the dynamic control, high inflammation and low inflammation groups after the means have been corrected for the change observed in the static control. By correcting for the static control, the change shown in Figure 215 is the change which can be attributed to flow through the device, and in the case of the high and low inflammation groups, flow and filtration. It can be seen that there is minimal change observed in Hb, Cellular Hb, MCH and MCHC suggesting that flow has minimal impact upon these parameters. However, there is a clear increase in plasma free haemoglobin in the dynamic control group and a lesser increase in the high inflammation group. By inspecting the absolute values of plasma free haemoglobin in Figure 215, it can be seen that the actual increase in plasma free haemoglobin after 60mins in the dynamic control group was very small and not significant but since the static control group (and indeed also the high and low inflammation groups) exhibited a decrease in plasma free haemoglobin in the same time period (albeit not a significant decrease) the 'corrected' percentage change for the dynamic control group is accentuated. Although this corrected percentage change is somewhat overstated considering there was no significant change in any of the groups after 60mins of MUF or no intervention, the implication of this observation is that flow through the MUF circuit may be more haemolytic than leaving the blood stationary which, based on the knowledge of haemolysis and particularly shear induced haemolysis would be entirely reasonable.

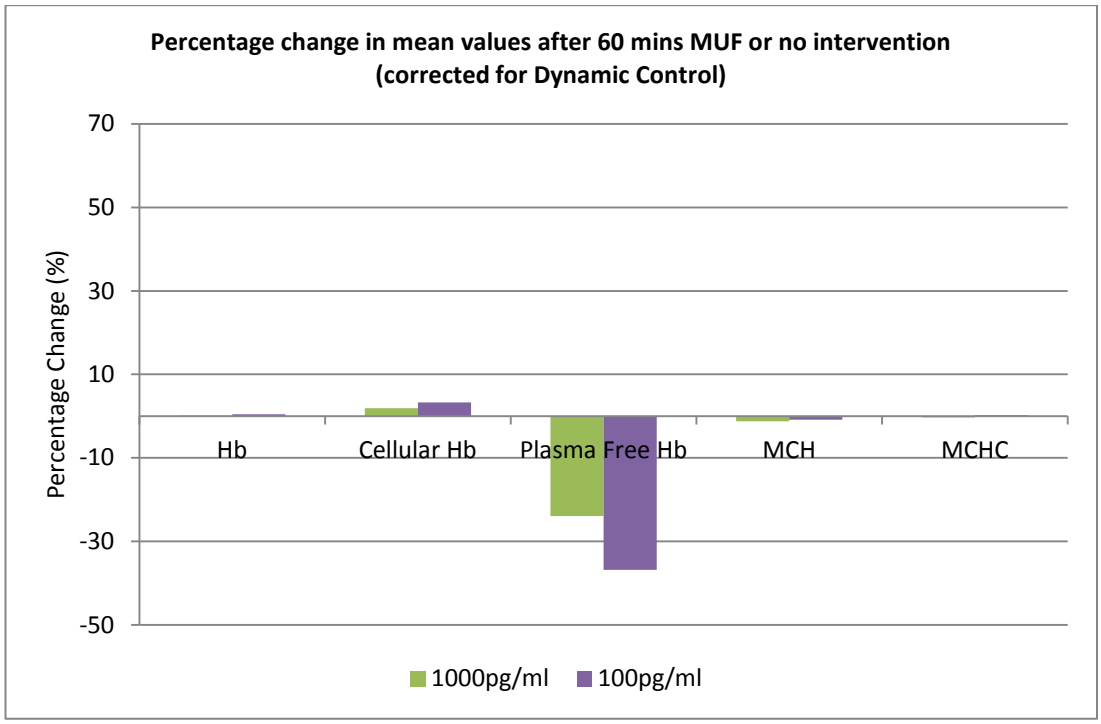


Figure 216: Percentage change in mean value (n=3) of the various haemoglobin parameters after 60mins of MUF or no intervention in the high inflammation and low inflammation groups after the means have been corrected for the change observed in the dynamic control.

Figure 216 shows the percentage change in mean value of the various haemoglobin parameters after 60mins of MUF or no intervention in the high inflammation and low inflammation groups after the means have been corrected for the change observed in the dynamic control. By correcting for the dynamic control, the change shown in Figure 216 is the change which can be attributed to filtration alone. Again, there is minimal change observed in Hb, Cellular Hb, MCH and MCHC suggesting that filtration has minimal impact upon these parameters. However, there appears to be a decrease in plasma free haemoglobin in this time period attributable to the filtration process. Again, it can be said that the change is accentuated by the correction for the dynamic control which, as discussed above, in terms of absolute plasma free haemoglobin levels exhibited a slight increase after 60mins of flow through the MUF circuit, however, since this percentage decrease in the high and low inflammation groups reflects the decrease seen previously in absolute plasma free haemoglobin levels (Figure 216) the notion of filtration being implicated in decrease plasma free haemoglobin levels is supported. The general implication of these observations appears to be that although flow through the MUF circuit may induce some insignificant levels of haemolysis the filtration procedure may also have some capacity to remove the plasma free haemoglobin which could temper the toxic effect of any haemoglobin that was released into the plasma which is a concern with haemolysis (Olsen 2000; Sakota et al. 2008; Gladwin et al. 2012).

4.3.6.1 Quantifying haemolysis

As described earlier (*3.3.3. Effect of flow on blood*), characterising haemolysis by relating increases in haemoglobin measurements to increased haemolysis is a common method, however some efforts have been made to go a step further and quantify haemolysis into indices which can make comparisons between the haemolytic effect of a broad range of devices and materials.

Gregoretti et al used a percentage haemolysis calculation for analysis of a suction based blood salvage system (Gregoretti 1996). *"The volume in ml of RBCs lysed by suction for each 100ml of RBCs present in the sample before suction was calculated by using the formula:*

$$\%Haemolysis = \frac{(plHb_A - plHb_B) \times (1 - Hct_B) \times 100}{Hb_B}$$

(Gregoretti 1996)

Where plHb and Hb are plasma free and total haemoglobin respectively in g/dl, Hct is haematocrit expressed as a decimal and A refers to the measurement after suction and B refers to the measurement before suction.

Using the Gregoretti percentage haemolysis calculation on the data from the complete blood counts presented earlier (*4.3.6 Haemolysis*), the percentage haemolysis after 60mins of MUF or no intervention are shown in Table 11. The values used are the Hct corrected values to eliminate changes due to dilution.

Group	Mean $plHb_A$	Mean $plHb_B$	Mean Hb_B	Hct_B	% Haemolysis
Static Control	0.66	0.93	15.6	0.47	-0.91
Dynamic Control	0.64	0.59	13.6	0.42	0.25
High Inflammation	0.76	0.88	15.6	0.47	-0.42
Low Inflammation	0.63	0.86	13.6	0.42	-0.99

Table 11: Mean %Haemolysis induced by 60mins of no intervention or MUF with the proposed device as calculated by the Gregoretti method

As can be seen from the results in Table 11, %haemolysis induced after the MUF period was negative in the static control, high inflammation and low inflammation groups. This is because plasma free haemoglobin decreased after the MUF period in these groups. In the dynamic control group the %haemolysis was relatively low at 0.25%.

Sowemimo-Coker also calculated percentage haemolysis during processing and storage of blood using a similar formula:

$$\%Haemolysis = \frac{(100 - Hct) \times plHb}{Hb}$$

(Sowemimo-Coker 2002)

Where plHb and Hb are plasma free and total haemoglobin respectively in g/dl, and Hct is haematocrit expressed as a percentage. Using the Sowemimo-Coker percentage haemolysis calculation on the data from the complete blood counts presented earlier (*4.3.6 Haemolysis*), the percentage haemolysis at the end of the experiment in each group is shown in Table 12. Again, the values used are the Hct corrected values to eliminate changes due to dilution.

Group	Mean Final plHb	Mean Final Hb	Mean Final Hct	% Haemolysis
Static Control	0.66	15.3	47.1	2.28
Dynamic Control	0.65	13.5	42.5	2.76
High Inflammation	0.76	15.6	47.2	2.56
Low Inflammation	0.63	13.6	41.8	2.68

Table 12: Mean %Haemolysis at the end of experiment the in each of the four groups as calculated by the Sowemimo-Coker method

As can be seen from the results in Table 12, %haemolysis after 60mins no intervention or MUF with the proposed device was similar between each group. Sowemimo-Coker et al suggest that haemolysis of 1% is clinically acceptable and although the values in the table for the MUF groups (high inflammation and low inflammation) are above this threshold, it can be seen that this is also true for the control groups and moreover from the Gregoretti %haemolysis calculations in Table 11 it can be seen that induced haemolysis in the worst case during the 60min period (0.25% in dynamic control) is below 1%; thus the proposed device does not appear to induce clinically problematic levels of haemolysis. Table 13 shows the %haemolysis at baseline, induced by dilution, and immediately before the MUF period. Again, the values used are the Hct corrected values to eliminate changes due to dilution rather than changes induced by the dilution procedure.

Group	Whole blood baseline (S)	Induced by dilution (G)	Pre-MUF (S)
Static Control	1.44%	1.65%	3.14%
Dynamic Control	1.49%	0.91%	2.49%
High Inflammation	1.32%	1.58%	2.98%
Low Inflammation	1.65%	1.91%	3.68%

Table 13: Mean %Haemolysis at the end of experiment the in each of the four groups at baseline, induced by dilution, and immediately before the MUF period S = Sowemimo-Coker method, G = Gregoretti method.

As can be seen in Table 13, percentage haemolysis levels were above the 1% threshold from baseline which indicates either a haemolytic event during the collection of the blood or a falsely elevated haemoglobin reading caused by high heparin concentrations (Fischbach et al. 2009). The quality of the blood from source is discussed later in this chapter (*4.5 Impact of Blood Age and Source*). Again, there appears to be evidence of haemolysis induced by the dilution procedure.

For continuous flow pumps (including implantable devices such as VADs) which may induce haemolysis 'indices of haemolysis' can be calculated from haemoglobin measurements to obtain a standardised and comparable assessment of the degree of haemolysis they cause. The indices are detailed by the *American Society for Testing and Materials (ASTM)* in standards 756-13, 1830-97, and 1841-97 and are present in the FDA *'Guidance for Cardiopulmonary Bypass Oxygenators 510(k)'* (FDA 2000b; ASTM International 2015a; ASTM International 2015b; ASTM International 2015c). There are three indices of haemolysis each calculated using a different formula as shown in Table 14 and which each quantify either the grams of Hb released per 100L of blood pumped (IH and NIH), a more convenient unit of measure for those devices, or the ratio of the amount of haemoglobin released into the plasma normalized by the total amount of haemoglobin pumped through the device (MIH) (FDA 2000b; Franco et al. 2003; ASTM International 2015a; ASTM International 2015b; ASTM International 2015c).

Index (g/100L)	Formula
Traditional index of haemolysis (IH)	$IH = \Delta FHb \times V \times \frac{100}{QT}$
Normalised index of haemolysis (NIH)	$NIH = \Delta FHb \times V \times \frac{(100 - Hct)}{100} \times \frac{100}{QT}$
Modified index of haemolysis (MIH)	$MIH = \Delta FHb \times V \times \frac{(100 - Hct)}{100} \times \frac{10^6}{(QTHb)}$

Table 14: Indices of haemolysis to quantify grams of Hb released per 100L of blood pumped (IH and NIH) or the ratio of the amount of haemoglobin released into the plasma normalized by the total amount of haemoglobin pumped through the device (MIH) and the corresponding formulae. ΔFHb = free plasma haemoglobin increase during testing period (g/dL), V = circuit volume (L), Q = flow rate (L/min), T = testing period duration (min), Hct = haematocrit (%)

Which of the three is most suitable depends on what information is available and under what scenario the data was obtained, however in some instances a mean of the three indices is calculated as a final index of haemolysis (Franco et al. 2003). MIH is beneficial when dealing with the range of Hct and Hb levels encountered when using bovine blood as it accounts for this variation, however, NIH can offer superior comparability across different haemolysis tests provided the level of Hb in the blood is close to the acceptable range (12±2g/dl) (Franco et al. 2003).

The range of clinically acceptable NIH values for continuous flow blood pumps is shown in Table 15 (Franco et al. 2003).

NIH	Clinical Outcome/Comments
>0.06	Increased free plasma haemoglobin levels
>0.04	No increase in free plasma but transfusion will be necessary
<0.04	Physiologically acceptable haemolysis
<0.02	Clinically acceptable haemolysis
<0.01	Design objective for continuous blood pumps

Table 15: range of clinically acceptable NIH values for continuous flow blood pumps (Franco et al. 2003)

Clearly these measures of haemolysis are not specifically designed for analysis of haemofiltration devices and the standard units of g/100L pumped are not appropriate, moreover, there has been some criticism of the suitability of these indices for paediatric scales (Kameneva et al. 2006). However there are sufficient similarities between the applications to make assessment of indices of haemolysis worthwhile as an intellectual exercise or indicator at the very least.

Indices of haemolysis were calculated for the proposed device and are shown in Table 16. Unlike with blood pumps and oxygenators, haemofilters will encounter varying Hcts, thus for calculation of the indices of haemolysis Hct corrected haemoglobin values were used to remove variation due to Hct changes.

Group	IH (g/100L)	NIH (g/100L)	MIH (mg/mg)
Static Control	-23.8	-12.6	-807.8
Dynamic Control	5.2	3.0	219.6
High Inflammation	-11.0	-5.8	-371.3
Low Inflammation	-20.6	-12.0	-880.2

Table 16: IH, NIH and MIH for each of the groups after 60mins of no intervention of MUF with the proposed device.

Due to the decrease in plasma free haemoglobin after the MUF period in the static control, high inflammation and low inflammation groups the IH, NIH and MIH indices for these groups are negative and thus difficult to analyse. The suggestion remains that the filtration procedure has some capacity to remove plasma free haemoglobin. For the dynamic control group NIH is much larger than the upper clinically acceptable level however, as described previously in this section, this threshold is intended for blood pumping systems including VADs which may be expected to pump up to approximately 10L/min for several months or more, whereas the proposed MUF device can run for a little as 40mins at a flow rate of 150ml/min meaning a total volume of 6L blood pumped during the procedure rendering these thresholds unsuitable for analysis of the proposed device (Slaughter et al. 2009; Slaughter et al. 2013).

4.3.7 Potential interfering factors

There are a number of factors which can influence the various measurements that form the flow cytometry complete blood count. For example; the lipid content of blood significantly affects the rate of haemolysis, it has been reported that in canines postalimentary lipemic blood is up to 50 times more fragile than blood from fasted animals and based on this observation some have suggested that fasted blood should be used for haemolysis testing (Franco et al. 2003).

One major factor, relevant to this study, which can influence measurements, is heparin concentration. Heparin is known to induce thrombocytopenia (low platelet counts) and in fact 'Heparin-Induced Thrombocytopenia' is a well documented and relatively common but potentially devastating immune mediated syndrome in which a hypercoagulable state is produced resulting in thrombocytopenia and thrombosis (Greinacher et al. 2003; Arepally et al. 2006; Ahmed et al. 2007; Linkins 2015). Freise el al found that heparin as an anticoagulant lowered platelet counts but did not significantly alter any other haematological parameter in umbilical cord blood (Freise et al. 2009). Moore et al found that in horses heparin treatment resulted in significantly increased MCV and significantly decreased Hct, RBC count and Hb concentration (Moore et al. 1987). Similarly, Duncan et al reported that heparin therapy in horses significantly decrease RBC count, Hct and Hb although free plasma Hb was unchanged (Duncan et al. 1983). Moreover, heparin resulted in a significant increase in MCV (associated with the decreased RBC count) and at high doses significantly reduced platelet counts (Duncan et al. 1983). As mentioned previously (*4.3.6 Haemolysis*), high heparin concentrations are known to falsely elevate MCH and MCHC readings (Fischbach et al. 2009).

Other interfering factors relevant to the current study are shown in Table 17:

Parameter	Interfering Factors
RBC Count	Age Exercise
Hct	Age Gender
Hb	Age Excessive fluid intake Falsely elevated by WBC counts >50×10^9/l
MCHC	Falsely elevated by high blood heparin concentration
MCH	Falsely elevated by high blood heparin concentration Falsely elevated by WBC counts >50×10^9/l
WBC Count	Hourly variation Age Exercise Pain Temperature
Neutrophil Count	Stress Exercise
Eosinophil Count	Daily variation (lowest in the morning, rises from noon until after midnight) Stress
Platelet Count	Stress Strenuous exercise Partially clotted specimen

Table 17: Relevant factors which can influence the various measurements that form the flow cytometry complete blood count (Fischbach et al. 2009).

As can be seen from Table 17, many of the interfering factors relate to the condition and welfare of the patient (or in the case of this experiment series the animal) immediately prior to blood collection, this is discussed later in this chapter (*4.5 Impact of Blood Age and Source*). As discussed (*4.3.1 Methodology*), high heparin concentration in the blood can falsely elevate certain parameters and it is possible this occurred during this study, thus other anticoagulation techniques may need to be explored for example Bivalirudin (Grubb et al. 2010).

4.3.8 Potential clinical consequences of observations

The results of the complete blood counts can be summarised in two segments; regarding those results pertaining to dilution of the blood, and regarding those results pertaining to the use of the proposed device.

4.3.8.1 Dilution

As expected the dilution procedure led to a significant decrease in Hct, but also induced a significant reduction in RBC count in each group and associated significant increase in MCV in each group and significant decrease in RDW in each group with the exception of the low inflammation group. This suggests the saline for dilution was hypotonic, causing the cells to

intake fluid leading an increase in RBC size but simultaneously the lysing of some RBCs. It should be noted, however that RBC count, MCV and RDW all remained within the reference ranges. After dilution there was no significant difference in the neutrophil, lymphocyte, monocyte, eosinophil or basophil counts however there was a significant decrease in the overall WBC count in the static control and high inflammation groups after dilution although the figures remained within the reference range. As expected after dilution, there was a significant decrease in PCT which reflected the observations of Hct after dilution, additionally there was also a significant decrease in both MPV and PDW which could be explained by low scale aggregation and activation of the more aggregable and reactive large platelets. Additionally, there was a significant increase in platelet count after dilution in the dynamic control group, it is unclear by what mechanism this could occur in vitro, however since this observation was not replicated in any of the other groups which underwent the same dilution procedure it is possible this result may be erroneous, although further investigation may be required. Total Hb levels significantly decreased in the static control, dynamic control and low inflammation groups and decreased slightly in the high inflammation group after dilution. Given that the Hb parameter is measured by the lyse method and thus includes plasma free haemoglobin, the decrease cannot be explained by dilution induced haemolysis. However, since Hb measurements can be falsely elevated by high blood concentrations of heparin, dilution may diminish this effect by reducing the heparin concentration in the blood, and consequently a reduction in false elevation may manifest as a significant reduction (Fischbach et al. 2009). CHb and MCHC both decreased significantly in three of the four groups after dilution which correlated with the earlier observations on MCV and RDW and the suggestion that RBCs underwent an intake of fluid and 'swelled' after the dilution procedure. MCH was seen to significantly decrease after dilution in the dynamic control group which can be a sign of subhaemolytic trauma (Sakota et al. 2008). There was a notable increase in plasma free haemoglobin after dilution in all groups although the change was only significant in the static control and high inflammation groups. This suggests some degree of haemolysis induced by the dilution procedure, which, based upon the significant decrease in RBC count, CHb, MCHC and RDW significant increase in MCV was most likely the result of RBC 'swelling' in a saline solution of adverse tonicity.

Based on these observations, it appears there was an issue with the dilution procedure, most likely a hypotonic saline solution which resulted in fluid intake by the RBCs. The saline used for these experiments was produced on-site, perhaps with insufficiently accurate method, and thus this issue should be easily avoidable in the future by implementing stricter production controls or sourcing saline externally.

4.3.8.2 Use of the proposed device

As intended, the proposed device successfully haemoconcentrated the blood leading to a significant increase in Hct. There was no significant change in RBC count, MCV or RDW after 60mins of MUF with the device in any of the study groups. Similarly, there were no significant changes in the WBC, neutrophil, monocyte or eosinophil counts after 60mins of MUF with the device in any of the study groups. Basophil counts were significantly reduced in the high inflammation group after MUF with the proposed device but since the basophil concentration were all particularly low, generally close to zero, and variable, the evidence for the device inducing loss of basophils is not strong, especially given that the effect was not replicated in the low inflammation MUF group. A significant increase in lymphocyte count after MUF with the device was observed in the high inflammation group. Since this observation was not replicated in the low inflammation group the cause may be related to the cytokine levels, although it is unclear by what mechanism this could occur in vitro and further investigation may be needed. PLT count and PCT did not change significantly after MUF with the device although there was a slight increase in PCT which reflected the Hct observations. There was a significant decrease in both MPV and PDW which could be explained by low scale aggregation and activation of the more aggregable and reactive large platelets, however the values seen remain within the aforementioned UCDavis reference ranges. After 60mins MUF with the device there was no significant change in total Hb, MCH, MCHC, CHB or plasma free Hb which is a strong indication that there was little to no haemolysis induced by the device.

Based on these observations, clinical use of the proposed device should result in no adverse outcomes for the patient. Changes in lymphocyte count, MPV and PDW may require further investigation but the current implication is that the changes observed are clinically insignificant.

4.4 Inflammatory Mediators

When discussing the objectives for the proposed device in terms of blood quality at the end of the previous chapter it was stated that "*demonstration of removal of some pro-inflammatory mediators would be advantageous".* As discussed in *1.3.10. Impact of MUF on Inflammatory Mediators*, the removal of inflammatory mediators by MUF has been studied at length with conflicting evidence. Clearly, the removal of pro-inflammatory mediators from the blood by filtration after CPB would be advantageous in the control and prevention of the inflammatory response and subsequent associated morbidities. It would be highly beneficial therefore to assess whether removal of pro-inflammatory mediators occurred with MUF using the proposed device.

4.4.1 Procedure

In order to assess whether the proposed device would indeed remove pro-inflammatory mediators from the blood, two pro-inflammatory cytokines were selected and their levels studied before and after MUF. The study was part of the same experiment series described in *4.3.1 Methodology*.

TNF-α and IL-6 were selected for this study due to their relevance to studies of CPB, MUF and MUF devices. TNF- α is acts as an initiator of the humoral inflammatory response whereas IL-6 coordinates the generalised systemic inflammatory response known as the 'acute-phase response' (Miller et al. 1997; Glogowski et al. 2001). After commencement of CPB plasma TNF- α levels increase at faster rate than all other cytokines and peak at 2hrs and again 18-24hrs whilst plasma IL-6 levels increase within 2hrs to a peak at 4hrs (Kawamura et al. 1993; Miller et al. 1997). Plasma TNF-α levels have been shown to rise in response to CPB whilst plasma IL-6 levels have been associated with the degree of tissue damage induced by CPB (Miller et al. 1997; Glogowski et al. 2001). Together TNF- α and IL-6 were the most commonly investigated cytokines in the studies discussed in *1.3.10. Impact of MUF on Inflammatory Mediators* regarding utilising MUF to remove inflammatory mediators in order to control the inflammatory response and improve clinical outcomes (Saatvedt et al. 1996; Wang et al. 1996; Wang et al. 1998; Portela et al. 1999; Tassani et al. 1999; Boga et al. 2000; Grunenfelder et al. 2000; Kiziltepe et al. 2001; Chew et al. 2002; Atkins et al. 2010). To demonstrate removal of these factors would be highly beneficial for any MUF device.

The cytokines used were both full length recombinant bovine proteins, supplied by *abcam Plc (Cambridge, UK)* and were reconstituted with sterile phosphate-buffered saline (PBS) containing at least 0.1% bovine serum albumin (BSA) and aliquoted and frozen for storage as instructed by the supplier.

As described previously (*4.3.1 Methodology*), there were four groups; static control, dynamic control, low inflammation and high inflammation and three experiments were performed per group. In each experiment the bovine blood was 'spiked' with a predetermined amount of both TNF-α and IL-6 using a micropipette. In the high inflammation group the 'spiked' amount was 1000pg/ml of both TNF-α and IL-6 to represent an elevated level, whereas, in the static control, dynamic control and low inflammation groups the 'spiked' amount was 100pg/ml, representing more normal level (Sekiyama et al. 1994).

Again, as described previously (*4.3.1 Methodology*), plasma samples were collected before dilution ('whole'), after dilution immediately before the commencement of MUF ('t=0') and then

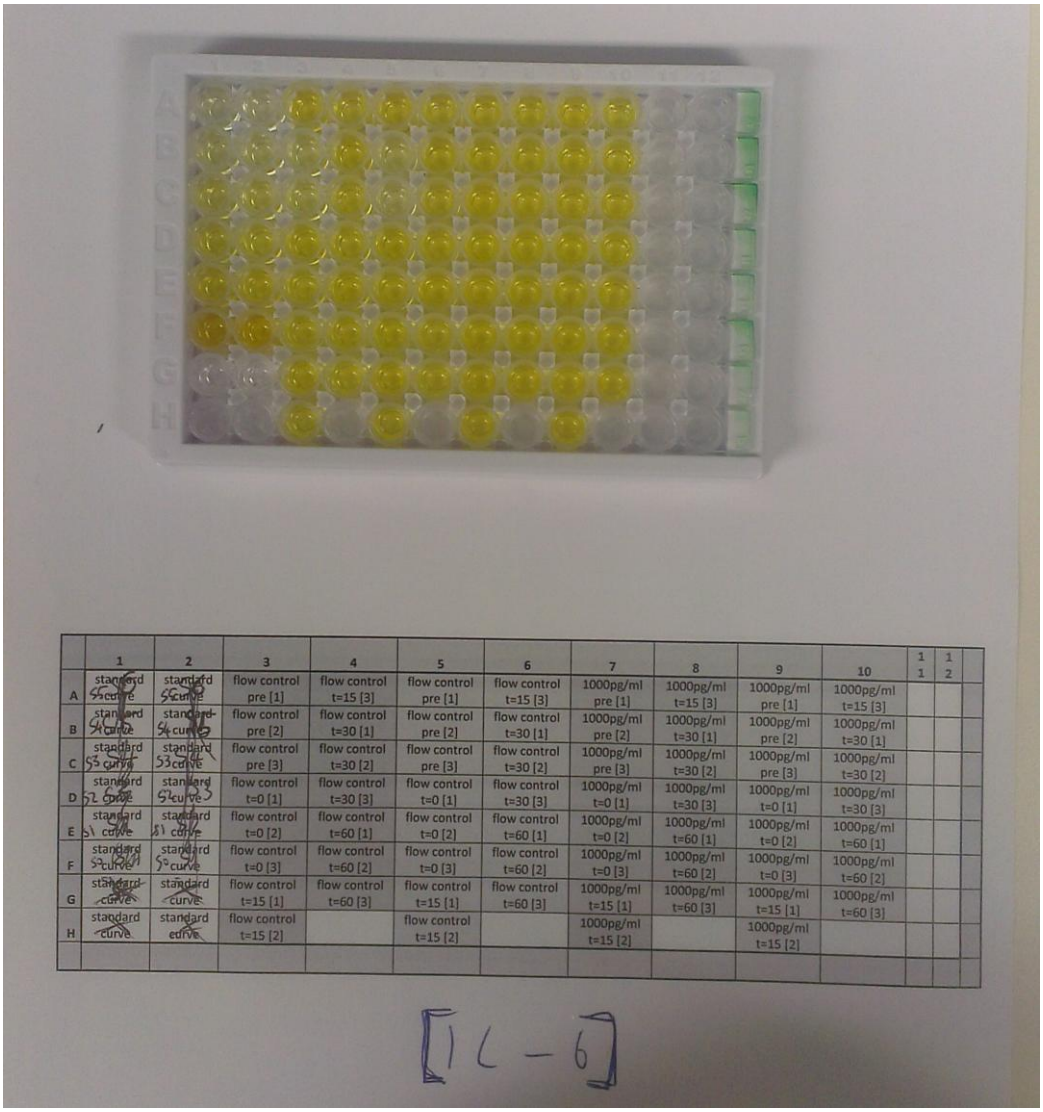
again after 15 ('t=15'), 30 ('t=30') and 60mins ('t=60'). The plasma samples were stored frozen for later analysis by ELISA.

ELISA analysis of TNF was performed using a bovine TNF-α ELISA kit from Cloud-Clone Corp *(Houston, TX)* with a detection range of 7.813-500pg/ml and a sensitivity of 2.5pg/ml, whilst ELISA analysis of IL-6 was performed using a bovine IL-6 ELISA kit from Cusabio (*Cusabio Biotech Co Ltd, Wuhan, China*) with a detection range of 5-1000 pg/ml and a sensitivity of 2.5pg/ml. Each plasma sample was added to two wells so that the absorbance could be measured in duplicate to improve the accuracy of results. Due to the number of samples, two 96-well plates were required for each cytokine analysis, meaning four plates were used in total. The samples were divided so there were two groups per plate, one plate with the samples from the static control and the low inflammation group, and one plate with samples from the dynamic control and high inflammation group.

The TNF- α assay involved first preparing all the reagent and samples including a standard curve (S0 = 7.813pg/ml, S1 = 15.625pg/ml, S2 = 31.25pg/ml, S3 = 62.5pg/ml, S4 = 125pg/ml, S5 = 125pg/ml, S6 = 500pg/ml) and adding 100µl of each sample to one of the 96 wells and incubating for 2 hours at 37°C. After incubation the solution in the cells was aspirated and 100µl of 'detection reagent A' was added to each well before incubating for a further hour at 37°C. After this incubation period the solution in the cells was aspirated and the plate was washed three times with the wash solution provided, before adding 100µl of 'detection reagent B' and incubating for 30mins at 37°C. After this incubation period the solution in the cells was aspirated and the plate was washed a further five times before adding 90µl of substrate solution and incubating for a final 15-25mins at 37°C. Finally, 50µl of stop solution was added to well and immediately after the plate was read at 450nm using a Labsystems Multiskan Ascent photometric plate reader (*Thermo Fischer Scientific Inc., Waltham, MA*).

The IL-6 assay involved first preparing all the reagent and samples including a standard curve (S0 = 0pg/ml, S1 = 5pg/ml, S2 = 20pg/ml, S3 = 80pg/ml, S4 = 250pg/ml, S5 = 1000pg/ml) and adding 50µl of each sample to one of the 96 wells followed by 50µl of HRP-conjugate and 50µl of antibody. After incubating for 60mins at 37°C the solution in the cells was aspirated and plate was washed three times with the wash solution provided and before adding 50µl of 'substrate A' and 50µl of 'substrate B' to each well and incubating for a further 15mins and 37°C. Finally, 50µl of stop solution was added to each well and within 10mins the plate was read at 450nm using the same Labsystems Multiskan Ascent photometric plate reader (*Thermo Fischer Scientific Inc., Waltham, MA*). Figure 217 shows a typical IL-6 plate immediately after use of the photometric plate reader, the colour differences denoting different concentration of IL-6 can be see clearly in

standard curves in columns 1 and 2 of the 96-well plate, whilst the locations of other samples are shown in the printed array.



	1	2	3	4	5	6	7	8	9	10	11	12	
A	standard curve	standard curve	flow control pre [1]	flow control t=15 [3]	flow control pre [1]	flow control t=15 [3]	1000pg/ml pre [1]	1000pg/ml t=15 [3]	1000pg/ml pre [1]	1000pg/ml t=15 [3]			
B	standard curve	standard curve	flow control pre [2]	flow control t=30 [1]	flow control pre [2]	flow control t=30 [1]	1000pg/ml pre [2]	1000pg/ml t=30 [1]	1000pg/ml pre [2]	1000pg/ml t=30 [1]			
C	standard curve	standard curve	flow control pre [3]	flow control t=30 [2]	flow control pre [3]	flow control t=30 [2]	1000pg/ml pre [3]	1000pg/ml t=30 [2]	1000pg/ml pre [3]	1000pg/ml t=30 [2]			
D	standard curve	standard curve	flow control t=0 [1]	flow control t=30 [3]	flow control t=0 [1]	flow control t=30 [3]	1000pg/ml t=0 [1]	1000pg/ml t=30 [3]	1000pg/ml t=0 [1]	1000pg/ml t=30 [3]			
E	standard curve	standard curve	flow control t=0 [2]	flow control t=60 [1]	flow control t=0 [2]	flow control t=60 [1]	1000pg/ml t=0 [2]	1000pg/ml t=60 [1]	1000pg/ml t=0 [2]	1000pg/ml t=60 [1]			
F	standard curve	standard curve	flow control t=0 [3]	flow control t=60 [2]	flow control t=0 [3]	flow control t=60 [2]	1000pg/ml t=0 [3]	1000pg/ml t=60 [2]	1000pg/ml t=0 [3]	1000pg/ml t=60 [2]			
G	standard curve	standard curve	flow control t=15 [1]	flow control t=60 [3]	flow control t=15 [1]	flow control t=60 [3]	1000pg/ml t=15 [1]	1000pg/ml t=60 [3]	1000pg/ml t=15 [1]	1000pg/ml t=60 [3]			
H	standard curve	standard curve	flow control t=15 [2]		flow control t=15 [2]		1000pg/ml t=15 [2]		1000pg/ml t=15 [2]				

Figure 217: A typical IL-6 plate immediately after use of the photometric plate reader, the colour differences denoting different concentration of IL-6 can be see clearly in standard curves in columns 1 and 2 of the 96-well plate. The locations of other samples are shown in the printed array.

The photometric plate reader produced a reading of absorbance for each well. This reading of absorbance was corrected by subtracting the absorbance reading from a 'blank' well containing none of the cytokine to be measured. After this, the absorbance reading for the samples in the standard curve was plotted against the known concentration of those samples and an appropriate trend line was added to produce a standard curve from which the concentration of the cytokine being studied could be ascertained from the absorbance measured in a given cell. Figure 218, Figure 219, Figure 220 and Figure 221 show the standard curves for the TNF-α and

IL-6 ELISAs from which the concentrations of the cytokines were calculated. Data analysis and trend lines were added using Microsoft Office Excel 2007 *(Microsoft Corporation, Redmond, WA)*.

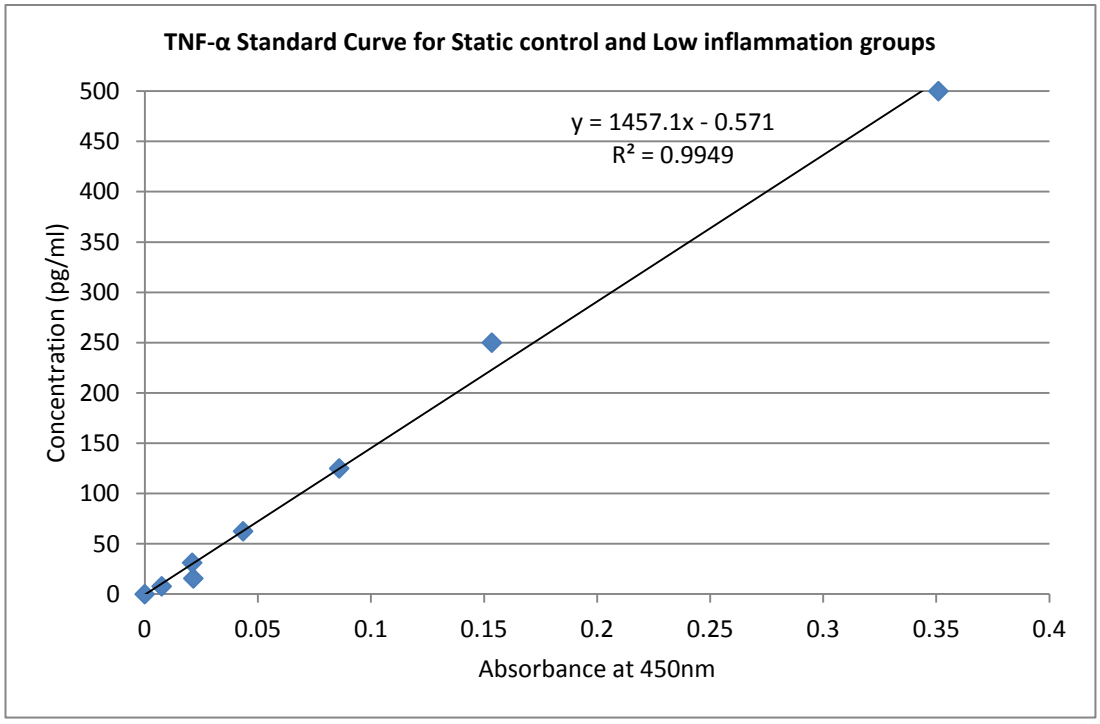


Figure 218: Standard curve from TNF-α ELISA showing relationship between concentration of TNF-α and absorbance at 450nm in the static control and low inflammation groups which was used to calculate TNF-α concentration from the absorbance measured in the sample wells.

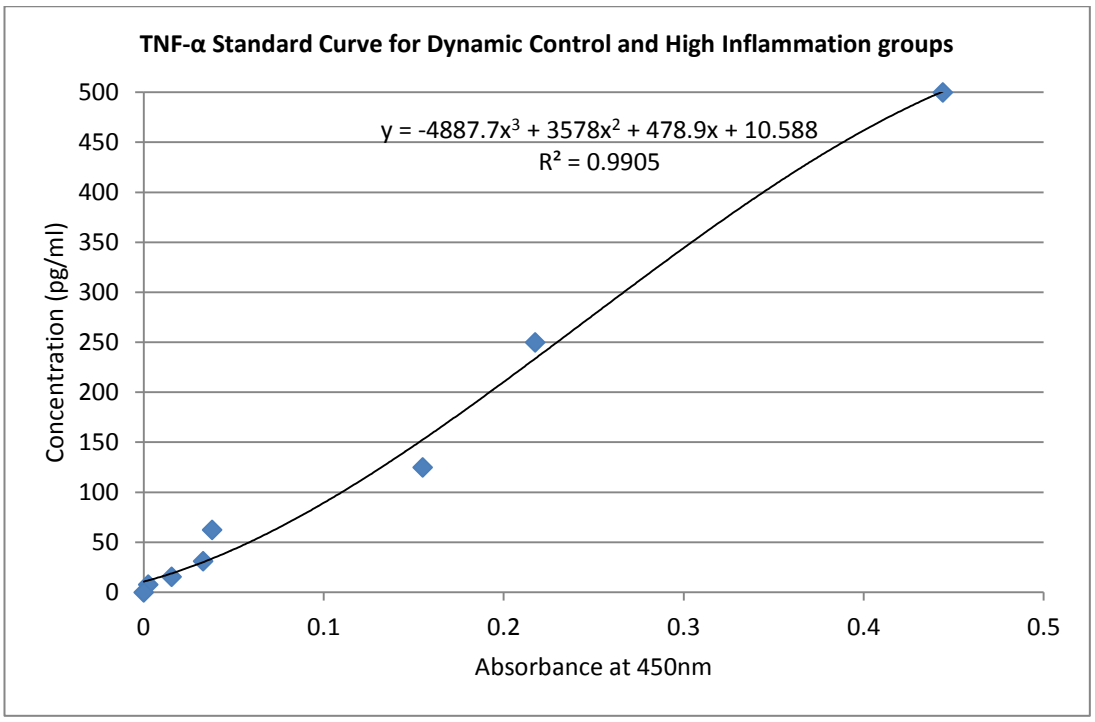


Figure 219: Standard curve from TNF-α ELISA showing relationship between concentration of TNF-α and absorbance at 450nm in the dynamic control and high inflammation groups which was used to calculate TNF-α concentration from the absorbance measured in the sample wells.

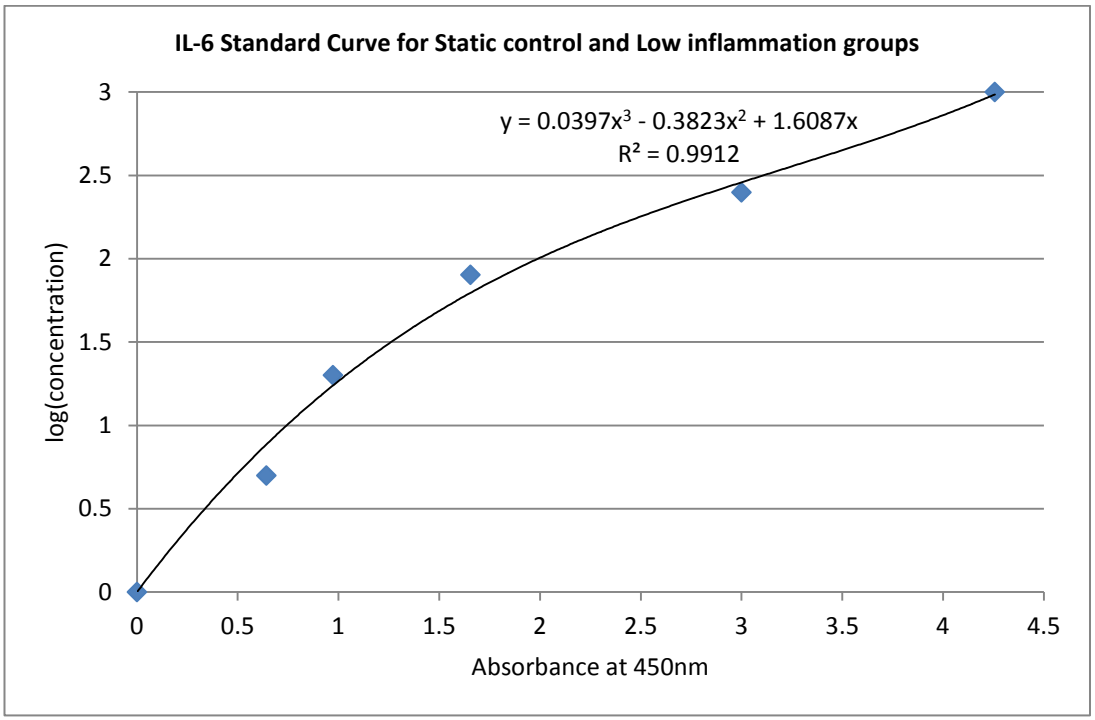


Figure 220: Standard curve from IL-6 ELISA showing relationship between concentration of IL-6 and absorbance at 450nm in the static control and low inflammation groups which was used to calculate IL-6 concentration from the absorbance measured in the sample wells.

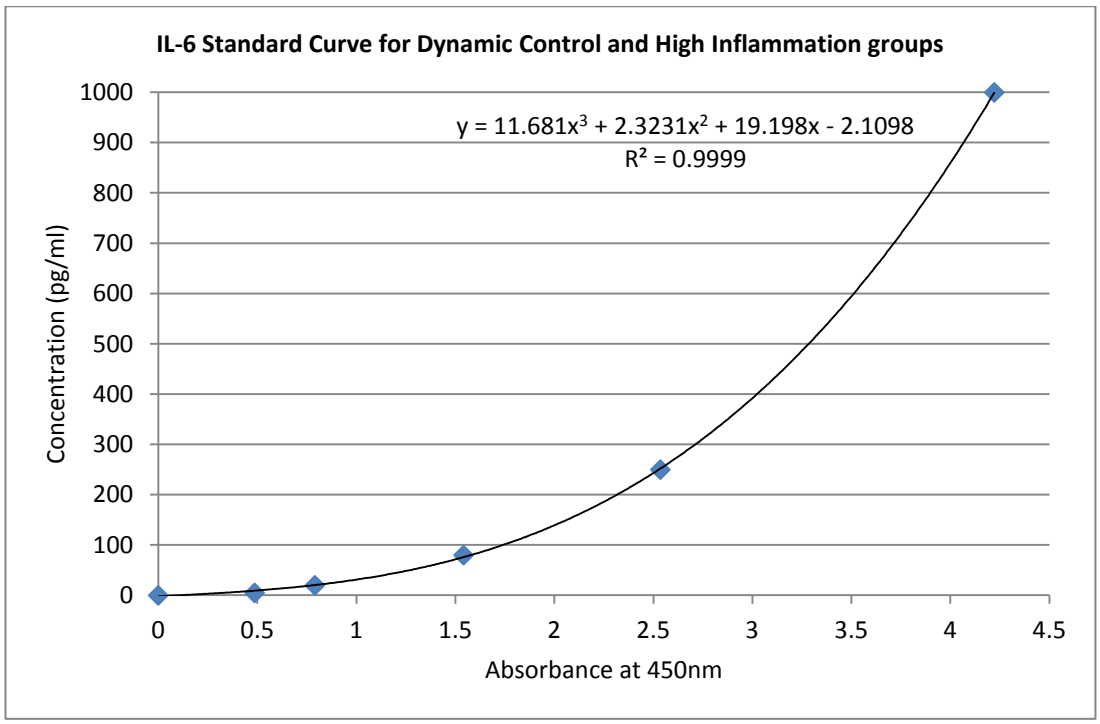


Figure 221: Standard curve from IL-6 ELISA showing relationship between concentration of IL-6 and absorbance at 450nm in the dynamic control and high inflammation groups which was used to calculate IL-6 concentration from the absorbance measured in the sample wells.

Figure 218, Figure 219, Figure 220 and Figure 221 show that in every assay the absorbance readings from the standards form a clear trend when plotted against the known concentrations. There no distinct outliers and the trend lines all have R^2 values greater that 0.99 indicating an excellent fit. This suggests that the concentrations readings from the trend line equation will be reliable and is also an indication that the ELISA procedure was performed correctly.

4.4.2 TNF-α

Figure 222 shows the mean concentration of TNF-α in each of the four groups (static control, dynamic control, high inflammation, low inflammation) at each of five measurement points (before dilution 'whole', after dilution but before MUF 't=0', 't=15', 't=30', and 't=60'). Significant differences between time intervals within the same group were calculated by a paired t-test with $p<0.05$ considered significant.

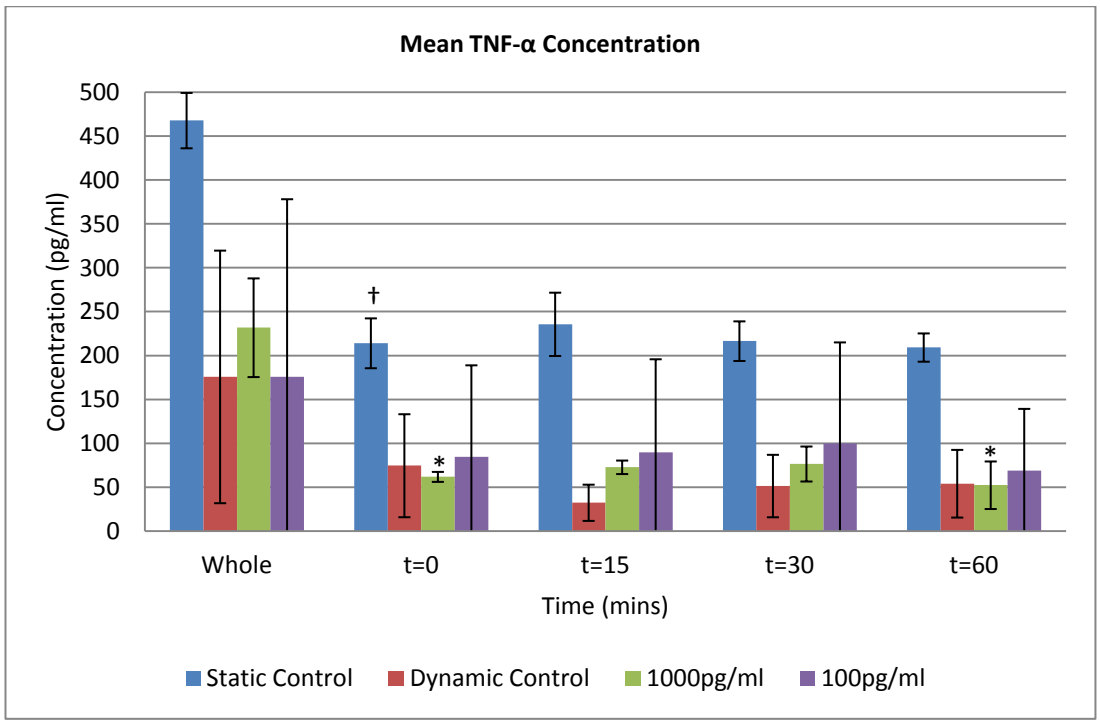


*Figure 222: Mean TNF-α concentration for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood 'whole', the diluted blood before MUF 't=0' and after 15, 30 and 60mins of either MUF or no intervention (t=15, t=30,t= 60). † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

As expected, in every group there is a decrease in TNF-α concentration after dilution, although the change was only significant in the static control and high inflammation groups. As indicated by the error bars of standard deviation there was a large degree of variation in the low inflammation group, but, with the exception of the 'whole' measurement for the dynamic control group, all other results show good consistency between experiments within the same group. The measured values are lower than would be expected (particularly in the high inflammation group) which could indicate an error in the spiking method or uneven distribution of the cytokines through the blood after spiking. Despite this, each group appears to follow a similar trend; the expected decrease in concentration after dilution, followed by no further changes in concentration during the remainder of the experiment period.

Given that this occurs in both control groups (where no plasma is removed) and MUF groups (where plasma is removed) this suggests that TNF-α was removed along with the plasma but only in proportion with the amount of plasma removed and not selectively filtered. If TNF-α was not removed it would have become concentrated in the blood with haemoconcentration, whereas if TNF-α was selectively filtered it would have been come less concentrated in the blood with haemoconcentration. One promising observation however is that between t=30 and t=60 in the high inflammation group, there was a significant decrease in TNF-α concentration.

This suggests that between these intervals, as the blood became more concentrated, TNF-α was indeed selectively removed. This can be seen in Figure 223.

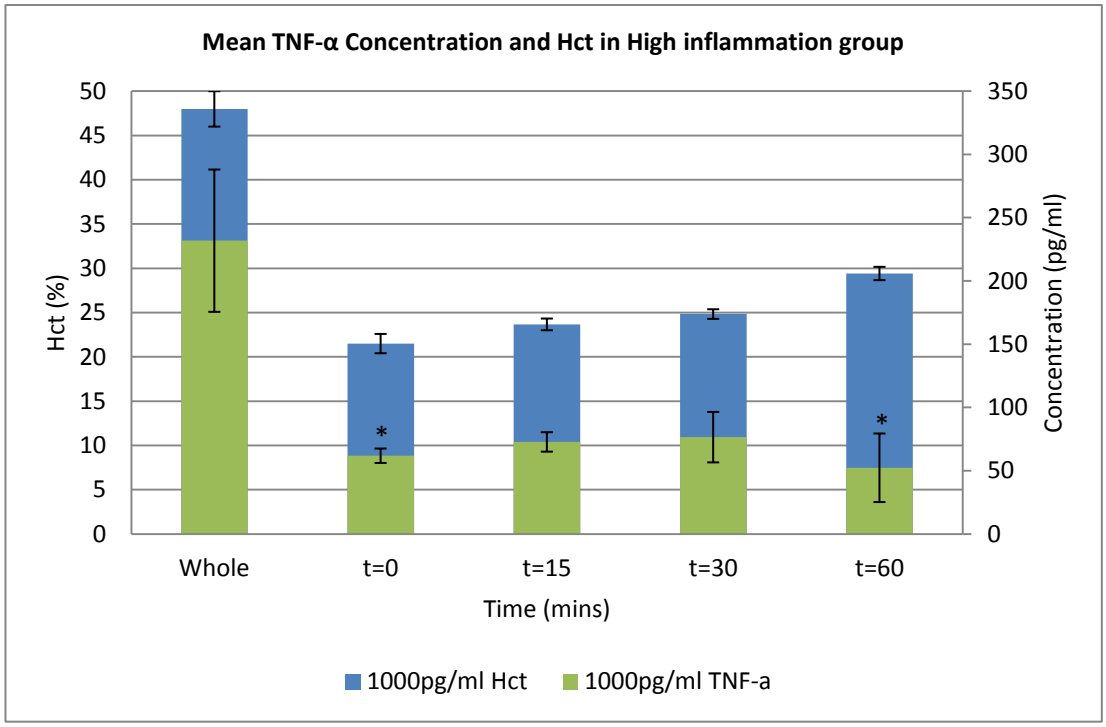


*Figure 223: Mean TNF-α concentration and Hct (n=3) for the high inflammation (1000pg/ml) group, in the initial whole blood 'whole', the diluted blood before MUF 't=0' and after 15, 30 and 60mins of either MUF or no intervention (t=15, t=30,t= 60). † = p<0.01 versus previous interval, * = p<0.05 versus previous interval.*

This observation was not seen in the low inflammation group but this effect may have been masked by the variation in measurements. This observation leaves open the possibility that TNF-α is selectively removed by the proposed device at higher Hct, or later in the MUF procedure, which could be confirmed by further investigation.

4.4.3 IL-6

Figure 224 shows the mean concentration of IL-6 in each of the four groups (static control, dynamic control, high inflammation, low inflammation) at each of five measurement points (before dilution 'whole', after dilution but before MUF 't=0', 't=15', 't=30', and 't=60'). Significant differences between time intervals within the same group were calculated by a paired t-test with $p<0.05$ considered significant.

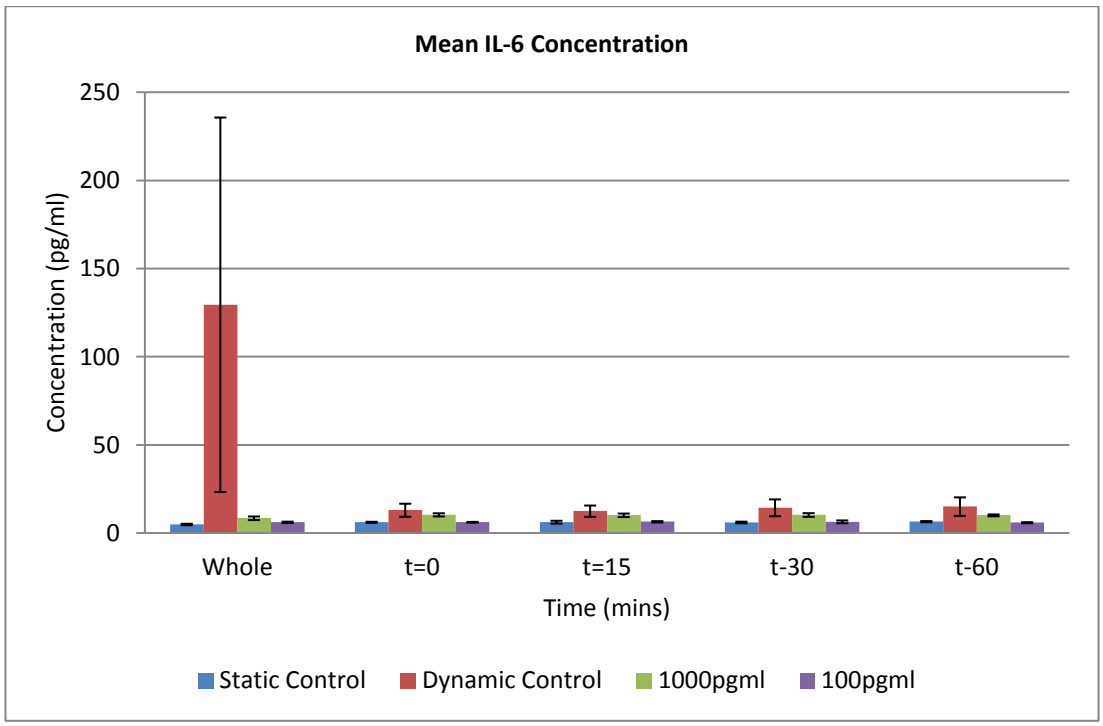


*Figure 224: Mean IL-6 concentration for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood 'whole', the diluted blood before MUF 't=0' and after 15, 30 and 60mins of either MUF or no intervention (t=15, t=30,t= 60). † = p<0.01 versus previous interval, * = p<0.05 versus previous interval (n=3).*

Although the good standard curves for the IL-6 ELISAs seemed to suggest the assay was performed correctly, the results obtained from the plasma samples are difficult to assess. With the exception of the 'whole' measurement in the dynamic control group the concentrations are very low in every group at every interval. Given that the groups were 'spiked' with much larger concentrations of IL-6 (particularly the high inflammation group) it is surprising that the measured concentrations are so low. As indicated by the error bars of standard deviation the variation in the results is low however, suggesting consistency in the method and measurements between experiments within the same group. This, in conjunction with the good standard curves suggests that the IL-6 concentrations in the samples are indeed low and that there was an issue with the spiking of the blood rather than the ELISA itself. There was no significant change in any group over the duration of the experiment even after dilution which again brings into question the validity of the measurements. Unfortunately therefore, it is difficult to draw any meaningful conclusions from this data.

Overall, based predominantly on the observations of TNF-α, it appears that pro-inflammatory mediators are not selectively filtered by the proposed device but are removed along with plasma in proportion to the amount of plasma removed. The significant decrease in TNF-α concentration between t=30 and t=60 observed in the high inflammation group suggests that at higher Hct, or later in the MUF procedure, TNF-α may be selectively removed but further

investigation is required. Clinically speaking, presently it appears that there would be no additional inflammatory reducing benefits of MUF with the proposed device, although there is some evidence to the contrary which in fact nicely mirrors the contrasting evidence regarding the impact of MUF on inflammatory mediators discussed in *1.3.10. Impact of MUF on Inflammatory Mediators*.

4.5 Impact of Blood Age and Source

As mentioned previously (*4.3.1 Methodology*), for logistical reasons, of the three experiments in each group, two were conducted with blood sourced a few hours earlier and one with blood sourced the previous day. Ideally, each experiment would have been conducted with identical blood, however this was not possible since experiments took place over a number of days and blood was collected at the point of slaughter. This scenario does however allow for some analysis of the impact of blood age (do the constituents of fresh blood differ significantly after one day storage?) and the blood source (how much variation in blood constituents is there between different cows?). Additionally, by studying the variation in the initial 'whole blood' measurements of the blood constituents from the same source but different experiments will allow us to assess the consistency of the complete blood count measurements. As these analyses were not the main focus of this study, n numbers are low and thus interpretation of the results should bear this in mind.

Table 18 shows the source and age of blood used in each experiment.

Blood Source	Blood Age	Experiments
COW 1	Fresh (<6hrs old)	SC1, SC2, HI1, HI2,
	Day old (24hrs old)	SC3, HI3, LI1
COW 2	Fresh (<6hrs old)	LI2, LI3
	Day old (24hrs old)	DC1
COW 3	Fresh (<6hrs old)	DC2, DC3

Table 18: Age and source of blood used in each experiment, SC=Static control group, DC=Dynamic control group, HI=High inflammation group, LI=Low inflammation group

4.5.1 Variations in blood from different bovine sources

When blood is sourced from different cows there will always be some degree of variation in the levels of the different constituents. Variation in the Hct and Hb levels of bovine blood used for testing medical devices is well known (Franco et al. 2003). Table 19 shows the bovine blood constituent reference range values from the Merck Veterinary Manual which gives some indication as to the wide range of variation possible amongst 'normal' animals (Merck et al. 2010).

Parameter	Unit	Reference Range
Red Blood Cell Count (RBC)	$\times 10^{12}$/l	5.0 – 10.0
Haemoglobin (Hb)	g/dl	8.0 – 15.0
Haematocrit (Hct)	%	24 – 46
Mean Corpuscular Volume (MCV)	fl	40.0 – 60.0
Mean Corpuscular Haemoglobin (MCH)	pg	11.0 – 17.0
Mean Corpuscular Haemoglobin Concentration (MCHC)	g/dl	30.0 – 36.0
White Blood Cell Count (WBC)	$\times 10^{9}$/l	4.0 – 12.0
Neutrophil Count	$\times 10^{9}$/l	0.6 – 4.12
Lymphocyte Count	$\times 10^{9}$/l	2.5 – 7.5
Monocyte Count	$\times 10^{9}$/l	0.0025 – 0.84
Eosinophil Count	$\times 10^{9}$/l	0.00 – 2.40
Platelet Count (PLT)	$\times 10^{9}$/l	100 – 800

Table 19: Bovine blood constituent reference range values from the Merck Veterinary Manual (Merck et al. 2010).

The nature of the data collected meant that comparisons could be made between the blood sourced from different cows and any significant differences in terms of those parameters measured as part of the complete blood count could be found. Table 20 shows the results of Single Factor Analysis of Variance (ANOVA) which was used to determine if there were significant differences between blood samples sourced from different animals in terms of the various haematological parameters.

Parameter	F	F_{crit}	p-value
Red Blood Cell Count (RBC)	178.60	5.78	***<0.001***
Haematocrit (Hct)	179.98	5.78	***<0.001***
Mean Corpuscular Volume (MCV)	228.79	5.78	***<0.001***
Red Blood Cell Distribution Width (RDW)	120.90	5.78	***<0.001***
White Blood Cell Count (WBC)	103.89	5.78	***<0.001***
Neutrophil Count	14.64	5.78	***<0.05***
Lymphocyte Count	93.42	5.78	***<0.001***
Monocyte Count	0.06	5.78	0.946
Eosinophil Count	4.83	5.78	0.068
Basophil Count	2.44	5.78	0.182
Haemoglobin (Hb)	262.37	5.78	***<0.001***
Cellular Haemglobin Concentration	278.89	5.78	***<0.001***
Plasma Free Haemoglobin Concentration	0.38	5.78	0.705
Mean Corpuscular Haemoglobin (MCH)	34.61	5.78	***<0.05***
Mean Corpuscular Haemoglobin Concentration (MCHC)	7.50	5.78	0.03

Table 20: Results of ANOVA for each of the complete blood count parameters. If $F>F_{crit}$ null hypothesis (mean is the same for all groups) is rejected, i.e. significant differences exist between the groups.

In order to determine between which animals the significant differences occurred for each parameter, a further ANOVA post-hoc statistical test is required. Namely, the Scheffe test to directly compare Cow 1 vs Cow 2, Cow 1 vs Cow 3, and Cow 2 vs Cow 3. Figure 225, Figure 226, Figure 227, Figure 228, Figure 229, and Figure 230 show the means of the initial 'whole blood'

measurements of each complete blood count parameter across experiments conducted with the same fresh blood (e.g. SC1, SC2, HI1 and HI2) from each of the three sources; Cow 1, Cow 2 and Cow 3. The error bars of standard deviation give an indication as to the consistency of the complete blood count measurements. Significant differences between the three groups were calculated by using a post-hoc ANOVA test called the Scheffe test. In a change from previous figures, only not significant differences are annotated on the figures. As mentioned previously (*4.3.5 Platelet levels and condition*), PLT, MPV, PCT and PDW were not measured in Cow 1 experiments due to aggregation and are omitted from the data presented below.

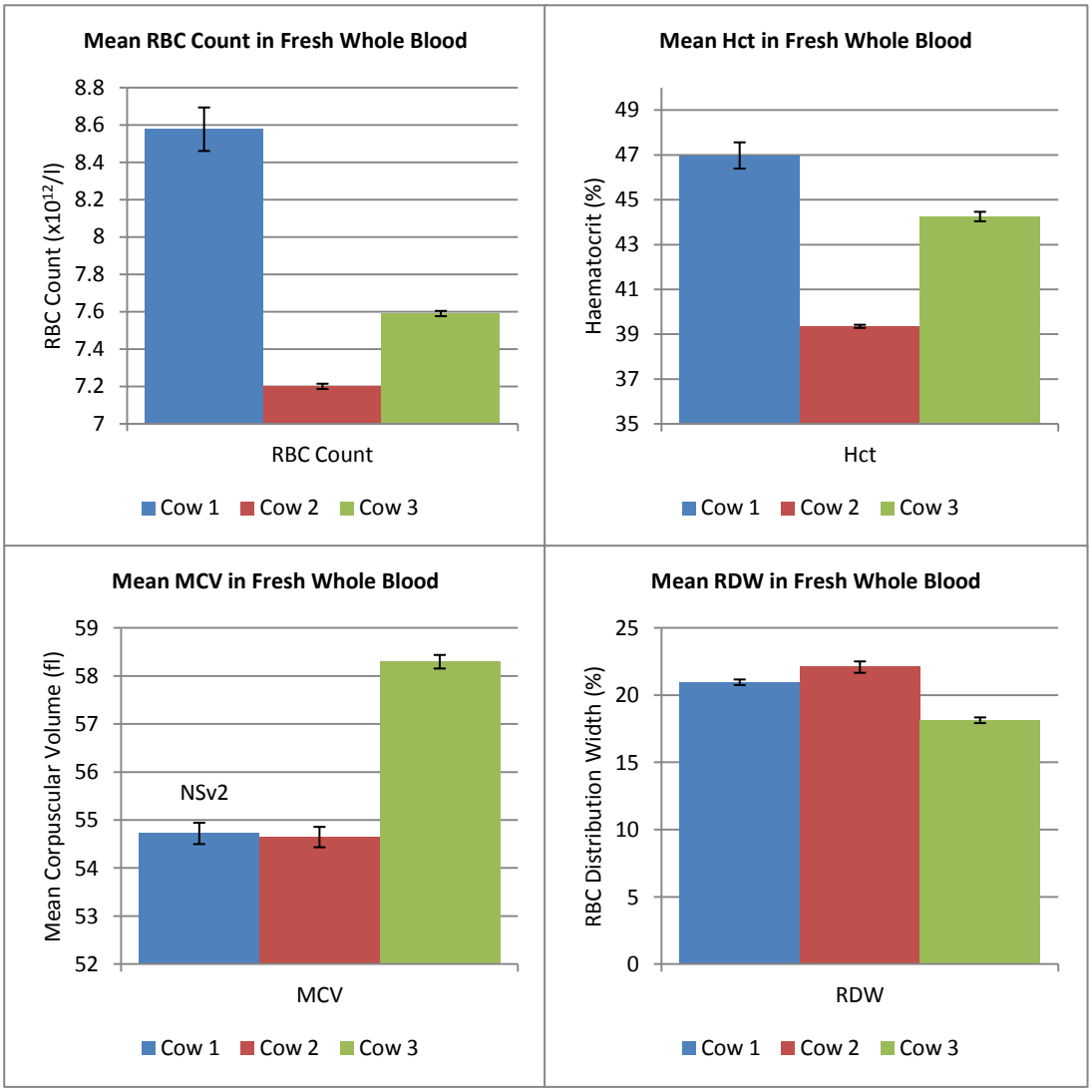


Figure 225: Mean RBC count (x10¹²/l), Hct (%), MCV (fl), and RDW (%) in freshly sourced whole (undiluted) blood for each of the three cows from which blood for these experiments was taken. NSv = Non-significant change versus indicated cow.

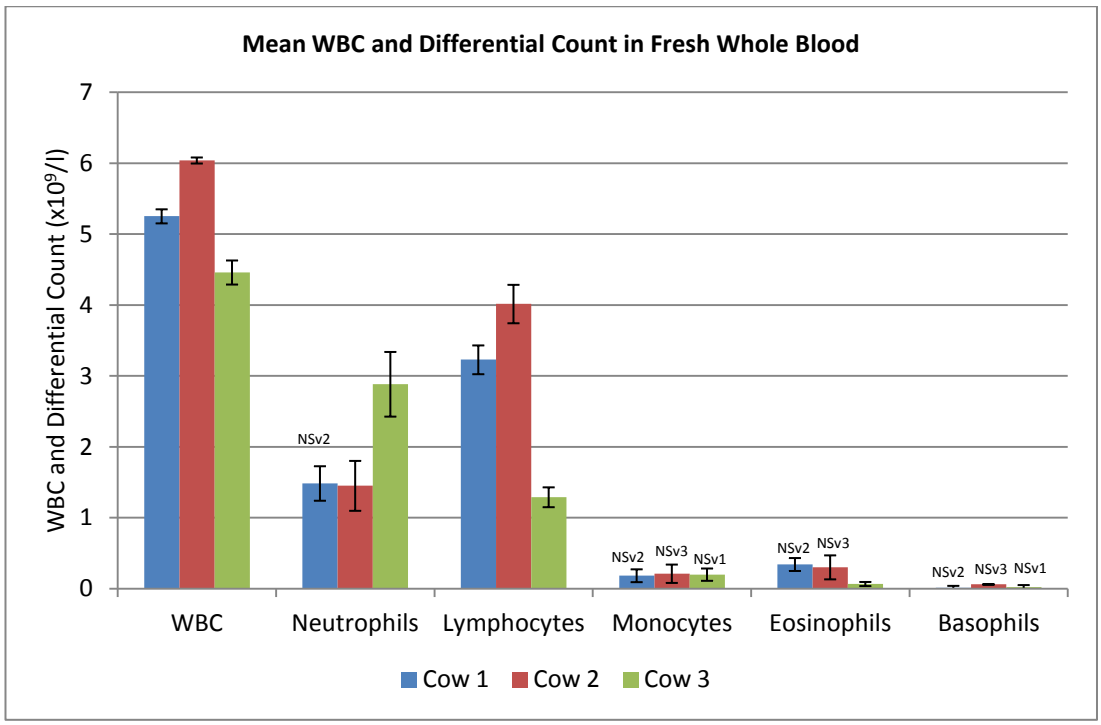


Figure 226: Mean WBC and Differential count (x10⁹/l) in freshly sourced whole (undiluted) blood for each of the three cows from which blood for these experiments was taken. NSv = Non-significant change versus indicated cow.

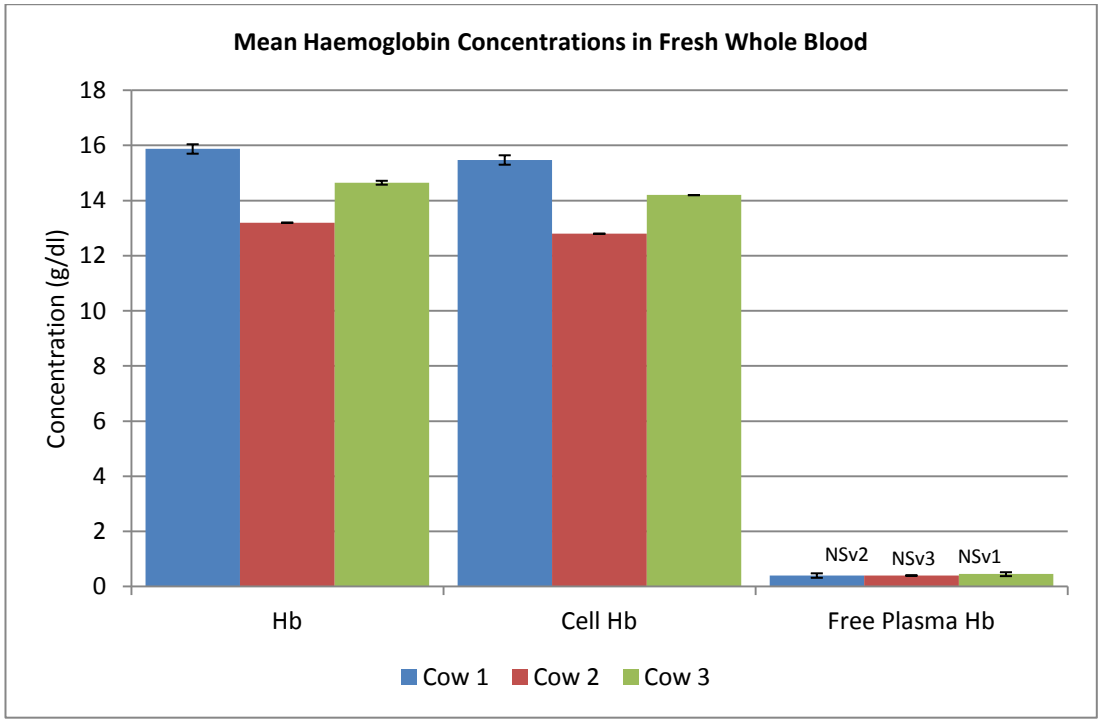


Figure 227: Mean Haemoglobin concentrations (g/dl) in freshly sourced whole (undiluted) blood for each of the three cows from which blood for these experiments was taken. NSv = Non-significant change versus indicated cow.

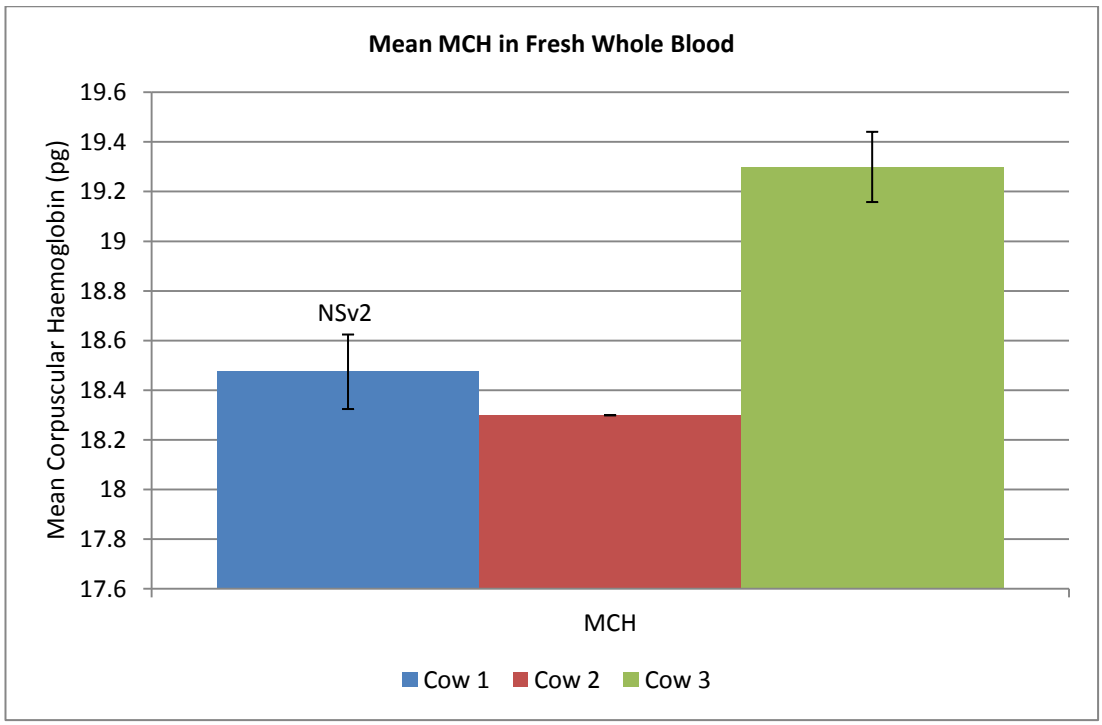


Figure 228: Mean MCH (pg) in freshly sourced whole (undiluted) blood for each of the three cows from which blood for these experiments was taken. NSv = Non-significant change versus indicated cow.

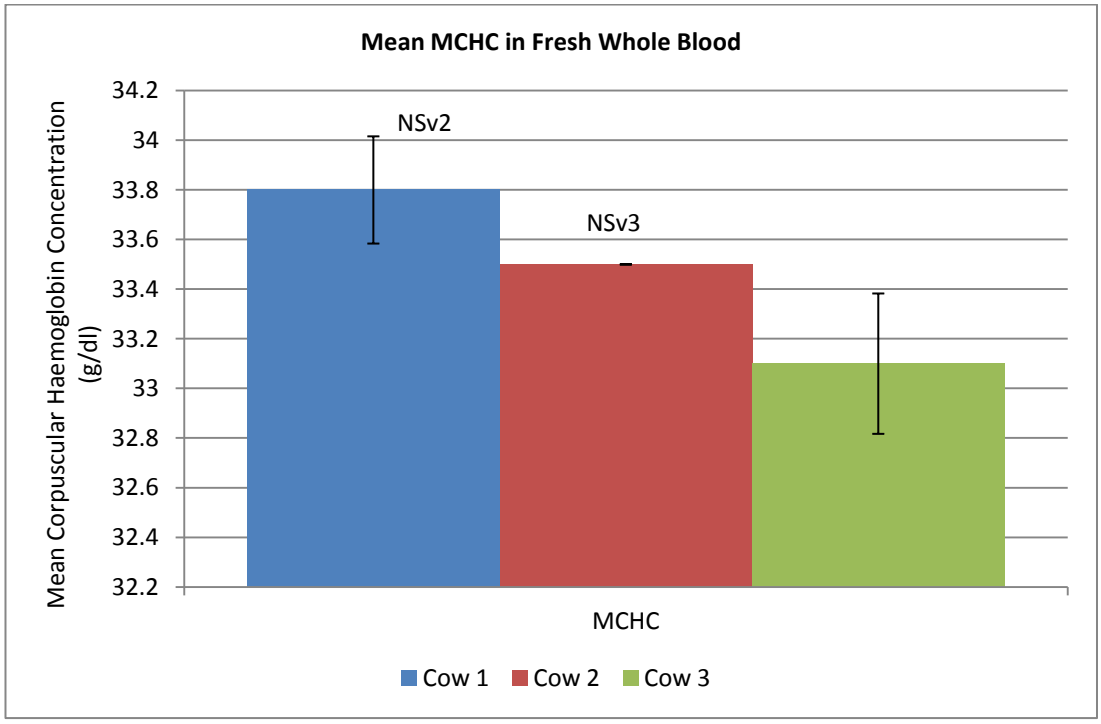


Figure 229: Mean MCHC (g/dl) in freshly sourced whole (undiluted) blood for each of the three cows from which blood for these experiments was taken. NSv = Non-significant change versus indicated cow.

For the remaining platlet parameters (PLT, MPV, PCT and PDW) where there was no data for Cow 1 due to aggregation an unpaired t-test was used with $p<0.05$ considered significant.

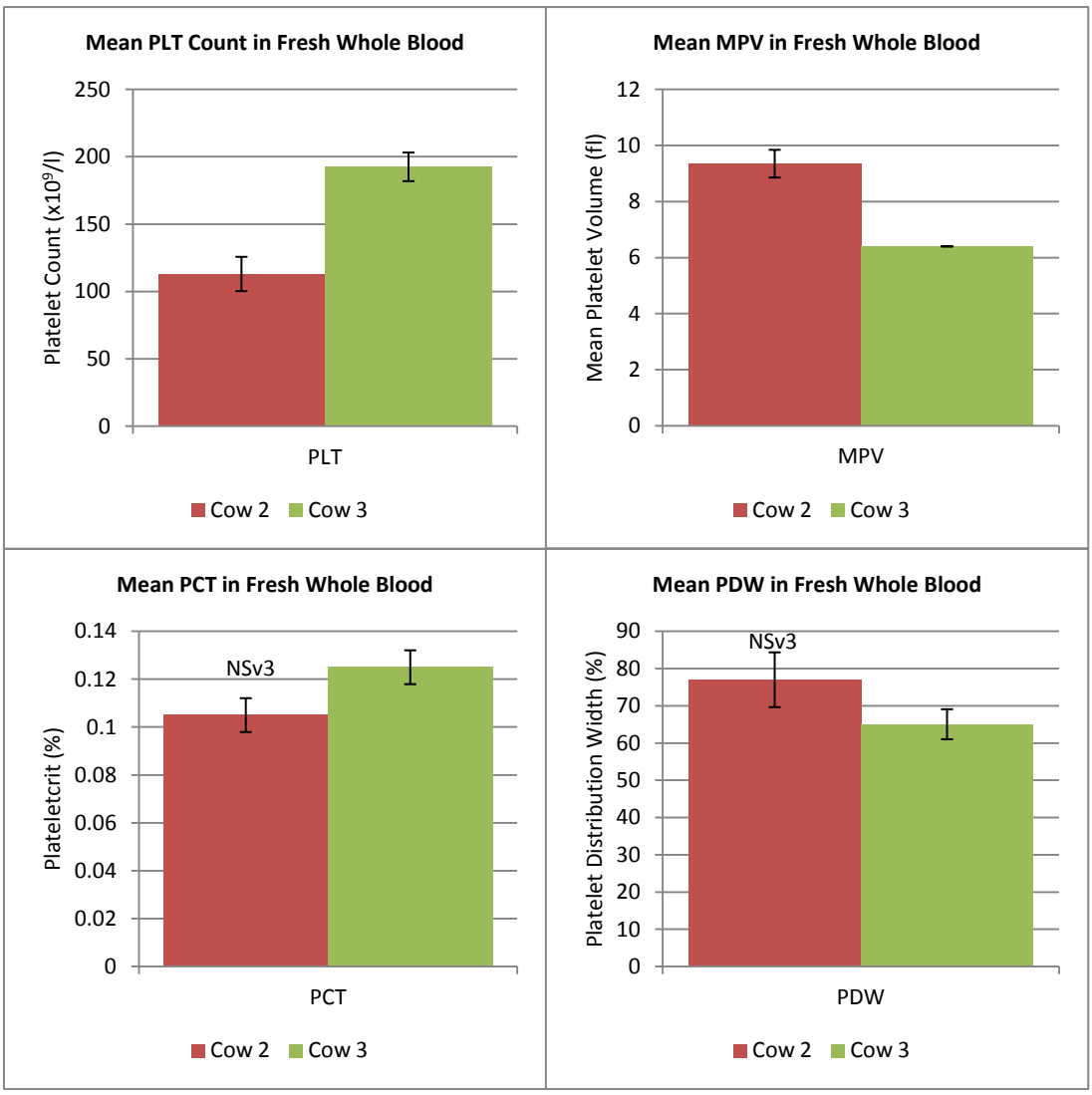


Figure 230: Mean PLT count (x10⁹/l), MPV (fl), PCT (%), and PDW (%) in freshly sourced whole (undiluted) blood for two of the cows from which blood for these experiments was taken and platelet data could be obtained. NSv = Non-significant change versus indicated cow.

It can be seen from Figure 225, Figure 226, Figure 227, Figure 228, Figure 229, and Figure 230 that for the majority of parameters, there were significant differences between the values measured in different cows. Monocyte count, free plasma Hb, PCT and PDW were the only parameters in which there was no significant difference between any of the cows studied. These results suggest that there is sufficient variation in the parameters of complete blood counts to warranty caution when comparing or collating results using blood from different cows. Fortunately, the results presented in this thesis are more concerned with the change observed from baseline levels rather than the absolute values of the various parameters. In the clinical setting it is reasonable to assume that there will be significant variation in the blood constituents of different patients and therefore the use of blood from different cows is at least in some way appropriate. The ability of the proposed device to work across a wide range of

baseline blood profiles is very important and thus inclusion of this variation in laboratory testing is not problematic provided researchers bear this in mind when analysing the data and drawing conclusions.

4.5.2 Blood age

Another question raised at the beginning of this section was do the constituents of fresh blood differ significantly after one day storage? Significant alterations in RBC membrane integrity and flow properties are known to occur during storage of blood as well as a significant increase in free haemoglobin, however these changes occur over durations much longer than the 24hr period of storage present in this study with blood donations for transfusions routinely stored for 35-42 days depending on anticoagulant and storage conditions (Sowemimo-Coker 2002). Additionally, older, denser, RBCs have been reported to aggregate to a significantly greater extent and stronger degree compared to younger, less dense cells (Rampling et al. 2004). Leukocytes can have a haemolytic effect on RBCs during storage as they can degrade, releasing proteases which have been reported to cause RBC lysis (Sowemimo-Coker 2002).

As mentioned previously (*4.3.1 Methodology*), for logistical reasons both fresh (<6hrs old) and day old (24hrs old) blood was used in this experiment set, and in each group two experiments involved fresh blood and one involved day old blood. Prior to this experiment set blood smears of fresh and day old blood were imaged under an optical microscope according to the method described earlier in this chapter. Figure 231 shows a comparison between these two smears.

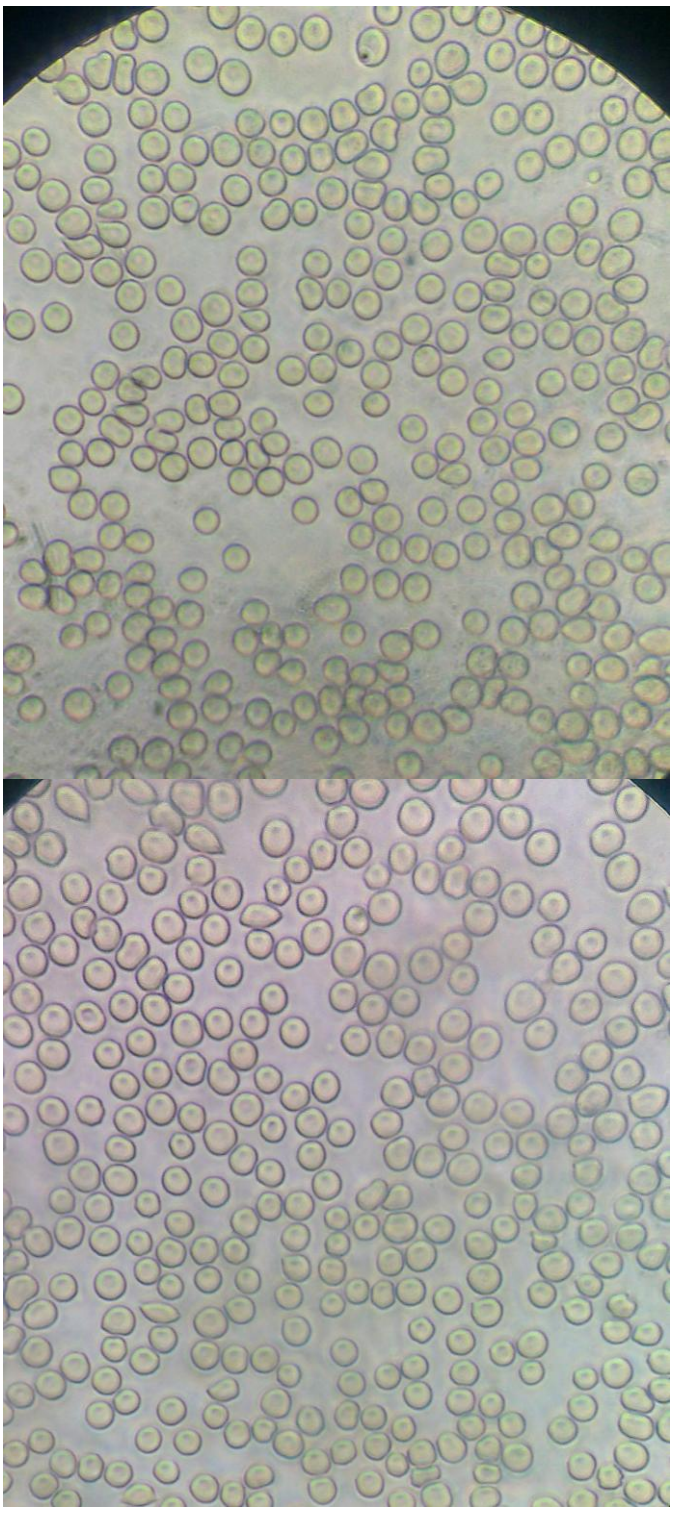


Figure 231: Optical microscope image at x100 magnification of a blood smear from a sample of: fresh blood (<6hrs old) (top) day old blood (24hrs old) (bottom).

There does not appear to be any clear difference in the numbers of poikilocytes (abnormally shaped RBCs) in these samples which suggests that one day of storage does not adversely affect RBCs however the complete blood count results will provide a more accurate assessment.

Figure 232, Figure 233, Figure 234 and Figure 235 show the means of the initial 'whole blood' measurements of each complete blood count parameter across experiments conducted with the either fresh blood (experiments SC1, SC2, HI1 and HI2) or day old blood (experiments SC3, HI3, LI1) from Cow 1. The blood from Cow 1 was used in four experiments when fresh and three experiments when a day old and therefore was selected for analysis on the basis that it was the cow for which most CBC data was collected. Significant differences between the fresh and day old blood were calculated by an unpaired t-test assuming equal variance with $p<0.05$ considered significant. As with earlier graphs, significant differences are marked as annotations where appropriate (* = $p<0.05$). As mentioned previously (*4.3.5 Platelet levels and condition*), PLT, MPV, PCT and PDW were not measured in Cow 1 experiments due to aggregation and are omitted from the data presented below.

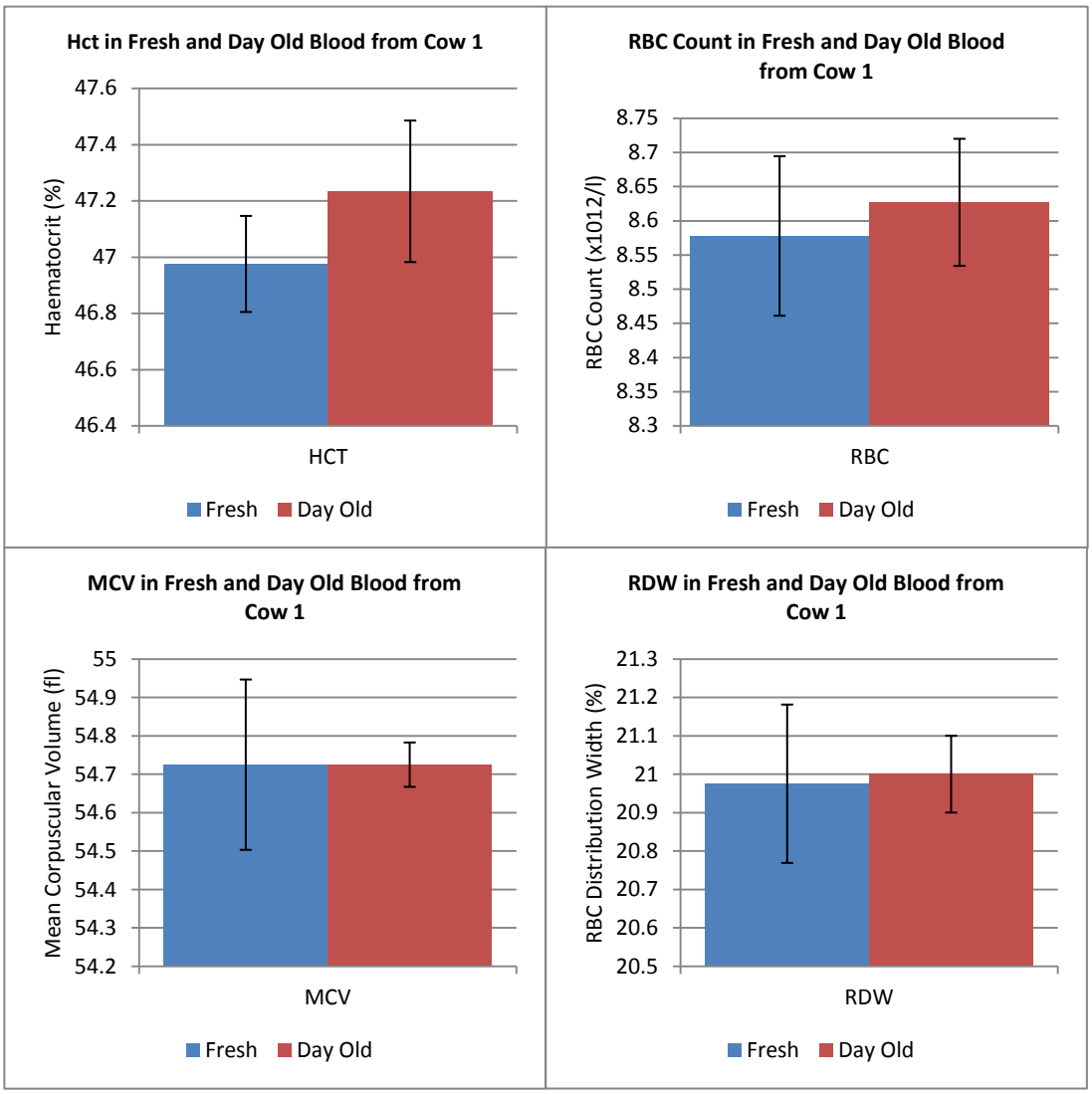


*Figure 232: Mean RBC count (x10^{12}/l), Hct (%), MCV (fl), and RDW (%) in fresh (<6hrs) and 'day old' (24hrs) blood from Cow 1. * = p<0.05.*

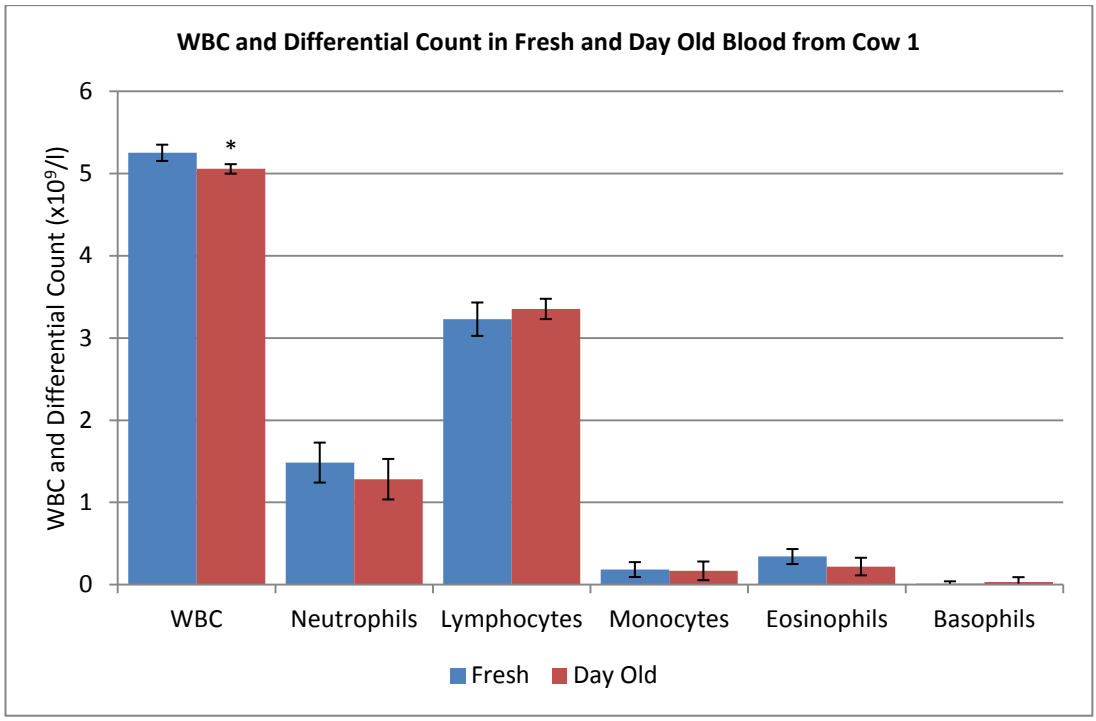


*Figure 233: Mean RBC count (x10⁹/l) in fresh (<6hrs) and 'day old' (24hrs) blood from Cow 1. * = p<0.05.*

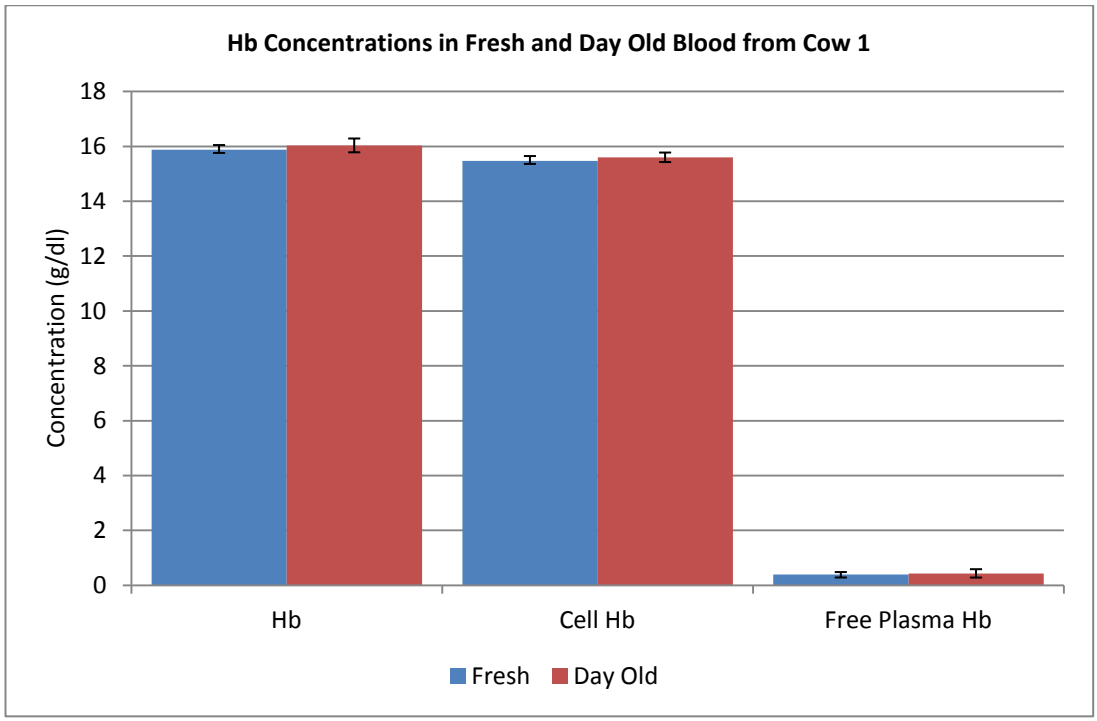


*Figure 234: Mean haemoglobin concentrations (g/dl) in fresh (<6hrs) and 'day old' (24hrs) blood from Cow 1. * = p<0.05.*

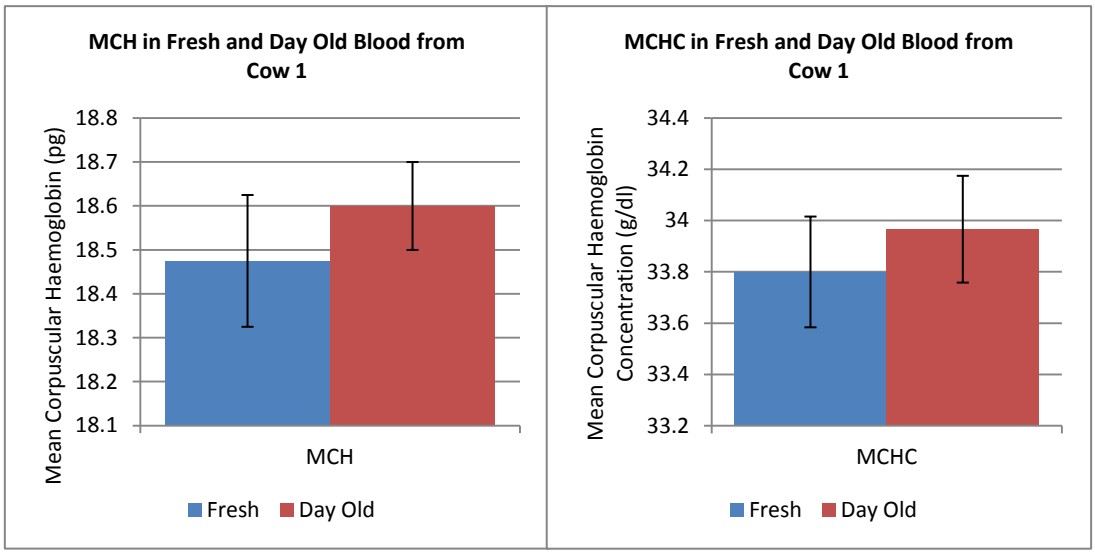


*Figure 235: Mean MCH (pg) and MCHC (g/dl) in fresh (<6hrs) and 'day old' (24hrs) blood from Cow 1. * = p<0.05.*

From Figure 232, Figure 233, Figure 234, and Figure 235 it can be seen that there was no significant difference between the fresh and day old blood in any of the complete blood count measured parameters with the exception of the WBC count which was significantly higher in the fresh blood. Although WBC was significantly lower in the old blood, there was no significant difference in any of the five WBC types suggesting that the significant WBC count difference was a cumulative effect of small differences in each of the five WBC types. The results suggest that, generally speaking, day old blood is not significantly different to fresh blood in terms of its constituents.

This, coupled with the previous results regarding blood from different cows, suggests that in order to minimise variation in baseline blood levels, it is better to use day old blood from the same source rather than use fresh blood from a new source.

4.5.3 Blood quality at source

It is important that the blood for testing is of good quality before it is used in experiments so that a fair assessment of the proposed device can be made. Table 21 shows the mean initial whole fresh blood measurements for all the blood received from the abattoir cows, which was used for the post-MUF blood quality analysis studies. Parameters for which there was no reference range have been omitted since no assessment of the mean fresh whole blood value can be made for these. Values are reported correct to the same number of decimal places as the original raw data that was provided by *Veterinary Diagnostic Services*.

Parameter	Unit	Reference Range	Mean Fresh Whole Blood Value
Red Blood Cell Count (RBC)	$\times 10^{12}$/l	5.0 – 10.0	7.99
Haemoglobin (Hb)	g/dl	8.0 – 15.0	14.9
Haematocrit (Hct)	%	24 – 46	44
Mean Corpuscular Volume (MCV)	fl	40.0 – 60.0	55.6
Mean Corpuscular Haemoglobin (MCH)	pg	11.0 – 17.0	18.6
Mean Corpuscular Haemoglobin Concentration (MCHC)	g/dl	30.0 – 36.0	33.6
White Blood Cell Count (WBC)	$\times 10^{9}$/l	4.0 – 12.0	5.25
Neutrophil Count	$\times 10^{9}$/l	0.6 – 4.12	1.826
Lymphocyte Count	$\times 10^{9}$/l	2.5 – 7.5	2.941
Monocyte Count	$\times 10^{9}$/l	0.0025 – 0.84	0.194
Eosinophil Count	$\times 10^{9}$/l	0.00 – 2.40	0.263
Platelet Count (PLT)	$\times 10^{9}$/l	100 – 800	153

Table 21: Mean initial whole fresh blood measurements for all the blood received from the abattoir cows which was used for the post-MUF blood quality analysis studies.

As can be seen from Table 21, all parameters were within the reference ranges with the exception of MCH which was above the normal range. Total haemoglobin (Hb) which was measured using the lysate method as described previously (*4.3.1 Methodology*), and is used to calculate MCH (*MCH = (10 x Hb) / RBC*) was very close to the upper limit of the reference range whereas the RBC count was more normal. Interestingly MCHC which is also calculated using Hb (*MCHC = (Hb x 100) / Hct*) was within the normal reference ranges, most likely due to the fact that Hct was also at the upper end of the reference range. However, as mentioned previously (*4.3.1 Methodology*), MCH can be falsely elevated by high blood concentrations of heparin and since each 5L batch of blood was heparinised with 10kIUs of heparin sodium from porcine mucosa it is possible that this was the case (Fischbach et al. 2009). A genuine elevated MCH in cows would suggest an increased number of reticulocytes which is a sign of haemolysis (Divers et al. 2008). Haemolysis in the blood received from the abattoir could be a result of the collection process and may negatively impact upon the accuracy of the haemolysis analysis. The mean %haemolysis for all the whole fresh bovine blood collected from the abattoir cows and used for the post-MUF blood quality analysis studies was calculated by the aforementioned 'Sowemimo-Coker method' to be 1.54% based on mean Hb of 14.9g/dl, a mean plasma free haemoglobin of 0.41g/dl, and a mean Hct of 44%. This is above the clinically acceptable 1% suggested by Sowemimo-Coker et al, and moreover, the Hb measurement is above the recommended Hb content of 12±1 g/dl for blood for device testing from the FDA guidance for CPB oxygenators (FDA 2000b; Sowemimo-Coker 2002). Again it must be stressed that high blood heparin concentration can influence measurements of haemoglobin content but the

suggestion appears to be that the blood received from the abattoir had undesirable levels of haemolysis prior to testing.

The suitability of bovine blood, and particularly abattoir bovine blood, for testing of this kind is an important consideration. Due to its availability and low cost bovine blood is recommended for early and comparative testing of devices although varying values of Hct and Hb can be expected (Franco et al. 2003). However, bovine blood exhibits different aggregation behaviours to human blood, showing negligible aggregation in dextran 70kDa which markedly aggregate human blood (Rampling et al. 2004). Additionally, human blood is more haemolytic than animal blood and is a better model for the clinical setting and consequently for the final assessment of a developmental device, fresh human blood is recommended (Franco et al. 2003).

There are potential disadvantages of using abattoir blood in these experiments. There are a number of factors which cannot be controlled which may influence the quality of the blood from the abattoir source. Firstly, there is no scope for control of the animals' age, gender, motility, or fluid intake which, as shown earlier in this chapter in Table 17 (*4.3.7 Potential interfering factors*), can affect Hct, Hb, and RBC, WBC and platelet counts. Secondly, the nature of the collection procedure does not allow for precise control of volume collected which, since heparin in saline is added to the collection container before the blood, can introduce variation in terms of heparin concentration in the blood. Another important factor is stress of the animals. It has been seen previously in this chapter in Table 17 (*4.3.7 Potential interfering factors*) how stress and pain can influence the WBC, neutrophil, eosinophil and platelet counts, but stress is known to influence the blood in other ways. Under acute stressful conditions, catecholamine discharge results in a significant reduction in the volume of the circulatory system and a significant increase in blood pressure leading to a fluid shift from the vascular space to the interstitial area and thus a rise in Hct (Baskurt et al. 2003). Concurrently, the protein content of the plasma increases leading to increased plasma viscosity and potentially increased RBC aggregation due to the increased fibrinogen concentration (Baskurt et al. 2003). Additionally, most mammals have a reserve volume of RBCs in the sphanchnic region which under stressful conditions can be rapidly introduced into the circulating bloodstream thus increasing Hct (Baskurt et al. 2003). In humans the reserve is small but in other mammals such as horses the reserve is much larger (Baskurt et al. 2003).

Despite these concerns, it was seen that the blood parameters for the abattoir bovine blood were generally within the normal reference ranges although there were some indications of unacceptable levels of haemolysis, yet these may have been falsely characterised by the high heparin content of the blood.

Chapter 5

Discussion

5. Discussion

5.1 Objectives

This thesis began with a discussion of the problem of post-CPB edema. Edema is particularly prevalent within paediatric patient populations and has been shown to cause organ dysfunction and lengthen procedure times by increasing the need for circulatory and respiratory support and delaying chest closure (Brans et al. 1981; Maehara et al. 1991; Seghaye et al. 1996; Neuhof et al. 2003; McGuinness et al. 2008). Edema is thought to be a combination of the inflammatory response and capillary leak brought about by exposure of the blood to the artificial surfaces of the CPB circuit (Miyaji et al. 2008), the extended subjection to hypothermic conditions and increased haemodilution (Ungerleider 2005). Several edema therapies aimed at controlling these factors have been devised, one of which, modified ultrafiltration (MUF), developed by Naik, Knight and Elliott of The Hospital for Sick Children, Great Ormond Street, London in 1991 was a modification of what is now termed 'conventional' ultrafiltration and involves haemofiltration immediately after CPB in a parallel circuit (Naik et al. 1991a). MUF has been studied extensively and has demonstrated benefits in cardiovascular function, pulmonary function, neurological function, total body water, transfusion requirements, coagulation and length of intensive care unit (ICU) stay. Additionally, there is some evidence to suggest MUF mitigates the inflammatory response by removing inflammatory mediators.

The objective of this thesis was to detail the process and outcomes of the development of a super-adsorber based haemofiltration technology for use in paediatric cardiopulmonary bypass. The development of the device in question involved the integration of the existing technique of modified ultrafiltration with a new superabsorber technology with the aim to create a novel device which has the ability to extract excess fluid from flowing blood, suitable for clinical use within a cardiopulmonary bypass circuit.

The device development objectives can be summarized as follows:

- Achieve passive chemically driven haemoconcentration of diluted bovine blood at a fixed flow rate
- Haemoconcentrate in a timely manner, comparable to current modified ultrafiltration procedures
- Output haemoconcentrated blood of good quality with minimal damage to the constituent parts of the blood, specifically avoiding haemolysis maintaining as much as possible the populations of red blood cells and platelets.

- Demonstrate selective removal of pro-inflammatory mediators by the haemofiltration method.
- Ensure procedures required to achieve the aforementioned objectives make reasonable expectations of, or where possible remove, input from clinical staff.

After extensive research into and discussion of the principles of ultrafiltration, ultrafilter systems and superabsorbent polymers it was decided that the device should be a cross flow system as this is most applicable to the continuous flow present in CPB circuits and will more successfully limit filter cake and protein adhesion to the membrane. Additionally, the device should comprise a single flat bed chamber as this configuration best allows for the swelling of the superabsorber and requires only a single surface to be managed, and moreover, this configuration is by far the simplest and cheapest to produce, and make modifications to, in-house and thus a rapid succession of prototypes can be developed.

Subsequently, development of the device involved iterative design improvements based on laboratory analysis.

5.2 Control membranes

After extensive research to improve understanding of various membrane characteristics and options, the polycarbonate track etched 'Cyclopore' membrane from Whatman was selected as the most appropriate for the single sheet flat bed membrane system to be used in the proposed device due to its suitable properties; hydrophilic whilst exhibiting very low levels of water adsorption and non-specific protein binding, biologically inert, applicability to blood filtration procedures, and regular topography arising from its uniform flat surface and straight through pores (Zydney et al. 1989; Fisher Scientific 2014). These characteristics provided the best anti-fouling properties and should have a minimal detrimental effect on the quality of the blood.

With early designs haemoconcentration was difficult to achieve and upon inspection of the polycarbonate membrane using scanning electron microscopy it was seen that a thick 'cake layer' deposit was obstructing the membrane pores. It was unclear at what point this deposit layer formed and how much of a restrictive effect it had upon the filtration capabilities of the device. Further investigation revealed that the deposit layer could be readily removed from membrane samples with washing with saline or water thus suggesting it was not the result of strong protein adsorption whilst inspection of the morphology of the deposit layer suggested it was not the result of widespread platelet adhesion. Moreover a similar deposit was observed after successful haemoconcentration in Hemosep and thus it was decided that although the presence of this deposit layer may indeed hinder haemoconcentration through membrane

fouling and concentration polarization mechanisms, it was unlikely that it was solely responsible for the lack of haemoconcentration achieved up to that point.

It was also observed that under high TMPs the polycarbonate membrane was susceptible to stretching which resulted in permanent deformation of the polycarbonate and an apparent change in the pore structure which would greatly impact upon the functionality of the proposed device. Consequently, it was noted that the proposed device must be designed to avoid reliance on high TMP for successful haemoconcentration.

5.3 Blood flow

In order to ensure good 'coverage' of the control membrane exchange surface and to help control potential membrane fouling it was important to ensure good bulk flow within the device. Simultaneously, it was important to ensure flow within the device did not induce haemolytic shear forces. In order to achieve this blood flow through the device was modelled using computational fluid dynamics (CFD) analysis. There were a large number of challenges in accurately modelling the blood flow; the two-phase, non-Newtonian, shear thinning nature of the blood increased complexity whilst the concentration of RBCs along with their varying aggregability and deformability makes modelling more difficult still. Moreover, the nature of the device adds an additional layer of complexity, in that, there will not only be a radial gradient of Hct (and hence viscosity) stemming from the naturally uneven distribution of RBCs when flowing through a pipe, but additionally, the chemically driven extraction of plasma by the superabsorber will introduce a viscosity gradient along the length of the device as well as an uplift force within the fluid. Additionally, the peristaltic flow induced by the roller pump and agitation of the entire device add yet more complexity. Accurate modelling of the blood flow through the proposed device during its operation would be an extremely difficult undertaking and beyond the scope of this thesis. Thus it was decided that the CFD model should focus on information, which could be realistically obtained and would best contribute towards validating the design of the proposed device with regards to producing good quality, haemoconcentrated blood, in a timely manner. Hence CFD modelling was utilised to inspect bulk flow of a simplified 'blood' fluid in the proposed device and ensure that there was consistent flow across the entire membrane exchange surface and that there were no areas of stasis in the flow channel and to give an insight into potential sites of elevated shear forces which maybe induce haemolysis and reduce the quality of the blood leaving the proposed device. The results of the CFD analysis suggested that there was good coverage of the control membrane exchange surface and that the shear forces would not be sufficient to induce haemolysis.

5.4 Fouling control strategies

Several strategies for limiting concentration polarisation and controlling membrane fouling were explored and it was found that the presence of air bubbles at the blood side of the membrane surface combined with orbital agitation of the device could disrupt formation of a fouling layer and via a combination of current fouling control strategies, namely; gas sparging, air scouring, dispersed phase disruption and more conventional mechanical stirring. The use of bubble surface mixing was assessed and resulted in significantly improved haemoconcentration and a reduction in MUF time. It was noted, however that the presence of bubbles in the blood phase could potentially be haemolytic and damaging to blood proteins, highlighting the importance of the blood quality analysis to come.

5.5 Agitation and Wave motion

The benefit of agitation has been demonstrated in Hemosep and it is required to drive the movement of the surface bubble mixing fouling control strategy. However, it was also suggested that if the control membrane was affixed so that it was not taut but rather allowed a wave motion induced by the agitator to propagate across its surface, this may further minimize concentration polarisation and membrane fouling whilst the uplift force in the waves could assist plasma transfer across the control membrane. The use of wave motion of a slack membrane was assessed and a slight but not significant improvement in haemoconcentration was observed. The demonstration of this slight improvement, coupled with the fact that agitation was already part of the MUF technique with the device, the use of a slack membrane in the proposed device was continued in subsequent designs of the device.

5.6 Superabsorber behaviour

The sodium polyacrylate superabsorber had been successfully implemented in Hemosep. Based on extensive research into the chemistry of the swelling of superabsorbent polymers it was noted, and then observed experimentally, that the capacity of the sodium polyacrylate superabsorber was greatly reduced if the fluid to be absorbed had an elevated salt concentration. This had the potential to be problematic since priming of the superabsorber with an isotonic solution (saline) was required to ensure good contact between itself and the control membrane and allow a path for plasma uptake. The reduction in capacity when absorbing saline was due to the reduced osmotic pressure between the gel phase and solution phase as a result of the higher salt concentration in the solution to be absorbed (Chatterjee et al. 2002; Peppas et al. 2010). Unfortunately, this reduced capacity is an unavoidable consequence of the chemistry

behind the large absorbance capacity of superabsorbent polymers and could not be avoided by using an alternative priming solution since the priming solution by its nature required a salt concentration equal to that of the blood in order to prevent haemolysis. Nevertheless, it was noted that the capacity of the superabsorber after priming with saline should still be sufficient to absorb a considerable amount of plasma as demonstrated by the success of the Hemosep system.

It was also observed that when the superabsorber swelled as it took up fluid, it often did so in a way that was non-uniform resulted in highly swelled areas adjacent to completely 'dry' areas. This can lead to loss of contact between the superabsorber and the control membrane and further hinder uniform swelling. Ideally, the superabsorber should swell in a uniform manner so that there is a uniform osmotic potential across the exchange surface. However, since the rate of fluid supply to the superabsorber is controlled by the membrane and its complex operating behaviour with regards to fouling, it is difficult to ensure uniform swelling. Additionally, the kinetics of superabsorbent polymer swelling mean that after an initial rapid swelling of dry particles the speed of absorption is limited by; the speed of diffusion through these swollen areas, the speed of relaxation of polymer chains, and the presence of 'gel blocking' in which swollen elements create a physical barrier between the fluid to be absorbed and the dry particles of the superabsorber. These restrictions can prevent uniform swelling by limiting distribution of the fluid to be absorbed. In order to encourage uniform swelling, expansion perpendicular to the control membrane and the associated disintegration was physically inhibited by utilising a retaining material. The retaining material was use to hold the superabsorber in contact with the membrane but allow some expansion perpendicular to the membrane surface. Consequently the retaining material needed to be able to stretch and expand with the superabsorber so that it did not hamper absorption but still exerted a force which maintained good contact between the superabsorber and the membrane. Initially a bandage mesh material was used but later a thin PVC film was utilised.

5.7 Value of design improvements

5.7.1 Impact on Haemoconcentration

The cumulative effect of the various design improvements made was assessed. The early system incorporated an exchange surface area of 110x200mm with and 2mm channel depth and an internal priming volume of approximately 172.8ml. Blood was pumped at 150ml/min and the device was agitated at 120rpm but the polycarbonate membrane was taut, there was no surface bubble mixing and no retaining film for the

superabsorber. The device concentrated 500ml of 17.5%Hct bovine blood to an Hct of 30.5% in 150mins removing approximately 250ml of plasma in that time resulting in a mean plasma extraction rate of 1.7ml/min.

The improved system had a larger exchange surface area of 155 x 200mm with a 2mm channel depth and a greatly reduced internal priming volume of approximately 76.3ml. This was achieved mainly by removing the filling chambers which were present in previous prototypes and allowing blood to enter the exchange channel from below via a 'scooped' inlet. Again blood was pumped at 150ml/min but the device was agitated at slower 100rpm and the polycarbonate membrane was slack. Surface bubble mixing was incorporated and a retaining film was in place above the superabsorber. The device concentrated a larger volume of blood, 800ml of 21.5%Hct bovine blood, to an Hct of 39.0% in 40mins removing approximately 360ml of plasma in that time resulting in a mean plasma extraction rate of 9ml/min, over five times greater than the initial device.

Clearly, the design improvements vastly improved the functioning of the device and the rate of haemoconcentration was sufficient that the MUF time was reduced to 40mins, close to what would be expected of a device in a clinical setting. As discussed previously (*3.1.10. Termination of MUF*), many groups do not apply a strict time limit on MUF but rather use a predetermined Hct or volume of filtrate removed as an endpoint, however, those groups which do apply a MUF time limit generally use 10-20 as a treatment duration (Aeba et al. 1998; Rivera et al. 1998; Luciani et al. 2001; Onoe et al. 2001; Kotani et al. 2008; Luciani et al. 2009). Clearly, there is still room for improvement in reducing the MUF time but it is anticipated that with further refinement and study and of the beneficial design improvements detailed in this thesis the MUF time can be reduced.

As described previously (*3.1.4. Level of dilution*), throughout testing of the proposed device Hct was measured manually using a Hawksley micro-haematocrit reader *(Hawksley and Sons Ltd., Brighton, UK)*. There are a number of errors associated with this method. Firstly, since the Hct is read, 'by eye' a degree of subjective human error is introduced, readings were recorded to an accuracy of 0.5% as this was the small fraction that could be reliably distinguished by eye. Measurements can also be affected by improper collection of the sample or inadequately mixed blood and by the speed and time of centrifugation (Estridge et al. 2000). The small size of the capillary tubes and the samples collected means that variations in the dimensions of the tubes can also introduce error into manual Hct readings, for example if the tubes taper or if the tube bore is inconsistent (Bain 2014). Additionally, errors can arise if the wax used to seal the capillary tube is not flat and level (Bain 2014). Although these errors will be minor by themselves, the cumulative effect of several error sources can have a large impact on

measurements. In order to minimise inaccuracies associated with the manual Hct method it is often recommended that two or more samples are collected simultaneously and the average Hct value recorded (Estridge et al. 2000; Bain 2014).

In experiments where flow two-dimensional laser scatter flow cytometry was used to analyse samples the Hct readings obtained from this method were used in place of the manually obtained readings. However, at certain time intervals Hct readings were obtained by both manual and flow cytometry methods which meant comparisons between the two could be made as can be seen in Figure 236.

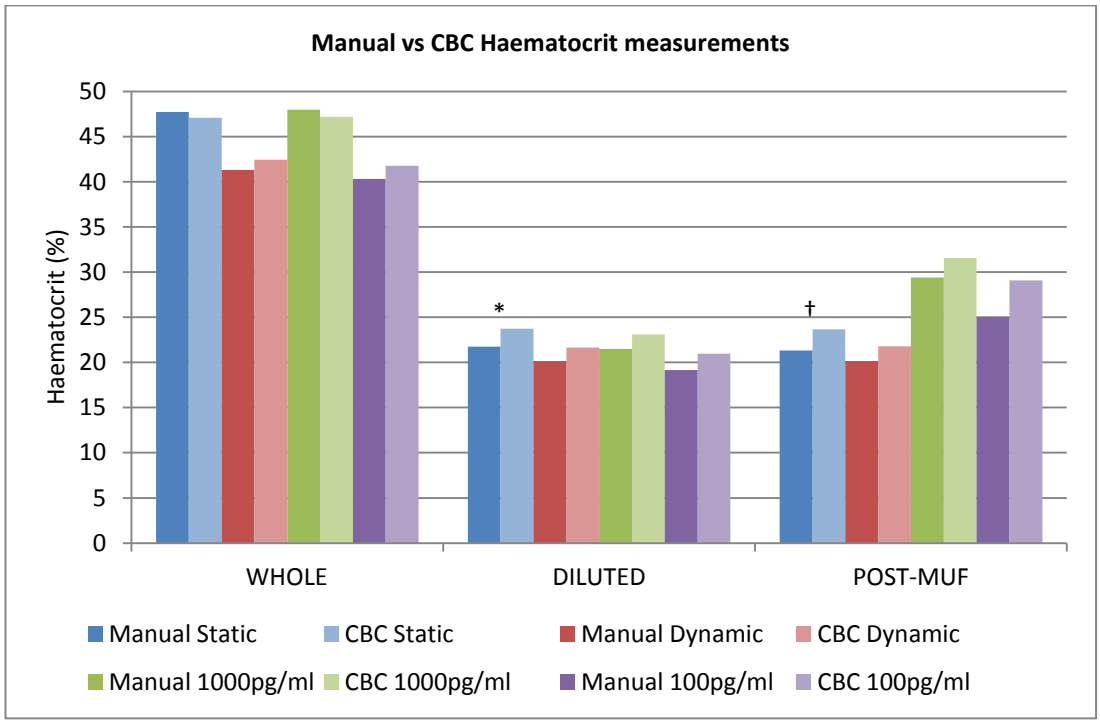


*Figure 236: C*omparison between the mean Hct readings obtained by the manual method and the complete blood count (CBC) flow cytometry method for each of the four groups: static control, dynamic control, high inflammation (1000pg/ml) and low inflammation (100pg/ml), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. † = $p<0.01$ versus previous interval, * = $p<0.05$ versus previous interval.

Figure 236 shows comparison between the mean Hct readings obtained by the manual method and the complete blood count (CBC) flow cytometry method for each of the four groups the (static control, dynamic control, high inflammation and low inflammation), in the initial whole blood, the diluted blood before MUF and after 60mins of either MUF or no intervention. As can be seen, there was no significant difference between the manual and CBC measurements in the dynamic control, high inflammation or low inflammation groups at any interval. However, in the static control group there was a significant difference between the manual and CBC readings after dilution and a more significant difference after MUF. One likely explanation for this is that the static control was the only group in which blood was not continually mixed by flow and thus,

although efforts were made to stir the blood before collection of samples, erythrocyte sedimentation may have affected the manual Hct measurements which, due to the method of filling the capillary tube, were collected from a shallower depth than the CBC Hct samples which were collected using a syringe. Generally, speaking however, provided there is sufficient mixing of the blood, the manual Hct method appears to be suitably accurate.

It should be noted however, that there can be questions of accuracy associated with any method of Hct measurement. As described previously (*3.3.4.1. Difficulties in modelling blood and haemofiltration*), RBCs tend to accumulate in the central region of a tube during flow and thus Hct levels are highest in this central zone and lowest at the periphery (Baskurt et al. 2003). One impact of this is that, since the shear forces are lowest and consequently velocities are highest in the central region, there is a higher average relative velocity for RBC in this region compared to the bulk and as a result the Hct of blood in such an environment is lower than that of blood collected from the outflow; this is known as the Fahraeus effect (Secomb et al. 1986; Baskurt et al. 2003).

5.7.2 Impact on Haematology

Once haemoconcentration using a clinically suitable method had been achieved within a clinically relevant time period, blood quality and the ability of the device to remove inflammatory mediators were assessed simultaneously.

Initial observations of RBC appearance using blood smears and optical microscopy indicated minimal change in poikilocytes after MUF with the proposed device thus suggesting minimal haemolysis and good blood quality after haemoconcentration. Manual platelet counts were conducted using a haemocytometer but were found to be unreliable. After these initial manual observations an experiment series was conducted in which complete blood counts including full haematology and five-part differential analyses were obtained using two-dimensional laser scatter flow cytometry. The full analysis included 18 haematological parameters: red blood cell count (RBC), total haemoglobin concentration (Hb), cellular haemoglobin concentration (CHb), haematocrit (Hct),mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cell count (WBC), Neutrophil count, Lymphocyte count, Monocyte count, Eosinophil count, Basophil count, platelet count (PLT) mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW).

In these experiments there were four study groups; a static control group, a dynamic control group, a low inflammation group, and a high inflammation group. The static control group, in

which blood was allowed to sit undisturbed in a polypropylene beaker for 60mins (the standardised MUF time for this experiment series), was used to characterise the changes in blood constituents and inflammatory mediator levels due to the MUF time alone. The dynamic control group, in which blood was circulated through the MUF circuit including the proposed device for 60mins with a single cellulose acetate sheet in place of the control membrane, was used to characterise the change in blood constituent parameters due to the MUF time and the flow through the MUF circuit and proposed device without filtration.

In the dynamic control a sheet of cellulose acetate was used in place of the polycarbonate membrane so that the blood would pass through a near identical circuit without filtration taking place. However, since the cellulose acetate was not an identical material to the polycarbonate membrane, the potential impact of cellulose acetate on the blood must be considered.

Cellulose based materials including cellulose acetate are considered biocompatible and induce negligible to mild inflammatory and foreign body responses (Miyamoto et al. 1989; Martson et al. 1997; Helenius et al. 2006). However, in studies on dialysers, blood contact with cellulose acetate membranes was shown to result in decreased WBC counts and increased C3a generation (Hakim et al. 1984; Falkenhagen et al. 1987). More recently, Ye et al stated that cellulose acetate membranes "induce serious protein adsorption and clot formation" and observed numerous platelets adhered to a cellulose acetate membrane, some of which exhibited aggregation and deformational change (Ye et al. 2003). We observed a no change in WBC, platelet or TNF-α levels in the dynamic control after 60mins which suggests that the cellulose acetate did not have a negative impact upon these factors as other studies found.

Blood samples for the measurement of these parameters were collected before dilution, after dilution but before MUF and immediately after 60mins MUF with the proposed device, or 60mins of no intervention in the controls.

Prior to collection of the first sample, blood was 'spiked' with 100pg/ml of TNF-α and IL-6 in the static control, dynamic control and low inflammation groups representing a normal level and with 1000pg/ml TNF-α and IL-6 in the high inflammation group representing an elevated level. The change in the concentration of these cytokines was assessed by collecting plasma samples before dilution, after dilution and at t=15, t=30 and t-60. TNF-α and IL-6 were selected for this study due to their relevance to studies of CPB, MUF and MUF devices and the change in their concentrations gave an insight in the ability of the device to remove inflammatory mediators.

The results of this experiment series not only gave an insight into the quality of blood after MUF with the proposed device and the ability of the device to remove inflammatory mediators but

also, due to the fact that each group utilised both blood sourced <6 hours earlier and one with blood sourced the previous day, gave insight into the impact of blood age on experiments of this type. Additionally, since blood was sourced from three different cows, the impact of this upon experiments of this type could also be assessed.

As intended, the proposed device successfully haemoconcentrated the blood leading to a significant increase in Hct. Similarly, PCT also increased but the change was not significant. There was no significant change in RBC count, MCV, RDW, WBC count, neutrophil count, monocyte count, eosinophil count, or platelet count, after 60mins of MUF with the device in any of the study groups indicating there was little to no impact upon the blood constituents. Moreover, after 60mins MUF with the device there was no significant change in total Hb, MCH, MCHC, CHB or plasma free Hb which is a strong indication that there was little to no haemolysis induced by the device.

Basophil counts were significantly reduced in the high inflammation group after MUF with the proposed device but since the basophil concentration were all particularly low, generally close to zero, and variable, the evidence for the device inducing loss of basophils is not strong, especially given that the effect was not replicated in the low inflammation MUF group. A significant increase in lymphocyte count after MUF with the device was observed in the high inflammation group. Since this observation was not replicated in the low inflammation group the cause may be related to the cytokine levels although it is unclear by what mechanism this could occur in vitro and further investigation may be needed. There was a significant decrease in both MPV and PDW which could be explained by low scale aggregation and activation of the more aggregable and reactive large platelets, however the values seen remain within the aforementioned UCDavis reference ranges. Some degree of platelet activation is to be expected with any blood contact with the foreign surfaces in biomedical devices and thus the observations regarding MPV and PDW are not concerning, particularly given platelet counts did not significantly decrease after MUF.

Due to the fact that blood samples were collected before and immediately after dilution of the blood, the impact of dilution upon the blood constituents could be assessed. Normally, dilution would be expected to have little to no impact upon the blood constituents in vitro, however this was not the case. As expected the dilution procedure led to a significant decrease in Hct and PCT but there were also unexpected changes in several other blood parameters.

Dilution led to a significant reduction in RBC in each along with a significant increase in MCV in each group and a significant decrease in RDW in each group (except the low inflammation group in which a slight decrease occurred). Additionally, there was a significant decrease in both CHb

and MCH in three of the four groups and a noticeable increase in plasma free haemoglobin in all groups although the change was only significant in the static control and high inflammation groups. Based upon these observations it appears that a degree of haemolysis was induced by the dilution procedure, most likely as a result of a hypotonic saline solution used for dilution which led to fluid intake by the RBCs and subsequent 'swelling' and bursting of a portion of the RBC population. It should be noted, however that RBC count, MCV and RDW all remained within the reference ranges and thus the degree of haemolysis was not catastrophic. The saline used for these experiments was produced on-site and thus this issue should be easily avoidable in the future by implementing stricter production controls or sourcing saline externally.

As with the post-MUF observations there was a significant decrease in both MPV and PDW observed which again could be explained low scale aggregation and activation of the more aggregable and reactive large platelets. Additionally, platelet count significantly increased after dilution in the dynamic control group however since this observation was not replicated in any of the other groups which underwent the same dilution procedure it is possible this result may be erroneous, although further investigation may be required.

Aside from these observations after dilution there was no significant difference in the neutrophil, lymphocyte, monocyte, eosinophil or basophil counts however there was a significant decrease in the overall WBC count in the static control and high inflammation groups although values remained within the reference range. Total Hb levels decreased significantly in the static control, dynamic control and low inflammation groups and slightly in the high inflammation group after dilution. Since this parameter is measured by the lyse method and thus includes plasma free haemoglobin, the decrease cannot be explained by dilution induced haemolysis mentioned previously. However, as discussed previously (*4.3.1 Methodology*), Hb measurements can be falsely elevated by high blood concentrations of heparin and if this was indeed the case, after dilution when heparin concentration is reduced this effect would be diminished, consequently what appears to be a significant reduction may merely be a reduction in the false elevation effect (Fischbach et al. 2009).

5.7.3 Impact on Inflammatory Mediators

The concentrations of TNF-α and IL-6 in the plasma samples collected from each group before dilution, after dilution and at t=15, t=30 and t-60 were assessed by ELISA.

As expected, there was a decrease in TNF-α concentration after dilution in each group although the change was only significant in the static control and high inflammation groups.

The concentration values seen were different to what was expected, for example the static control group was spiked with 100pg/ml of TNF-α but the measured concentration was consistently above this, whereas, the high inflammation group was spiked with 1000pg/ml of TNF-α but the measured concentration was consistently below this level. In the low inflammation group there was a large degree of variation between concentration measurements whilst interestingly, the static control group and high inflammation groups showed the most consistency. The standard curves for both TNF-α and IL-6 assays were satisfactory as the trend lines used to calculate concentration from absorbance at 450nm were an excellent fit with an R^2 value greater than 0.99 in each case. This suggests that the ELISAs were performed correctly and thus any unexpected results cannot be reasonably attributed to simple experimental error.

Despite the unexpected concentration levels observed, the TNF-α levels in each group followed a similar trend; an initial large decrease after dilution followed by a stable concentration throughout the remainder of the experiment. This was to be expected in the static and dynamic control groups where no filtration occurred and thus no TNF-α was removed, but the fact that the TNF-α concentration remained relatively stable in both the high inflammation and low inflammation groups, where large volume of plasma were removed, suggests that TNF-α was removed by the device but only in proportion with the amount of plasma removed and not selectively filtered. Were TNF-α selectively removed a decrease in its plasma concentration levels would have been observed and similarly, if TNF-α were not removed during MUF its plasma concentration levels would have increased.

One promising observation however was that between t=30 and t=60 in the high inflammation group, there was indeed a significant decrease in TNF-α concentration, suggesting that between these intervals, as the blood became more concentrated, TNF-α was indeed selectively removed. Although this observation was not seen in the low inflammation group the effect may have been masked by the variation in measurements. Although the mechanism remains unclear, this observation leaves open the possibility that TNF-α is selectively removed by the proposed device at higher Hct, or later in the MUF procedure, which could be confirmed by further investigation.

For IL-6 plasma concentrations good standard curves were achieved suggesting that the assays were performed correctly, however the results from the plasma samples were difficult to assess. There was very little variation between measurements implying consistency across repeat experiments but the values are much lower than would be expected and there was no significant change observed even after dilution which should have resulted in a clear decrease in concentration. It is likely therefore that the IL-6 concentration results are unreliable and that, given the EILSA appears to have been performed correctly resulting in consistent

measurements and good standard curves; this arose from an issue with the blood spiking procedure rather than the ELISA itself.

Overall, based predominantly on the observations of TNF-α, it appears that pro-inflammatory mediators are not selectively filtered by the proposed device but are removed along with plasma in proportion to the amount of plasma removed. The significant decrease in TNF-α concentration between t=30 and t=60 observed in the high inflammation group suggests that at higher Hct, or later in the MUF procedure, TNF-α may be selectively removed but further investigation is required. Clinically speaking, presently it appears that there would be no additional inflammatory reducing benefits of MUF with the proposed device, although there is some evidence to the contrary which in fact nicely mirrors the contrasting current evidence regarding the impact of MUF on inflammatory mediators discussed in *1.3.10. Impact of MUF on Inflammatory Mediators*.

5.7.4 Impact of Blood Age and Source

Since, for logistical reasons, blood in these experiments came from a total of three different cows and was used when both fresh (<6hrs) and day old (24hrs) (two experiments with fresh blood and one with day old blood in each group) analysis regarding the impact of these factors could be made using the data collected. It was seen that for blood sourced from different cows there was a significant difference between the values for the majority of blood parameters measured, the exceptions being monocyte count, free plasma Hb, PCT and PDW. These results suggests that there is sufficient variation in the parameters of complete blood counts to warranty caution when comparing or collating results using blood from different cows. This observation does not have ramifications for the accuracy of the previous results since the important observations are the changes in these parameters after dilution and MUF and no the absolute baseline values themselves, moreover, the ability of the device to function across a wide range of baseline blood profiles, typical in the clinical setting, is an important test of robustness. It was also observed that there was no significant difference between the blood parameters measured in the blood from a given cow when fresh (<6hrs) or when a day old (24hrs). The only exception to this was the WBC count, which was significantly higher in the fresh blood, although there was no significant difference between any of the five WBC types suggesting that the significant difference in WBC count was a cumulative effect of small differences in each of those types. The overall results suggest that, generally speaking, day old blood is not significantly different fresh blood in terms of its constituents. These observations, coupled with the previous observation regarding blood from different cows, suggests that in order to minimise variation in baseline blood levels, it would be preferential to make use of day old blood from the same source rather than fresh blood from a new source.

As an aside, the quality of abattoir blood was analysed as the lack of any control over the age, gender, motility, fluid intake, or stress levels of animals, and lack of *sufficient* control over volume collected or degree of blood heparinisation, were a concern. Despite this, it was seen that the blood parameters for the abattoir bovine blood were generally within the normal reference ranges although there were some indications of unacceptable levels of haemolysis, yet these may have been falsely characterised by the high heparin content of the blood.

Chapter 6

Conclusions

6. Conclusions

The objective of this thesis was to detail the process and outcomes of the development of a super-adsorber based haemofiltration technology for use in paediatric cardiopulmonary bypass. More specifically the device involved the integration of the existing technique of modified ultrafiltration with a new superabsorber technology with the aim to create a novel device which has the ability to extract excess fluid from flowing blood, suitable for clinical use within a cardiopulmonary bypass circuit. This particular design has the potential to broaden access to the MUF technique to patients in low resource settings. Regarding the development of the aforementioned device, the following conclusions can be made:

- The device is able achieve passive, chemically driven extraction of this excess fluid at a fixed blood flow rate, with little to no adjustment of other necessary inputs.

- Haemoconcentration with the device occurs in a timely manner as measured by achieving a suitably rapid rise in haematocrit. The haemoconcentration time is close to current modified ultrafiltration procedures but still requires some improvement to match them, which, with further refinement of the system (as described in *9. Future Work*) is certainly achievable.

- The blood haemoconcentrated by the device was of good quality with no change in RBC count, MCV, RDW, WBC count, neutrophil count, monocyte count, eosinophil count, or platelet count, after 60mins of MUF with the device in any of the study groups indicating there was little to no impact upon the blood constituents. Moreover, after 60mins MUF with the device there was no significant change in total Hb, MCH, MCHC, CHB or plasma free Hb which is a strong indication that there was little to no haemolysis induced by the device.

- The device did not demonstrate selective removal of pro-inflammatory mediators but there was also no increase in concentration of pro-inflammatory mediators observed. Further investigation of this is recommended however since data regarding IL-6 was unreliable and a follow-up study with the experience gained in this study would likely yield better results.

- The design of the device is such that the associated MUF technique requires minimal input from clinical staff and is suitable for deployment in a clinical setting.

- Regarding the use of abattoir blood for studies, the blood parameters for the abattoir bovine blood were generally within the normal reference ranges although there were some indications of unacceptable levels of haemolysis, yet these may have been falsely characterised by the high heparin content of the blood. Additionally, in order to minimise variation in baseline bovine blood levels and potentially improve accuracy of result, it is preferential to make use of day old blood from the same source rather than fresh blood from a new source.

Chapter 7

Considerations for medical devices in low resource settings

7. Considerations for Medical Devices in Low Resource Settings

One potential application for the device highlighted in *2.4. Applications for the proposed device* is deployment in low resource settings. If this were the case, there are a number of additional factors which should be considered as its development progresses in the future.

The environment in which the device could be deployed may be harsh, and thus the design must be sufficiently robust to withstand adverse temperatures and humidity, the presence of particulates, physical impacts and vibrations. Moreover, the nature of low resource settings and their infrastructure means it is likely there will be a period of transportation and storage in which the device may encounter such environments. Additionally, this need for transportation means the design should facilitate this need by being as lightweight, small and regularly shaped as possible. The simplistic single sheet flat bed cross-flow design in conjunction with the passive chemically driven extraction of plasma means that the device is naturally lightweight and dimensionally low-profile. Additionally, the lack of any complex mechanisms means the device should not be sensitive to physical impact, whilst the sodium polyacrylate superabsorber should not be affected by high temperatures. High humidity may lead to fluid intake by the superabsorber and thus this should be assessed in the future.

The clinical setting in which the device could be deployed is likely to be considerably inferior to those encountered in the UK and other developed nations. In medical centres in developing countries, even basic equipment can be lacking, similarly in disaster relief scenarios equipment could be unavailable for a variety of reasons (Razzak et al. 2002; Hodges et al. 2007). It is important therefore that the device is designed so that there is minimal reliance on secondary equipment and additional consumables for correct functioning. This is one area in which the device outperforms current haemofiltration systems in that there is no requirement for a vacuum pump to generate the pressure gradient that drives plasma removal. Presently, the device does make us of an orbital agitator but with further development it is anticipated that this could be achieved manually or removed entirely if flow patterns are carefully designed to achieve fouling control.

As with the potential lack of equipment, there is also the potential of encountering insufficiently trained or skilled clinical staff in developing countries (Razzak et al. 2002; Hodges et al. 2007). It was mentioned in the thesis objectives that the development of device would aim to remove input for clinical staff where possible and this is particularly important in low resource settings where clinicians may not be adequately trained. Set-up and operation of the device should require minimal instruction, primarily to avoid incorrect use of the device, but also to remove

the need for specialist training which can be particularly problematic in developing countries where there may be a language barrier and/or knowledge gap.

Furthermore, a device which is clinically simple to run and operate will likely reduce the cost of the device over its entire life cycle. Naturally, with devices deployed in developing countries cost is a highly important factor, however many devices developed for this setting are overly focussed on the 'headline' cost of the device and ignore costs associated with entire life cycle (Cordero 2014). Life cycle costs are numerous and include; shipping, distribution, approval by regulatory bodies, training of clinicians, installation, operating and consumable costs, preventive maintenance, repairs, technical support, decommissioning and disposal (Cordero 2014). Moreover there are a number of ownership costs which can include; labour, service contracts, test equipment, licenses, storage, downtime, depreciation and upgrades (Cordero 2014). Again, the nature of the device means that several of these costs will be avoided or will not be applicable, but going forward considerations of this kind must be at the forefront of development.

Deployment of medical devices in developing countries also means encountering different regulatory requirements to those of the FDA and EU. Several groups exist to attempt to standardise medical device regulation across global regions such as the *Asian Harmonisation Working Party (AHWP)* which includes China, India, Pakistan, Indonesia and several Middle Eastern countries in its membership as well as many less developed nations (Lamph 2012). Similarly, the *Pan American Health Organization (PAHO)* includes representatives from the majority of the countries in the Caribbean, Central and South American area (Lamph 2012). These groups are not regulatory bodies in themselves but are focussed on improving safety by harmonising regulation within their respective regions. Lamph et al state that: *"In 2005, the WHO reported that only 7% of the 46 sub-Saharan African countries had National Medicines Regulatory Authorities (NMRA) in place. Of the remaining countries, 63% had minimal regulation and 30% had no regulation."* (Lamph 2012). Lack of regulation of medical devices in developing countries can clear the way for irresponsible practices, however, it is an important ethical point that medical devices for low resource settings should still be designed to meet the high regulatory standards present in more developed nations and not deployed simply because less developed nation's regulatory systems are not sophisticated enough to reject it (Cordero 2014).

Chapter 8

Limitations

8. Limitations

Although the overriding objectives of this thesis were met, as with any study there are a number of limitations which impacted upon the scope and quality of the work which are discussed in the following sections.

8.1 Experiment size

For testing of the proposed, device experiments were generally repeated to n=3. Ideally, the number of repeated experiments would have been larger to improve the reliability of the results. Although with n=3 some statistical analysis as to the variation in parameters and significant differences between groups and time intervals could be obtained, the data would be more robust with more repetitions. Time was a factor in the decision to limit repetition to n=3 given that it generally took several hours to complete each experiment including collection of blood, construction and sealing of the test rig, sealant drying time, MUF time, careful deconstruction and cleaning of the test rig and other apparatus, and sealant removal, but the overriding factor was cost. Complete blood counts could not be performed 'in-house' and consequently were outsourced to *Veterinary Diagnostic Services* at the *School of Veterinary Medicine* at the *University of Glasgow* which carried a cost per sample. Bovine proteins and ELISA kits are generally less commonly used than those used for human or rat samples and consequently are more expensive, moreover the need to analyse these samples in duplicate further increased costs. The polycarbonate membrane and sodium polyacrylate superabsorber can only be used once and thus there is an elevated consumable cost for each experiment.

8.2 Limits on design

As discussed in *2.2. Ultrafilters*, there are a number of possible ultrafilter design configurations including plate and frame modules, spiral wound modules, tubular modules, and hollow fibre modules. Some of these systems may have been advantageous in addressing certain issues which were met during the development of the device e.g. controlling superabsorber disintegration and membrane fouling and may have improved the ultimate performance of the device but realistically the choice of ultrafilter configuration was limited by the resources available. In-house construction of tubular and hollow fibre modules would have been practically impossible and outsourcing production of these would have been extremely expensive. Similarly, production of plate and frame modules, though more feasible, would still have presented several design and manufacture complexities which would have increased both prototype production and experiment set-up time considerably. A single flat bed cross-flow

system was selected as this was the simplest basis for a prototype which could reasonably be manufactured in-house at low cost. Moreover, a single flat bed cross-flow system allowed for easier observations of the filtration process and meant that incremental changes in the design were more straightforward, for example altering the exchange surface area. In addition, due to the aforementioned elevated consumable costs, efforts were made to make economical use of both the membrane and superabsorber. A typical example would be selecting dimensions of the exchange surface area whilst taking into consideration the dimensions in which the polycarbonate membrane could be supplied to reduce wasteful off-cuts. Though this was somewhat limiting, management of resources in this way, particularly with regards to the supply of raw materials, is highly applicable to the mass production of any medical device and an important skill in reducing production costs, particularly pertinent to medical devices for low resource settings. Use of the superabsorber was central to the device design but this limited our ability to directly measure filtrate volume as filtered fluid was captured. An indirect measure of filtrate volume could be made by measuring the weight increase in the superabsorber before and after filtrate absorption.

8.3 Blood source

Due to its availability and low cost bovine blood is recommended for early and comparative testing of devices, although varying values of Hct and Hb can be expected and it is less haemolytic than human blood and exhibits different aggregation behaviours (Franco et al. 2003). Ideally, human blood would have been used for testing of this device, if only during later phases, but this was not possible due to the extremely high cost. Bovine blood sourced from a local abattoir was used as it was low cost and more readily available, although on occasions blood was not available and generally collection was time consuming. The concerns regarding the abattoir blood source were discussed previously (*5.7.3*) and regard the lack of any control over the age, gender, motility, fluid intake, or stress levels of animals, and lack of sufficient control over volume collected or degree of blood heparinisation. The accuracy of results could be affected by these factors, although complete blood count analysis of the blood showed values are within reference ranges.

8.4 Departmental resources

The scope of work was somewhat limited by the resources available, mostly due to time consuming procedures. For example, complete blood counts had to be outsourced which meant samples needed to be delivered and there was a waiting period for results. Similarly, collection of blood from the abattoir was time consuming and could only be done at certain times which

often delayed laboratory work. Additionally, in-house manufacture of prototypes was preferred due to convenience but often involved long lead times. Ideally, the device would have been tested in an animal model, but this was not possible due to the resources available at the time. Also, as is the case when performing any new technique, some time was required to learn and become competent at a number of the procedures involved in this study, however, this limitation did result in the development of laboratory skills.

Chapter 9

Future work

9. Future Work

Investigation into the removal of inflammatory mediators yielded promising results for TNF-α, suggesting that TNF-α is removed proportionally to its concentration in the plasma, and may, at high Hct or later in the intervention period, be removed selectively as it was seen that between 30mins and 60mins of MUF there was a significant decrease in TNF- α concentration in the high inflammation group. The mechanism by which this occurred is not clear. Further study into the removal of TNF-α (and other inflammatory mediators) in this late phase could confirm this result and may indentify conditions under which selective removal of cytokines occurs. The IL-6 study, however, was less successful most likely as a result of a problem during the spiking procedure. Consequently no meaningful conclusions could be drawn regarding the removal of IL-6 by the device. It would be advantageous to repeat this study to gain an insight into the ability of the device to remove of a broader range of inflammatory mediators.

Bubble surface mixing resulted in a significant improvement in haemofiltration rates, but since this is a new fouling control strategy, further investigation into the mechanisms and optimal conditions of this technique may well yield further improvements in the performance of the device. Additionally, it may be possible to identify substances which could replace the role of the the risk associated with the blood-air interface described previously (*3.4.3.4. Potential problems with bubble mixing*). Similarly, wave motion of the membrane slightly improved the performance of the device and is a new fouling control strategy. Given that the wave motion is currently generated by orbital agitation and is rather chaotic it would be interesting to study the impact of different wave patterns on filtration rates for example more regularly waves propagating in the direction of flow only. The use of ladder-like cellulose acetate baffles adjacent to the membrane surface as turbulence promoters demonstrated some success in encouraging plasma removal (see Figure 142). Turbulence promotion and the introduction of secondary flow is a better established fouling control strategy and with more investigation could well be incorporated into the device to further improve performance.

The superabsorber performance was limited by the need to prime it with an isotonic solution to establish good contact with the control membrane. The use of an alternate priming method or removal of this step entirely would increase the capacity of the superabsorber, possibly leading to lower costs as less superabsorber would be required, reduce the time of the intervention, and simplify the technique. This is an area of study which could therefore be highly beneficial to the performance and success of the device. Any improvement in the performance of the superabsorber would achieve these benefits and it was noted previously (*3.6.2.1. Kinetics of superabsorbent hydrogel swelling*) that good distribution of fluid directly to 'dry' areas was very

important. As described previously (*3.6.2.1. Kinetics of superabsorbent hydrogel swelling*), good distribution of fluid in superabsorbent polymers is generally achieved by the addition of 'fluff' (non-swelling materials). By increasing the fraction of fluff in the superabsorber or by changing its position and structure within the superabsorber, better distribution could be achieved which may improve the fluid uptake rate.

As described previously (*4.5.3 Blood quality at source*), since it is more haemolytic than and exhibits different aggregation behaviours to animal blood, fresh human blood is recommended for the final assessment of a haemofiltration device (Franco et al. 2003). If the problem of the high cost could be overcome, it would be beneficial to repeat this study with human blood. Similarly, as mentioned in the limitations, it was not possible to conduct animal studies with the time and resources available, however if this could be overcome, a study in a porcine model would provide a essential measure of the robustness and 'whole body' impact of the device and procedure in a clinically analogous setting.

Additional testing related to the deployment of the device in low resource settings would be required if this was identified as an application for the device including assessing the robustness of the system in adverse environments. Of particular interest would be assessing the affect of high humidity levels on the sodium polyacrylate superabsorber as high moisture levels could lead to fluid intake which may reduce performance.

Chapter 10

References

10. References

Absolom, D. R., W. Zingg, et al. (1987). "Protein adsorption to polymer particles: role of surface properties." Journal of biomedical materials research **21**(2): 161-171.

Ad, N., E. Snir, et al. (1996). "Use of the modified technique of ultrafiltration in pediatric open-heart surgery: a prospective study." Isr J Med Sci **32**(12): 1326-31.

Advantec MFS Inc (2014). Membrane Filters Catalog, Available at: http://www.advantecmfs.com/catalog/filt/membrane.pdf .

Aeba, R., T. Katogi, et al. (2000). "Modified ultrafiltration improves carbon dioxide removal after cardiopulmonary bypass in infants." Artificial Organs **24**(4): 300-304.

Aeba, R., T. Matayoshi, et al. (1998). "Speed-controlled venovenous modified ultrafiltration for pediatric open heart operations." Annals of Thoracic Surgery **66**(5): 1835-1836.

Ahmad, A. L., K. K. Lau, et al. (2005). "Integrated CFD simulation of concentration polarization in narrow membrane channel." Computers & chemical engineering **29**(10): 2087-2095.

Ahmed, I., A. Majeed, et al. (2007). "Heparin induced thrombocytopenia: diagnosis and management update." Postgraduate medical journal **83**(983): 575-582.

Alkhamis, T. M., R. L. Beissinger, et al. (1990). "Artificial surface effect on red blood cells and platelets in laminar shear flow." Blood **75**(7): 1568-75.

Ameri, M., H. A. Schnaars, et al. (2011). "Stability of hematologic analytes in monkey, rabbit, rat, and mouse blood stored at 4 C in EDTA using the ADVIA 120 hematology analyzer." Veterinary Clinical Pathology **40**(2): 188-193.

American Association of Blood Banks Standards Committee, A. (1989). Standards for Blood Banks and Transfusion Services, American Association of Blood Banks.

Anand, G., S. Sharma, et al. (2010). "Conformational transitions of adsorbed proteins on surfaces of varying polarity." Langmuir **26**(13): 10803-10811.

Anderson, J. M., T. L. Bonfield, et al. (1990). "Protein adsorption and cellular adhesion and activation on biomedical polymers." The International journal of artificial organs **13**(6): 375-382.

Anderson, J. M., A. Rodriguez, et al. (2008). Foreign body reaction to biomaterials. Seminars in immunology, Elsevier.

Andrade, J. D. and V. Hlady (1986). Protein adsorption and materials biocompatibility: a tutorial review and suggested hypotheses. Biopolymers/Non-Exclusion HPLC, Springer**:** 1-63.

Andrade, J. D. and V. Hlady (1987). "Plasma protein adsorption: the big twelve." Annals of the New York Academy of Sciences **516**(1): 158-172.

Andrew, M., B. Paes, et al. (1988). "Development of the human coagulation system in the healthy premature infant." Blood **72**(5): 1651-7.

Apel, P. (2001). "Track etching technique in membrane technology." Radiation Measurements **34**(1): 559-566.

Arepally, G. M. and T. L. Ortel (2006). "Heparin-induced thrombocytopenia." New England Journal of Medicine **355**(8): 809-817.

Asada, S. and M. Yamaguchi (1971). "Fine structural change in the lung following cardiopulmonary bypass. Its relationship to early postoperative course." Chest **59**(5): 478-83.

Asimakopoulos, G., P. L. Smith, et al. (1999). "Lung injury and acute respiratory distress syndrome after cardiopulmonary bypass." Ann Thorac Surg **68**(3): 1107-15.

ASTM International (2015a). Standard Practice for Assessment of Hemolytic Properties of Materials. **F756-13:** Available at: http://www.astm.org/Standards/F756.htm.

ASTM International (2015b). Standard Practice for Selection of Blood for in vitro Evaluation of Blood Pumps. **F1830-97:** Available at: http://www.astm.org/Standards/F1830.htm.

ASTM International (2015c). Standard Practice for Assessment of Hemolysis in Continuous Flow Blood Pumps. **F1841-97:** Available at: http://www.astm.org/Standards/F1841.htm.

Atkins, B. Z., D. S. Danielson, et al. (2010). "Modified ultrafiltration attenuates pulmonary-derived inflammatory mediators in response to cardiopulmonary bypass." Interactive Cardiovascular and Thoracic Surgery **11**(5): 599-603.

Atkins, P. and J. de Paula (2014). Atkins' Physical Chemistry, OUP Oxford.

Auddy, K., S. De, et al. (2004). "Flux enhancement in nanofiltration of dye solution using turbulent promoters." Separation and purification technology **40**(1): 31-39.

Bacher, R. P. and M. C. Williams (1970). "Hemolysis in capillary flow." J Lab Clin Med **76**(3): 485-96.

Baggiolini, M., A. Walz, et al. (1989). "Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils." J Clin Invest **84**(4): 1045-9.

Bain, B. J. (2014). Blood Cells: A Practical Guide, Wiley.

Baker, R. W. (2004). Membrane Technology and Applications, Wiley.

Balasubramanian, V., N. K. Grusin, et al. (1999). "Residence time dependent changes in fibrinogen adsorbed to polymeric biomaterials." Journal of biomedical materials research **44**(3): 253-260.

Bando, K., M. W. Turrentine, et al. (1998a). "Effect of modified ultrafiltration in high-risk patients undergoing operations for congenital heart disease." Annals of Thoracic Surgery **66**(3): 821-827.

Bando, K., P. Vijay, et al. (1998b). "Dilutional and modified ultrafiltration reduces pulmonary hypertension after operations for congenital heart disease: A prospective randomized study." Journal of Thoracic and Cardiovascular Surgery **115**(3): 517-525.

Baskurt, O. K. and H. J. Meiselman (2003). "Blood rheology and hemodynamics." Semin Thromb Hemost **29**(5): 435-50.

Basu, O. D. and P. M. Huck (2005). "Impact of support media in an integrated biofilter-submerged membrane system." Water research **39**(17): 4220-4228.

Baue, A. E. (1975). "Multiple, progressive, or sequential systems failure. A syndrome of the 1970s." Arch Surg **110**(7): 779-81.

Bauer, N. and A. Moritz (2008). "Evaluation of three methods for measurement of hemoglobin and calculated hemoglobin parameters with the ADVIA 2120 and ADVIA 120 in dogs, cats, and horses." Veterinary Clinical Pathology **37**(2): 173-179.

Beaudoin, G. and M. Y. Jaffrin (1986). "High efficiency plasmapheresis using rotating membrane device." Life support systems: the journal of the European Society for Artificial Organs **5**(3): 273-278.

Beckman Coulter (2007). Red Cell Distribution Parameters - (1) RDW – SD (2) RDW (CV). Technical Information Bulletin 9617: Hematology, Available at: https://www.beckmancoulter.com/wsrportal/bibliography?docname=9617_Red_Cell_Parameters.pdf.

Beers, M. H., R. Berkow, et al. (1999). The Merck Manual of Diagnosis and Therapy. Whitehouse Station, N.J., Merck Research Laboratories.
A compendium of facts and information on hundreds of diseases, including etiology, symptoms and signs, diagnosis and treatment.

Belfort, G., R. H. Davis, et al. (1994). "The behavior of suspensions and macromolecular solutions in crossflow microfiltration." Journal of Membrane Science **96**(1): 1-58.

Bellara, S. R., Z. F. Cui, et al. (1996). "Gas sparging to enhance permeate flux in ultrafiltration using hollow fibre membranes." Journal of membrane science **121**(2): 175-184.

Bellhouse, B. J. (1976a). A High-Performance Membrane Lung. Artificial Organs, Proceedings of a seminar on the clinical applications of membrane oxygenators and sorbent-based systems in kidney and liver failure and drug overdose, held at the University of Strathclyde, Glasgow in August 1976. R. M. Kenedi, J. M. Courtney, J. D. S. Gaylor and T. Gilchrist. Baltimore, University Park Press**:** 61-69.

Bellhouse, B. J. (1976b). Design of the Oxford Membrane Oxygenator and its Performance During Prolonged Pulmonary Support of Newborn Lambs. Artificial Lungs for Acute Respiratory Failure: theory and practice. W. M. Zapol and J. Qvist. New York, Academic Press**:** 197-210.

Bellhouse, B. J. and R. W. Lewis (1988). "A high efficiency membrane separator for donor plasmapheresis." ASAIO Trans **34**(3): 747-54.

Bellhouse, B. J., I. J. Sobey, et al. (1994). "Enhanced filtration using flat membranes and standing vortex waves." Bioseparation **4**(2): 127-138.

Bellhouse, D. R. (1992). David Bellhouse and Sons, Manchester, David Bellhouse, 1992.

Benjamin, M. M. and D. F. Lawler (2013). Water quality engineering: physical/chemical treatment processes, John Wiley & Sons.

Bergdahl, L. and V. O. Bjork (1980). "The effect of a nylon mesh blood filter in the arterial line during extracorporeal circulation." Scandinavian Cardiovascular Journal **14**(3): 263-266.

Bernstein, E. F., P. L. Blackshear, Jr., et al. (1967). "Factors influencing erythrocyte destruction in artificial organs." Am J Surg **114**(1): 126-38.

Berube, P. R. and E. Lei (2006). "The effect of hydrodynamic conditions and system configurations on the permeate flux in a submerged hollow fiber membrane system." Journal of membrane science **271**(1): 29-37.

Betteridge, D. J. (2000). "What is oxidative stress?" Metabolism **49**(2): 3-8.

Beugeling, T. (1979). The interaction of polymer surfaces with blood. Journal of polymer science: Polymer symposia, Wiley Online Library.

Bian, R., K. Yamamoto, et al. (2000). "The effect of shear rate on controlling the concentration polarization and membrane fouling." Desalination **131**(1): 225-236.

Binazadeh, M., H. Zeng, et al. (2013a). "Inhibiting Nonspecific Protein Adsorption: Mechanisms, Methods, and Materials." Biomaterials Surface Science: 45-61.

Binazadeh, M., H. Zeng, et al. (2013b). "Effect of peptide secondary structure on adsorption and adsorbed film properties." Acta biomaterialia **9**(5): 6403-6413.

Bixler, H. J. and G. C. Rappe (1970). Ultrafiltration process, Google Patents.

Blackshear Jr, P. L. and R. J. Anderson (1978). Hemolysis thresholds in microporous structures. Red Cell Rheology, Springer**:** 373-391.

Blackshear, P. L., Jr., F. D. Dorman, et al. (1966). "Shear, wall interaction and hemolysis." Trans Am Soc Artif Intern Organs **12**: 113-20.

Blatt, W. F., A. Dravid, et al. (1970). Solute polarization and cake formation in membrane ultrafiltration: causes, consequences, and control techniques. Membrane science and technology, Springer**:** 47-97.

Boga, M., F. Islamoglu, et al. (2000). "The effects of modified hemofiltration on inflammatory mediators and cardiac performance in coronary artery bypass grafting." Perfusion-Uk **15**(2): 143-150.

Bohnert, J. L. and T. A. Horbett (1986). "Changes in adsorbed fibrinogen and albumin interactions with polymers indicated by decreases in detergent elutability." Journal of colloid and interface science **111**(2): 363-377.

Born, G. V. (1962). "Aggregation of blood platelets by adenosine diphosphate and its reversal." Nature **194**: 927-9.

Bouhabila, E. H., R. B. Aim, et al. (1998). "Microfiltration of activated sludge using submerged membrane with air bubbling (application to wastewater treatment)." Desalination **118**(1): 315-322.

Brannon-Peppas, L. and R. S. Harland (2012). Absorbent Polymer Technology, Elsevier Science.

Brans, Y. W., H. S. Dweck, et al. (1981). "Effect of Open-Heart Surgery on the Body-Composition of Infants and Young-Children." Pediatric Research **15**(7): 1024-1028.

Brash, J. L. and P. Ten Hove (1984). "Effect of plasma dilution on adsorption of fibrinogen to solid surfaces." Thrombosis and haemostasis **51**(3): 326-330.

Brodbeck, W. G., E. Colton, et al. (2003). "Effects of adsorbed heat labile serum proteins and fibrinogen on adhesion and apoptosis of monocytes/macrophages on biomaterials." Journal of Materials Science: Materials in Medicine **14**(8): 671-675.

Bronzino, J. D. and D. R. Peterson (2006). Tissue Engineering and Artificial Organs, Taylor & Francis.

Brujan, E. (2010). Cavitation in Non-Newtonian Fluids: With Biomedical and Bioengineering Applications, Springer Science & Business Media.

Brust, M., C. Schaefer, et al. (2013). "Rheology of human blood plasma: viscoelastic versus Newtonian behavior." Phys Rev Lett **110**(7): 078305.

Bullinger, H. J. and L. Behlau (2009). Technology Guide: Principles - Applications - Trends, Springer Berlin Heidelberg.

Burton (2000). Physiology by Numbers: An Encouragement to Quantitative Thinking, Cambridge University Press.

Butler, J., G. M. Rocker, et al. (1993). "Inflammatory Response to Cardiopulmonary Bypass." Annals of Thoracic Surgery **55**(2): 552-559.

Cabassud, C., S. Laborie, et al. (2001). "Air sparging in ultrafiltration hollow fibers: relationship between flux enhancement, cake characteristics and hydrodynamic parameters." Journal of Membrane Science **181**(1): 57-69.

Cadroy, Y., J. P. Bossavy, et al. (2000). "Early potent antithrombotic effect with combined aspirin and a loading dose of clopidogrel on experimental arterial thrombogenesis in humans." Circulation **101**(24): 2823-8.

Caldin, M., E. Carli, et al. (2005). "A retrospective study of 60 cases of eccentrocytosis in the dog." Veterinary Clinical Pathology **34**(3): 224-231.

Casey, L. C. (1993). "Role of cytokines in the pathogenesis of cardiopulmonary-induced multisystem organ failure." Ann Thorac Surg **56**(5 Suppl): S92-6.

Chandavarkar, A. S. (1990). Dynamics of fouling of microporous membranes by proteins, Massachusetts Institute of Technology.

Chandy, T. and C. P. Sharma (1985). "Changes in protein adsorption on polycarbonate due to L-ascorbic acid." Biomaterials **6**(6): 416-420.

Chang, I.-S. and S. J. Judd (2002). "Air sparging of a submerged MBR for municipal wastewater treatment." Process Biochemistry **37**(8): 915-920.

Chang, J. B., K. Prasad, et al. (2012). Textbook of Angiology, Springer New York.

Chatterjee, P. K. and B. S. Gupta (2002). Absorbent Technology, Elsevier Science.

Chaturvedi, R. R., D. F. Shore, et al. (1999). "Modified ultrafiltration improves global left ventricular systolic function after open-heart surgery in infants and children." European Journal of Cardio-Thoracic Surgery **15**(6): 742-746.

Chen, J., H. Park, et al. (1999). "Synthesis of superporous hydrogels: hydrogels with fast swelling and superabsorbent properties." Journal of Biomedical Materials Research(44): 53-62.

Chew, M. S., V. Brix-Christensen, et al. (2002). "Effect of modified ultrafiltration on the inflammatory response in paediatric open-heart surgery: a prospective, randomized study." Perfusion-Uk **17**(5): 327-333.

Chin, K. J., J. Macachor, et al. (2006). "A comparison of 5% dextrose in 0.9% normal saline versus non-dextrose-containing crystalloids as the initial intravenous replacement fluid in elective surgery." Anaesthesia and intensive care **34**(5): 613-617.

Choi, J.-H. and H. Y. Ng (2008). "Effect of membrane type and material on performance of a submerged membrane bioreactor." Chemosphere **71**(5): 853-859.

Chua, H. C., T. C. Arnot, et al. (2002). "Controlling fouling in membrane bioreactors operated with a variable throughput." Desalination **149**(1): 225-229.

Ciesla, B. (2011). Hematology in practice, FA Davis.

Cinar, Y., G. Demir, et al. (1999). "Effect of hematocrit on blood pressure via hyperviscosity." Am J Hypertens **12**(7): 739-43.

Clarkson, J. R., Z. F. Cui, et al. (1999a). "Protein denaturation in foam: I. Mechanism study." Journal of colloid and interface science **215**(2): 323-332.

Clarkson, J. R., Z. F. Cui, et al. (1999b). "Protein denaturation in foam: II. Surface activity and conformational change." Journal of colloid and interface science **215**(2): 333-338.

Clarkson, J. R., Z. F. Cui, et al. (2000). "Effect of solution conditions on protein damage in foam." Biochemical engineering journal **4**(2): 107-114.

Collier, T. O. and J. M. Anderson (2002). "Protein and surface effects on monocyte and macrophage adhesion, maturation, and survival." Journal of biomedical materials research **60**(3): 487-496.

Colville, T. P. and J. M. Bassert (2015). Clinical Anatomy and Physiology for Veterinary Technicians, Elsevier Science Health Science.

Connes, P. (2011). Blood Rheology. Encyclopedia of Exercise Medicine in Health and Disease. F. C. Moreen, Springer Berlin Heidelberg**:** 128-131.

Coraim, F. J., H. P. Coraim, et al. (1986). "Acute Respiratory-Failure after Cardiac-Surgery - Clinical-Experience with the Application of Continuous Arteriovenous Hemofiltration." Critical Care Medicine **14**(8): 714-718.

Cordero, I. (2014). Innovation for medical devices for low resource settings- how limiting the scope of work to creating low cost attention grabbing designs is not enough, Institution of Engineering and Technology.

Corrigan, A., L. Gorski, et al. (2011). Infusion Nursing: An Evidence-Based Approach, Elsevier Health Sciences.

Costigan, G., B. J. Bellhouse, et al. (2002). "Flux enhancement in microfiltration by corkscrew vortices formed in helical flow passages." Journal of membrane science **206**(1): 179-188.

Courtney, J. M., B. M. Matata, et al. (1996). "The influence of biomaterials on inflammatory responses to cardiopulmonary bypass." Perfusion **11**(3): 220-228.

Courtney, J. M., X. Zhao, et al. (1999). "Biomaterials in cardiopulmonary bypass." Perfusion **14**(4): 263-267.

Courtney, J. M., X. B. Zhao, et al. (2003). "Modification of polymer surfaces: optimization of approaches." Perfusion **18**(1 suppl): 33-39.

Cui, Z. F., S. Chang, et al. (2003). "The use of gas bubbling to enhance membrane processes." Journal of Membrane Science **221**(1): 1-35.

Cui, Z. F. and K. I. T. Wright (1996). "Flux enhancements with gas sparging in downwards crossflow ultrafiltration: performance and mechanism." Journal of Membrane Science **117**(1): 109-116.

Daggett, C. W., A. J. Lodge, et al. (1998). "Modified ultrafiltration versus conventional ultrafiltration: A randomized prospective study in neonatal piglets." Journal of Thoracic and Cardiovascular Surgery **115**(2): 336-341.

Darling, E., K. Nanry, et al. (1998). "Techniques of paediatric modified ultrafiltration: 1996 survey results." Perfusion **13**(2): 93-103.

Darup, J., N. Bleese, et al. (1979). "Hemofiltration during extracorporeal circulation (ECC)." Thorac Cardiovasc Surg **27**(4): 227-30.

Davies, M. J., K. Nguyen, et al. (1998). "Modified ultrafiltration improves left ventricular systolic function in infants after cardiopulmonary bypass." Journal of Thoracic and Cardiovascular Surgery **115**(2): 361-369.

De Leval, M. and J. Stark (1974). "Open heart surgery in the first year of life." Acta Chirurgica Belgica **73**(5): 481-493.

De, S., S. Mondal, et al. (2013). Stevioside: technology, applications and health, John Wiley & Sons.

de Simone, G., R. B. Devereux, et al. (1990). "Relation of blood viscosity to demographic and physiologic variables and to cardiovascular risk factors in apparently normal adults." Circulation **81**(1): 107-17.

Ding, L. H., M. Y. Jaffrin, et al. (1986). "A model of hemolysis in membrane plasmapheresis." ASAIO Trans **32**(1): 330-3.

Divers, T. J. and S. F. Peek (2008). Rebhun's Diseases of Dairy Cattle, Saunders Elsevier.

Dobson, A. (1984). "Blood flow and absorption from the rumen." Q J Exp Physiol **69**(3): 599-606.

Draaisma, A. M., M. G. Hazekamp, et al. (1997). "Modified ultrafiltration after cardiopulmonary bypass in pediatric cardiac surgery." Annals of Thoracic Surgery **64**(2): 521-525.

Draper, N. and C. Hodgson (2008). Adventure Sport Physiology, Wiley.

Dubitsky, A. and S. Perreault (2014). "A model for binding of DNA and proteins to transfer membranes." Pall Corporation Tech Note.

Ducom, G. and C. Cabassud (2003). "Possible effects of air sparging for nanofiltration of salted solutions." Desalination **156**(1): 267-274.

Duncan, S. G., K. M. Meyers, et al. (1983). "Reduction of the red blood cell mass of horses: toxic effect of heparin anticoagulant therapy." American journal of veterinary research **44**(12): 2271-2276.

Durney, C. H., H. Massoudi, et al. (1986). Radiofrequency radiation dosimetry handbook, DTIC Document.

Edwards, C. J. and J. Fuller (1996). "Oxidative stress in erythrocytes." Comparative Haematology International **6**(1): 24-31.

Elliott, M. (1999a). "Modified ultrafiltration and open heart surgery in children." Paediatric Anaesthesia **9**(1): 1-5.

Elliott, M. (2004). "Superabsorbent polymers." Product development scentist for SAP. BASF Aktiengesellschaft ss **13**.

Elliott, M. J. (1993). "Ultrafiltration and Modified Ultrafiltration in Pediatric Open-Heart Operations." Annals of Thoracic Surgery **56**(6): 1518-1522.

Elliott, M. J. (1999b). "Recent advances in paediatric cardiopulmonary bypass." Perfusion-Uk **14**(4): 237-246.

Elofsson, U. M., M. A. Paulsson, et al. (1997). "Adsorption of B-lactoglobulin A and B: Effects of ionic strength and phosphate ions." Colloids and Surfaces B: Biointerfaces **8**(3): 163-169.

Elwing, H., A. Askendal, et al. (1987). "Competition between adsorbed fibrinogen and high-molecular-weight kininogen on solid surfaces incubated in human plasma (the vroman effect): Influence of solid surface wettability." Journal of biomedical materials research **21**(8): 1023-1028.

Eppinger, M. J., P. A. Ward, et al. (1996). "Regulatory effects of interleukin-10 on lung ischemia-reperfusion injury." J Thorac Cardiovasc Surg **112**(5): 1301-5; discussion 1305-6.

Essemiani, K., G. l. Ducom, et al. (2001). "Spherical cap bubbles in a flat sheet nanofiltration module: experiments and numerical simulation." Chemical engineering science **56**(21): 6321-6327.

Estridge, B. H., A. P. Reynolds, et al. (2000). Basic medical laboratory techniques, Cengage Learning.

EUROMBRA (2006). Membrane bioreactor technology (MBR) with an EU perspective for advanced municipal wastewater treatment strategies for the 21st century. D5 - Literature Review on Aeration and main Operating Conditions in Membrane Bioreactors.

Evans, E. A. and P. L. La Celle (1975). "Intrinsic material properties of the erythrocyte membrane indicated by mechanical analysis of deformation." Blood **45**(1): 29-43.

Falkenhagen, D., T. Bosch, et al. (1987). "A clinical study on different cellulosic dialysis membranes." Nephrology Dialysis Transplantation **2**(6): 537-545.

Fang, F. and I. Szleifer (2003). "Competitive adsorption in model charged protein mixtures: equilibrium isotherms and kinetics behavior." The Journal of chemical physics **119**(2): 1053-1065.

Faraday, N., R. B. Scharpf, et al. (2001). "Leukocytes can enhance platelet-mediated aggregation and thromboxane release via interaction of P-selectin glycoprotein ligand 1 with P-selectin." Anesthesiology **94**(1): 145-51.

Farstad, M., J. K. Heltne, et al. (2004). "Can the use of methylprednisolone, vitamin C, or alpha-trinositol prevent cold-induced fluid extravasation during cardiopulmonary bypass in piglets?" Journal of Thoracic and Cardiovascular Surgery **127**(2): 525-534.

FDA (2000a). Guidance for Cardiopulmonary Bypass Arterial Line Blood Filter 510(k) Submissions. Food and Drug Administration**:** 1-5.

FDA (2000b). Guidance for Cardiopulmonary Bypass Oxygenators 510(k) Submissions; Final Guidance for Industry and FDA Staff. F. a. D. Administration**:** 1-20.

Field, R. W., D. Wu, et al. (1995). "Critical flux concept for microfiltration fouling." Journal of Membrane Science **100**(3): 259-272.

Finn, A., S. Naik, et al. (1993). "Interleukin-8 release and neutrophil degranulation after pediatric cardiopulmonary bypass." J Thorac Cardiovasc Surg **105**(2): 234-41.

Fischbach, F. T. and M. B. Dunning (2009). A manual of laboratory and diagnostic tests, Lippincott Williams & Wilkins.

Fisher Scientific (2014). Membrane Filters. Filtration, Available at: https://webshop.fishersci.com/webfiles/uk/web-docs/2013_EN_LAB_0744.PDF.

Flory, P. J. and J. Rehner Jr (1943). "Statistical mechanics of cross-linked polymer networks II. Swelling." The Journal of Chemical Physics **11**(11): 521-526.

Foley, G. (2013). Membrane Filtration: A Problem Solving Approach with MATLAB, Cambridge University Press.

Franco, K. L. and E. D. Verrier (2003). Advanced therapy in cardiac surgery, PMPH-USA.

Freise, K. J., R. L. Schmidt, et al. (2009). "The effect of anticoagulant, storage temperature and dilution on cord blood hematology parameters over time." International journal of laboratory hematology **31**(5): 496-504.

Friesen, R. H., D. N. Campbell, et al. (1997). "Modified ultrafiltration attenuates dilutional coagulopathy in pediatric open heart operations." Annals of Thoracic Surgery **64**(6): 1787-1789.

Gaell, C. and R. H. Davis (1996). "Membrane fouling during microfiltration of protein mixtures." Journal of Membrane Science **119**(2): 269-284.

Gaeta, M. G., L. Martinelli, et al. (2012). "Uplift Forces on Wave Exposed Jetties: Scale Comparison and Effect of Venting." Coastal Engineering Proceedings **1**(33): structures. 34.

Galletti, P. M., P. D. Richardson, et al. (1983). "Oscillating blood flow enhances membrane plasmapheresis." ASAIO Journal **29**(1): 279-282.

Garrison, T. (2006). Enhanced Essentials of Oceanography, Cengage Learning.

Ghosh, R. (2002). "Study of membrane fouling by BSA using pulsed injection technique." Journal of membrane science **195**(1): 115-123.

Ghosh, R. and Z. F. Cui (1998). "Fractionation of BSA and lysozyme using ultrafiltration: effect of pH and membrane pretreatment." Journal of membrane science **139**(1): 17-28.

Ghosh, S., F. Falter, et al. (2009). Cardiopulmonary bypass, Cambridge University Press.

Gibbon, J. J. H., J. R. Engstrom, et al. (1953). Extracorporeal circulation device, Google Patents.

Giersiepen, M., L. J. Wurzinger, et al. (1990). "Estimation of shear stress-related blood damage in heart valve prostheses--in vitro comparison of 25 aortic valves." Int J Artif Organs **13**(5): 300-6.

Ginés Escolar (2015). Platelet Function. www.platelet-research.org. Ginés Escolar, Platelet Research Laboratory.

Gladwin, M. T., T. Kanias, et al. (2012). "Hemolysis and cell-free hemoglobin drive an intrinsic mechanism for human disease." The Journal of clinical investigation **122**(4): 1205.

Glogowski, K. R., A. H. Stammers, et al. (2001). "The effect of priming techniques of ultrafiltrators on blood rheology: an in vitro evaluation." Perfusion-Uk **16**(3): 221-228.

Goldsmith, H. L., G. R. Cokelet, et al. (1989). "Robin Fahraeus: evolution of his concepts in cardiovascular physiology." Am J Physiol **257**(3 Pt 2): H1005-15.

Gomaa, H. G. (2012). "Flux characteristics at oscillating membrane equipped with turbulent promoters." Chemical Engineering Journal **191**: 541-547.

Gomaa, H. G. and A. M. A. Taweel (2007). "Intensification of inter-phase mass transfer: the combined effect of oscillatory motion and turbulence promoters." Heat and mass transfer **43**(4): 371-379.

Gooch, J. W. (2010). Biocompatible Polymeric Materials and Tourniquets for Wounds, Springer.

Goto, S., N. Tamura, et al. (2003). "Different effects of various anti-GPIIb-IIIa agents on shear-induced platelet activation and expression of procoagulant activity." J Thromb Haemost **1**(9): 2022-30.

Gourlay, T. (2011). Fluid processing device, Patent number US20110297615 A1, University of Strathclyde.

Gourlay, T., I. Samartzis, et al. (2003). "The effect of haemodilution on blood-biomaterial contact-mediated CD11b expression on neutrophils: ex vivo studies." Perfusion **18**(2): 87-93.

Gourlay, T., D. C. Stefanou, et al. (2001). "The effect of circuit surface area on CD11b(mac-1) expression in a rat recirculation model." Artif Organs **25**(6): 475-9.

Gourlay, T. and K. M. Taylor (2000). Pulsatile Cardiopulmonary Bypass. Cardiopulmonary Bypass: Principles and Practice. Glenn P. Gravlee, Richard F. Davis, Mark Kurusz and J. R. Utley, Lippincott Williams & Wilkins.

Gray, J. J. (2004). "The interaction of proteins with solid surfaces." Current opinion in structural biology **14**(1): 110-115.

Greeley, W. J., V. A. Bracey, et al. (1991a). "Recovery of cerebral metabolism and mitochondrial oxidation state is delayed after hypothermic circulatory arrest." Circulation **84**(5 Suppl): III400-6.

Greeley, W. J., F. H. Kern, et al. (1991b). "The effect of hypothermic cardiopulmonary bypass and total circulatory arrest on cerebral metabolism in neonates, infants, and children." J Thorac Cardiovasc Surg **101**(5): 783-94.

Gregoretti, S. (1996). "Suction-induced hemolysis at various vacuum pressures: implications for intraoperative blood salvage." Transfusion **36**(1): 57-60.

Greinacher, A., N. Lubenow, et al. (2003). "Heparin-induced thrombocytopenia." Orphanet Encyclopedia. December.

Grigioni, M., U. Morbiducci, et al. (2005). "A novel formulation for blood trauma prediction by a modified power-law mathematical model." Biomech Model Mechanobiol **4**(4): 249-60.

Grinnell, F. and M. K. Feld (1981). "Adsorption characteristics of plasma fibronectin in relationship to biological activity." Journal of biomedical materials research **15**(3): 363-381.

Groom, R. C., B. F. Akl, et al. (1994). "Alternative Method of Ultrafiltration after Cardiopulmonary Bypass." Annals of Thoracic Surgery **58**(2): 573-574.

Grubb, K. J., P. Salehi, et al. (2010). "Bivalirudin: alternative anticoagulation during cardiopulmonary bypass in patients with heparin-induced thrombocytopenia." Recent patents on cardiovascular drug discovery **5**(1): 20-24.

Gruber EM, J. R., Newburger JW, Zurakowski D, and L. P. Hansen DD (1999). "The effect of hematocrit on cerebral blood flow velocity in neonates and infants undergoing deep hypothermic cardiopulmonary bypass." Anesthesia and Analgesia **89**(2): 322-327.

Grunenfelder, J., G. Zund, et al. (2000). "Modified ultrafiltration lowers adhesion molecule and cytokine levels after cardiopulmonary bypass without clinical relevance in adults." European Journal of Cardio-Thoracic Surgery **17**(1): 77-83.

Gu, Y. J. and P. W. Boonstra (2006). "Selection of priming solutions for cardiopulmonary bypass in adults." Multimedia Manual of Cardio-Thoracic Surgery **2006**(0109): mmcts. 2005.001198.

Guell, C. and R. H. Davis (1996). "Membrane fouling during microfiltration of protein mixtures." Journal of Membrane Science **119**(2): 269-284.

Guglielmi, G., D. Chiarani, et al. (2007). "Flux criticality and sustainability in a hollow fibre submerged membrane bioreactor for municipal wastewater treatment." Journal of Membrane Science **289**(1): 241-248.

Gunaydin, S. and T. Gourlay (2013). "Novel ultrafiltration technique for blood conservation in cardiac operations." Ann Thorac Surg **95**(6): 2148-51.

Gupta, B. B. (1996). "Use of hydrodynamic methods for pressure driven membrane operations: Recent advances in membrane-based separation science and technology." Indian journal of chemical technology **3**(3): 156-163.

Gupta, B. B., J. A. Howell, et al. (1995). "A helical baffle for cross-flow microfiltration." Journal of Membrane Science **102**: 31-42.

Gupta, B. B., M. Y. Jaffrin, et al. (1986). "Membrane plasma separation through small-area, hollow-fiber filters." Artif Organs **10**(1): 45-51.

Gupta, B. S., S. Hasan, et al. (2005). "Effects of colloidal fouling and gas sparging on microfiltration of yeast suspension." Bioprocess and biosystems engineering **27**(6): 407-413.

Gupta, S. C. (2003). The classical Stefan problem: basic concepts, modelling and analysis, Elsevier.

Gurbuz, A. T., W. M. Novick, et al. (1998). "Impact of ultrafiltration on blood use for atrial septal defect closure in infants and children." Annals of Thoracic Surgery **65**(4): 1105-1108.

Hakim, R. M., D. T. Fearon, et al. (1984). "Biocompatibility of dialysis membranes: Effects of chronic complement activation." Kidney Int **26**(2): 194-200.

Hansard, S. L., W. O. Butler, et al. (1953). "Blood Volume of Farm Animals." Journal of Animal Science **12**(2): 402-413.

Harig, F., C. Meier, et al. (2006). "Does the additional use of heparin-coated extracorporeal circuits (ECC) optimize the effect of modified ultrafiltration (MUF) in pediatric perfusion?" Thorac Cardiovasc Surg **54**(3): 168-72.

Harris, D. N., S. M. Bailey, et al. (1993). "Brain swelling in first hour after coronary artery bypass surgery." Lancet **342**(8871): 586-7.

Harris, D. N., A. Oatridge, et al. (1998). "Cerebral swelling after normothermic cardiopulmonary bypass." Anesthesiology **88**(2): 340-5.

Hastings, G. W. (2012). Cardiovascular Biomaterials, Springer London.

Haynes, C. A. and W. Norde (1994). "Globular proteins at solid/liquid interfaces." Colloids and Surfaces B: Biointerfaces **2**(6): 517-566.

Helenius, G., H. Backdahl, et al. (2006). "In vivo biocompatibility of bacterial cellulose." Journal of Biomedical Materials Research Part A **76**(2): 431-438.

Heltne, J. K., J. Bert, et al. (2002). "Temperature-related fluid extravasation during cardiopulmonary bypass: An analysis of filtration coefficients and transcapillary pressures." ACTA Anaesthesiologica Scandinavica **46**(1): 51-56.

Hennein, H. A. (2000). "Venovenous modified ultrafiltration after cardiopulmonary bypass in children: a prospective randomized study." J Thorac Cardiovasc Surg **119**(3): 630.

Henry, M., C. Dupont-Gillain, et al. (2003). "Conformation change of albumin adsorbed on polycarbonate membranes as revealed by ToF-SIMS." Langmuir **19**(15): 6271-6276.

Heuberger, R., G. Sukhorukov, et al. (2005). "Biofunctional polyelectrolyte multilayers and microcapsules: control of non-specific and bio-specific protein adsorption." Advanced Functional Materials **15**(3): 357-366.

Hilal, N., O. O. Ogunbiyi, et al. (2005). "Methods employed for control of fouling in MF and UF membranes: a comprehensive review." Separation Science and Technology **40**(10): 1957-2005.

Hillegass, E. and H. S. Sadowsky (2010). Essentials of Cardiopulmonary Physical Therapy, Elsevier Health Sciences.

Himmelfarb, J., E. McMonagle, et al. (1999). "Increased susceptibility to erythrocyte C5b-9 deposition and complement-mediated lysis in chronic renal failure." Kidney Int **55**(2): 659-66.

Hindman, B. J., N. Funatsu, et al. (1990). "Differential Effect of Oncotic Pressure on Cerebral and Extracerebral Water-

Content During Cardiopulmonary Bypass in Rabbits." Anesthesiology **73**(5): 951-957.

Hiramatsu, T., Y. Imai, et al. (2002). "Effects of dilutional and modified ultrafiltration in plasma endothelin-1 and pulmonary vascular resistance after the Fontan procedure." Ann Thorac Surg **73**(3): 861-5.

Hirleman, E. and D. F. Larson (2008). "Cardiopulmonary bypass and edema: physiology and pathophysiology." Perfusion-Uk **23**(6): 311-322.

Hlady, V. and J. Buijs (1996). "Protein adsorption on solid surfaces." Current Opinion in Biotechnology **7**(1): 72-77.

Hlady, V., J. Buijs, et al. (1999). "Methods for studying protein adsorption." Methods in enzymology **309**: 402.

Hlavacek, M. and F. Bouchet (1993). "Constant flowrate blocking laws and an example of their application to dead-end microfiltration of protein solutions." Journal of membrane science **82**(3): 285-295.

Hodges, S. C., C. Mijumbi, et al. (2007). "Anaesthesia services in developing countries: defining the problems." Anaesthesia **62**(1): 4-11.

Hodges, U. M., S. Berg, et al. (1994). "Filtration of fentanyl is not the cause of the elevation of arterial blood pressure associated with post-bypass ultrafiltration in children." J Cardiothorac Vasc Anesth **8**(6): 653-7.

Hollinger, J. O. (2011). An introduction to biomaterials, CRC press.

Holmes, J. H., N. C. Connolly, et al. (2002). "Magnitude of the inflammatory response to cardiopulmonary bypass and its relation to adverse clinical outcomes." Inflammation Research **51**(12): 579-586.

Holt, D. W., G. H. Landis, et al. (1982). "Hemofiltration as an adjunct to cardiopulmonary bypass for total oxygenator volume control." J Extracorp Tech **14**: 373-378.

Hong, S. P., T. H. Bae, et al. (2002). "Fouling control in activated sludge submerged hollow fiber membrane bioreactors." Desalination **143**(3): 219-228.

Honjo, O., S. Osaki, et al. (2010). "Diagnosis-Based Differences in Response of Global Ventricular Performance to Modified Ultrafiltration in Children." Circulation Journal **74**(1): 86-92.

Hornick, P. and K. Taylor (1997). "Pulsatile and nonpulsatile perfusion: the continuing controversy." Journal of cardiothoracic and vascular anesthesia **11**(3): 310-315.

Howell, J. A., R. W. Field, et al. (1993). "Yeast cell microfiltration: flux enhancement in baffled and pulsatile flow systems." Journal of Membrane Science **80**(1): 59-71.

Howie, S. R. C. (2011). "Blood sample volumes in child health research: review of safe limits." Bulletin of the World Health Organization **89**(1): 46-53.

Hu, H., D. Varon, et al. (2003). "Platelet-leukocyte aggregation under shear stress: differential involvement of selectins and integrins." Thromb Haemost **90**(4): 679-87.

Hu, W.-J., J. W. Eaton, et al. (2001). "Molecular basis of biomaterial-mediated foreign body reactions." Blood **98**(4): 1231-1238.

Huang, H., T. Yao, et al. (2003). "Continuous ultrafiltration attenuates the pulmonary injury that follows open heart surgery with cardiopulmonary bypass." Ann Thorac Surg **76**(1): 136-40.

Huang, N.-P., R. Michel, et al. (2001). "Poly (L-lysine)-g-poly (ethylene glycol) layers on metal oxide surfaces: surface-analytical characterization and resistance to serum and fibrinogen adsorption." Langmuir **17**(2): 489-498.

Huotari, H. M., G. Tragardh, et al. (1999). "Crossflow membrane filtration enhanced by an external DC electric field: a review." Chemical Engineering Research and Design **77**(5): 461-468.

Hwu, T.-H., I. J. Sobey, et al. (1996). "Observation of concentration dispersion in unsteady deflected flows." Chemical engineering science **51**(13): 3373-3390.

Hwu, T.-H., I. J. Sobey, et al. (1997). "Simulation of concentration dispersion in unsteady deflected flows." Chemical engineering science **52**(19): 3233-3242.

Iaizzo, P. A. (2010). Handbook of Cardiac Anatomy, Physiology, and Devices, Humana Press.

Ikeda, U., F. Ohkawa, et al. (1992). "Serum interleukin 6 levels become elevated in acute myocardial infarction." J Mol Cell Cardiol **24**(6): 579-84.

Ikeda, Y., M. Handa, et al. (1991). "The role of von Willebrand factor and fibrinogen in platelet aggregation under varying shear stress." J Clin Invest **87**(4): 1234-40.

Iritani, E., Y. Mukai, et al. (2000). "Effects of electric field on dynamic behaviors of dead-end inclined and downward ultrafiltration of protein solutions." Journal of membrane Science **164**(1): 51-57.

Jackson, M. J. (2006). Surface Engineering: Proceedings of the 5th International Surface Engineering Congress - May 15-17, 2006, Washington State Convention Center, Seattle, Washington, USA, A S M International.

Jacobs, C. (1996). Replacement of Renal Function by Dialysis, Springer Netherlands.

Jaffrin, M. Y. (1989). "Innovative processes for membrane plasma separation." Journal of Membrane Science **44**(1): 115-129.

Jaffrin, M. Y. (2008). "Dynamic shear-enhanced membrane filtration: a review of rotating disks, rotating membranes and vibrating systems." Journal of Membrane Science **324**(1): 7-25.

Jaffrin, M. Y. (2012). "Hydrodynamic techniques to enhance membrane filtration." Annual Review of Fluid Mechanics **44**: 77-96.

Jaffrin, M. Y., L. H. Ding, et al. (1992). "Kinetics of concentration-polarization formation in crossflow filtration of plasma from blood: experimental results." Journal of membrane science **72**(3): 267-275.

Jaishankar, A., V. Sharma, et al. (2011). "Interfacial viscoelasticity, yielding and creep ringing of globular protein-surfactant mixtures." Soft Matter **7**(17): 7623-7634.

Jeffree, M. A., J. Peacock, et al. (1981). "Gel layer limited haemofiltration rates can be increased by vortex mixing." Renal Failure **5**(4): 373-380.

Jenney, C. R. and J. M. Anderson (2000). "Adsorbed serum proteins responsible for surface dependent human macrophage behavior." Journal of Biomedical Materials Research **49**(4): 435-447.

Jensen, E., S. Andreasson, et al. (2004). "Changes in hemostasis during pediatric heart surgery: Impact of a biocompatible heparin-coated perfusion system." Annals of Thoracic Surgery **77**(3): 962-967.

Jerner, R. C. (2015). Hurricane Surge Corrosion Failure Analysis. Metallurgist.com. J.E.I. Metallurgical Inc, Available at: http://www.metallurgist.com/files/2014/04/Corrosion1.jpg.

Jockusch, S., N. J. Turro, et al. (2009). "Photoinduced surface crosslinking of superabsorbent polymer particles." Journal of Applied Polymer Science **111**(5): 2163-2170.

Johir, M. A. H., R. Aryal, et al. (2011). "Influence of supporting media in suspension on membrane fouling reduction in submerged membrane bioreactor (SMBR)." Journal of Membrane Science **374**(1): 121-128.

Johnston, B. M., P. R. Johnston, et al. (2006). "Non-Newtonian blood flow in human right coronary arteries: transient simulations." J Biomech **39**(6): 1116-28.

Jones, K. L. and C. R. O'Melia (2000). "Protein and humic acid adsorption onto hydrophilic membrane surfaces: effects of pH and ionic strength." Journal of Membrane Science **165**(1): 31-46.

Jones, T. J. and M. J. Elliott (2006). "Paediatric CPB: bypass in a high risk group." Perfusion-Uk **21**(4): 229-233.

Journois, D., D. Israel-Biet, et al. (1996). "High-volume, zero-balanced hemofiltration to reduce delayed inflammatory response to cardiopulmonary bypass in children." Anesthesiology (Hagerstown) **85**(5): 965-976.

Journois, D., P. Pouard, et al. (1994). "Hemofiltration During Cardiopulmonary Bypass in Pediatric Cardiac-Surgery - Effects on Hemostasis, Cytokines, and Complement Components." Anesthesiology **81**(5): 1181-1189.

Jung, S.-Y., S.-M. Lim, et al. (2003). "The Vroman effect: a molecular level description of fibrinogen displacement." Journal of the American Chemical Society **125**(42): 12782-12786.

Kakishita, E., M. Higuchi, et al. (1989). "A New Index for Collagen Induced Platelet-Aggregation." Thrombosis Research **56**(3): 465-475.

Kakkar, N. and M. Makkar (2009). "Red cell cytograms generated by an ADVIA 120 automated hematology analyzer: Characteristic patterns in common hematological conditions." Lab Medicine **40**(9): 549-555.

Kalasin, S. and M. M. Santore (2009). "Non-specific adhesion on biomaterial surfaces driven by small amounts of protein adsorption." Colloids and Surfaces B: Biointerfaces **73**(2): 229-236.

Kameneva, M., P. Marascalco, et al. (2006). "Validity of Normalized Index of Hemolysis in Pediatric Mechanical Circulatory Assist Devices." ASAIO Journal **52**(2): 41A.

Kameneva, M. V., J. F. Antaki, et al. (1997). "Plasma protective effect on red blood cells exposed to mechanical stress." Asaio J **43**(5): M571-5.

Kameneva, M. V., G. W. Burgreen, et al. (2004). "Effects of turbulent stresses upon mechanical hemolysis: experimental and computational analysis." Asaio J **50**(5): 418-23.

Kameneva, M. V., A. Undar, et al. (1999). "Decrease in red blood cell deformability caused by hypothermia, hemodilution, and mechanical stress: factors related to cardiopulmonary bypass." Asaio J **45**(4): 307-10.

Karliczek, G. F., I. Tigchelaar, et al. (1986). "How much additional blood trauma is caused by haemofiltration during cardiopulmonary bypass?" Life Support Syst **4 Suppl 1**: 167-73.

Kawamura, T., R. Wakusawa, et al. (1993). "Elevation of cytokines during open heart surgery with cardiopulmonary bypass: participation of interleukin 8 and 6 in reperfusion injury." Canadian journal of anaesthesia **40**(11): 1016-1021.

Keenan, H. T., R. Thiagarajan, et al. (2000). "Pulmonary function after modified venovenous ultrafiltration in infants: A prospective, randomized trial." Journal of Thoracic and Cardiovascular Surgery **119**(3): 501-505.

Kelly, S. T., W. S. Opong, et al. (1993). "The influence of protein aggregates on the fouling of microfiltration membranes during stirred cell filtration." Journal of membrane science **80**(1): 175-187.

Kelly, S. T. and A. L. Zydney (1995). "Mechanisms for BSA fouling during microfiltration." Journal of Membrane Science **107**(1): 115-127.

Kern, F. H., N. J. Morana, et al. (1992). "Coagulation defects in neonates during cardiopulmonary bypass." Ann Thorac Surg **54**(3): 541-6.

Keuren, J. F. W., S. J. H. Wielders, et al. (2002). "Fibrinogen adsorption, platelet adhesion and thrombin generation at heparinized surfaces exposed to flowing blood." Thromb Haemost **87**(4): 742-747.

Kim, J.-P., J.-J. Kim, et al. (2002). "Effect of glass ball insertion on vortex-flow microfiltration of oil-in-water emulsion." Desalination **143**(2): 159-172.

Kim, Y. R. and L. Ornstein (1983). "Isovolumetric sphering of erythrocytes for more accurate and precise cell volume measurement by flow cytometry." Cytometry **3**(6): 419-427.

Kirshbom, P. M., B. E. Miller, et al. (2006). "Failure of surface-modified bypass circuits to improve platelet function during pediatric cardiac surgery." Journal of Thoracic and Cardiovascular Surgery **132**(3): 675-680.

Kirshbom, P. M., S. O. Page, et al. (1997). "Cardiopulmonary bypass and circulatory arrest increase endothelin-1 production and receptor expression in the lung." J Thorac Cardiovasc Surg **113**(4): 777-83.

Kiser, E. J. and J. A. Latty (1981). Coated membranes, Google Patents.

Kiziltepe, U., A. Uysalel, et al. (2001). "Effects of combined conventional and modified ultrafiltration in adult patients." Annals of Thoracic Surgery **71**(2): 684-693.

Klineberg, P. L., C. A. Kam, et al. (1984). "Hematocrit and Blood-Volume Control During Cardiopulmonary Bypass with the Use of Hemofiltration." Anesthesiology **60**(5): 478-480.

Konrad, J. (2015). Water, waves, and ripples, Available at: http://www.scienceisart.com/B_Waves/Waves.html.

Konstantopoulos, K., S. G. Kamat, et al. (1995). "Shear-induced platelet aggregation is inhibited by in vivo infusion of an anti-glycoprotein IIb/IIIa antibody fragment, c7E3 Fab, in patients undergoing coronary angioplasty." Circulation **91**(5): 1427-31.

Konstantopoulos, K., S. Neelamegham, et al. (1998). "Venous levels of shear support neutrophil-platelet adhesion and neutrophil aggregation in blood via P-selectin and beta(2)-integrin." Circulation **98**(9): 873-882.

Kopman, E. A. and T. B. Ferguson (1978). "Pulmonary Edema Following Cardiopulmonary Bypass." Anesthesia & Analgesia **57**(3): 367-371.

Kotani, Y., O. Honjo, et al. (2008). "Effect of modified ultrafiltration on postoperative course in neonates with complete transposition of the great arteries undergoing arterial switch operation." Circulation Journal **72**(9): 1476-1480.

Kotz, J., P. Treichel, et al. (2009). Chemistry and Chemical Reactivity, Enhanced Edition, Cengage Learning.

Koutlas, T. C., J. W. Gaynor, et al. (1997). "Modified ultrafiltration reduces postoperative morbidity after cavopulmonary connection." Ann Thorac Surg **64**(1): 37-42; discussion 43.

Koutsoukos, P. G., W. Norde, et al. (1983). "Protein adsorption on hematite (a-Fe2O3) surfaces." Journal of colloid and interface science **95**(2): 385-397.

Kroner, K. H. and V. Nissinen (1988). "Dynamic filtration of microbial suspensions using an axially rotating filter." Journal of membrane science **36**: 85-100.

Kucera, J. (2015). Reverse Osmosis: Design, Processes, and Applications for Engineers, Wiley.

Kunicka, J. E., G. Fischer, et al. (2000). "Improved platelet counting using two-dimensional laser light scatter." Am J Clin Pathol **114**(2): 283-9.

Kuypers, F. A. (1998). "Red cell membrane damage." J Heart Valve Dis **7**(4): 387-95.

Kyllonen, H. M., P. Pirkonen, et al. (2005). "Membrane filtration enhanced by ultrasound: a review." Desalination **181**(1): 319-335.

Lameire, N. and R. Mehta (2000). Complications of dialysis, CRC Press.

Lampert, R. H. and M. C. Williams (1972). "Effect of surface materials on shear-induced hemolysis." J Biomed Mater Res **6**(6): 499-532.

Lamph, S. (2012). "Regulation of medical devices outside the European Union." Journal of the Royal Society of Medicine **105**(suppl 1): S12-S21.

Leber, E. (2004). Protein Adsorption. University of Washington Engineered Biomaterials, Available at: http://www.uweb.engr.washington.edu/research/tutorials/proteinadsorbtion.html.

Leckband, D. and J. Israelachvili (2001). "Intermolecular forces in biology." Q Rev Biophys **34**(2): 105-267.

Lee, C.-K., W.-G. Chang, et al. (1993). "Air slugs entrapped cross-flow filtration of bacterial suspensions." Biotechnology and bioengineering **41**(5): 525-530.

Lee, M. (2013). Basic Skills in Interpreting Laboratory Data, ASHP.

Lev, E. I., J. I. Osende, et al. (2001). "Administration of abciximab to patients receiving tirofiban or eptifibatide: effect on platelet function." J Am Coll Cardiol **37**(3): 847-55.

Leverett, L. B., J. D. Hellums, et al. (1972). "Red blood cell damage by shear stress." Biophys J **12**(3): 257-73.

Levitzky, M. G. (2008). "Using the pathophysiology of obstructive sleep apnea to teach cardiopulmonary integration." Advances in Physiology Education **32**(3): 196-202.

Levy, B. I., E. L. Schiffrin, et al. (2008). "Impaired tissue perfusion: a pathology common to hypertension, obesity, and diabetes mellitus." Circulation **118**(9): 968-76.

Leyh, R. G., C. Bartels, et al. (2001). "Influence of modified ultrafiltration on coagulation, fibrinolysis and blood loss in adult cardiac surgery." European Journal of Cardio-Thoracic Surgery **19**(2): 145-151.

Li, N. N., A. G. Fane, et al. (2011). Advanced Membrane Technology and Applications, Wiley.

Li, Q. Y., Z. F. Cui, et al. (1997a). "Effect of bubble size and frequency on the permeate flux of gas sparged ultrafiltration with tubular membranes." Chemical Engineering Journal **67**(1): 71-75.

Li, Q. Y., R. Ghosh, et al. (1998). "Enhancement of ultrafiltration by gas sparging with flat sheet membrane modules." Separation and purification technology **14**(1): 79-83.

Li, Y., T. Chang, et al. (2006). "CFD analysis of particle deposition in the spacer-filled membrane module." Journal of Water Supply: Research and Technology - AQUA **55**(7-8): 589-601.

Lin, L. and J. T. Guthrie (2000). "Preparation and characterisation of novel, blood-plasma-separation membranes for use in biosensors." Journal of Membrane Science **173**(1): 73-85.

Linderkamp, O., D. Berg, et al. (1980). "Blood-Volume and Hematocrit in Various Organs in Newborn Piglets." Pediatric Research **14**(12): 1324-1327.

Linkins, L.-A. (2015). "Heparin induced thrombocytopenia." BMJ **350**: g7566.

Lisitsin, D., D. Hasson, et al. (2005). "Critical flux detection in a silica scaling RO system." Desalination **186**(1): 311-318.

Liu, J., B. Ji, et al. (2007). "Comparative effectiveness of methylprednisolone and zero-balance ultrafiltration on inflammatory response after pediatric cardiopulmonary bypass." Artificial Organs **31**(7): 571-575.

Longmore, D. B. and G. Wright (1981). Haematological effects of cardiotomy suction. Towards Safer Cardiac Surgery, Springer Netherlands**:** 313-323.

Lord, M. S., M. Foss, et al. (2010). "Influence of nanoscale surface topography on protein adsorption and cellular response." Nano Today **5**(1): 66-78.

Lowe, G. D. O., J. C. Barbenel, et al. (2012). Clinical Aspects of Blood Viscosity and Cell Deformability, Springer London.

Lu, D. R. and K. Park (1991). "Effect of surface hydrophobicity on the conformational changes of adsorbed fibrinogen." Journal of colloid and interface science **144**(1): 271-281.

Lu, J. R., X. Zhao, et al. (2007). "Protein adsorption studied by neutron reflection." Current Opinion in Colloid & Interface Science **12**(1): 9-16.

Luciani, G. B., T. Menon, et al. (2001). "Modified ultrafiltration reduces morbidity after adult cardiac operations: A prospective, randomized clinical trial." Circulation **104**(12 Supplement): I-253-I-259.

Luciani, R., M. Goracci, et al. (2009). "Reduction of Early Postoperative Morbidity in Cardiac Surgery Patients Treated With Continuous Veno-Venous Hemofiltration During Cardiopulmonary Bypass." Artificial Organs **33**(8): 654-U1.

Lunderquist, A. (1952). "On the Artificial Kidney XXI: The Efficacy of the Dialyser-Ultrafilter Intended for Human Use. Including a Preliminary Report on Treatment of Oedemic Patients by Means of Ultrafiltration." Acta Medica Scandinavica **143**(4): 307-314.

Ma, H., L. F. Hakim, et al. (2001). "Factors affecting membrane fouling reduction by surface modification and backpulsing." Journal of Membrane Science **189**(2): 255-270.

MacDonald, G. (2005). Massage for the hospital patient and medically frail client, Lippincott Williams & Wilkins.

Mackarel, A. J., K. J. Russell, et al. (2000). "Interleukin-8 and leukotriene-B(4), but not formylmethionyl leucylphenylalanine, stimulate CD18-independent migration of neutrophils across human pulmonary endothelial cells in vitro." Am J Respir Cell Mol Biol **23**(2): 154-61.

Maehara, T., I. Novak, et al. (1991). "Perioperative Monitoring of Total-Body Water by Bioelectrical Impedance in Children Undergoing Open-Heart-Surgery." European Journal of Cardio-Thoracic Surgery **5**(5): 258-265.

Magilligan, D. J. (1985). "Indications for Ultrafiltration in the Cardiac Surgical Patient." Journal of Thoracic and Cardiovascular Surgery **89**(2): 183-189.

Magilligan, D. J. and C. Oyama (1984). "Ultrafiltration During Cardiopulmonary Bypass - Laboratory Evaluation and Initial Clinical-Experience." Annals of Thoracic Surgery **37**(1): 33-39.

Mahmoud, A. B. S., M. S. Burhani, et al. (2005). "Effect of modified ultrafiltration on pulmonary function after cardiopulmonary bypass." Chest **128**(5): 3447-3453.

Malinauskas, R. A., R. M. Sade, et al. (1988). "Blood damaging effects in cardiotomy suction return. 1988." The Journal of extra-corporeal technology **44**(2): 84-89.

Mallubhotla, H. and G. Belfort (1997). "Flux enhancement during Dean vortex microfiltration. 8. Further diagnostics." Journal of membrane science **125**(1): 75-91.

Malmsten, M. (1998). "Formation of adsorbed protein layers." Journal of colloid and interface science **207**(2): 186-199.

Maluf, M. A. (2003). "Modified ultrafiltration in surgical correction of congenital heart disease with cardiopulmonary bypass." Perfusion-Uk **18**: 61-68.

Manning, G. S. (1969). "Limiting laws and counterion condensation in polyelectrolyte solutions I. Colligative properties." The journal of chemical Physics **51**(3): 924-933.

Marchant, A., J. Deviere, et al. (1994). "Interleukin-10 production during septicaemia." Lancet **343**(8899): 707-8.

Martin, L. (1999). All You Really Need to Know to Interpret Arterial Blood Gases, Lippincott Williams & Wilkins.

Martson, M., J. Viljanto, et al. (1997). "Biocompatibility of cellulose sponge with bone." European surgical research. Europaische chirurgische Forschung. Recherches chirurgicales europeennes **30**(6): 426-432.

Masoodi, R. and K. M. Pillai (2012). Wicking in Porous Materials: Traditional and Modern Modeling Approaches, Taylor & Francis.

Matsuura, T. (1993). Synthetic Membranes and Membrane Separation Processes, Taylor & Francis.

Mavroudis, C., C. Backer, et al. (2012). Pediatric cardiac surgery, John Wiley & Sons.

McConnell, T. H. (2007). The nature of disease: Pathology for the Health professions, Lippincott Williams & Wilkins.

McDonald, C. I., J. F. Fraser, et al. (2014). "Oxidative stress during extracorporeal circulation." European Journal of Cardio-Thoracic Surgery **46**(6): 937-943.

McGuinness, J., D. Bouchier-Hayes, et al. (2008). "Understanding the inflammatory response to cardiac surgery." Surgeon-Journal of the Royal Colleges of Surgeons of Edinburgh and Ireland **6**(3): 162-171.

McKay, C. B., O. Linderkamp, et al. (1993). "Fahraeus and Fahreaus-Lindqvist effects for neonatal and adult red blood cell suspensions." Pediatr Res **34**(4): 538-43.

McMaster, P. D. and R. J. Parsons (1938). "The Effect of the Pulse on the Spread of Substances Through Tissues." The Journal of Experimental Medicine **68**(3): 377-400.

Mechtcherine, V. and H. W. Reinhardt (2012). Application of Super Absorbent Polymers (SAP) in Concrete Construction: State-of-the-Art Report Prepared by Technical Committee 225-SAP, Springer.

Meintker, L., J. r. Ringwald, et al. (2011). "Comparison of automated differential blood cell counts from Abbott Sapphire, Siemens Advia 120, Beckman Coulter DxH 800, and Sysmex XE-2100 in normal and pathologic samples." American journal of clinical pathology **139**(5): 641-650.

Meireles, M., P. Aimar, et al. (1991). "Effects of protein fouling on the apparent pore size distribution of sieving membranes." Journal of membrane science **56**(1): 13-28.

Meng, F., S.-R. Chae, et al. (2009). "Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material." Water Research **43**(6): 1489-1512.

Mercier-Bonin, M., C. Lagane, et al. (2000). "Influence of a gas/liquid two-phase flow on the ultrafiltration and microfiltration performances: case of a ceramic flat sheet membrane." Journal of Membrane Science **180**(1): 93-102.

Mercier, M., C. Fonade, et al. (1997). "How slug flow can enhance the ultrafiltration flux in mineral tubular membranes." Journal of Membrane Science **128**(1): 103-113.

Merck, E., C. M. Kahn, et al. (2010). The Merck veterinary manual, Merck, Incorporated.

Merck Millipore Corporation (2014). Membrane 101. Merck Millipore R&D Poster Podcasts. Mike Mansfield and A. Baly;, Available at: http://www.podcasting.org.uk/millipore-posters-podcast-119422/membrane-101-part-1-what-is-a-membrane-show-193370256/.

Merrill, E. W. (1969). "Rheology of blood." Physiol. Rev **49**(4): 863-888.

Merten, M., T. Chow, et al. (2000). "A new role for P-selectin in shear-induced platelet aggregation." Circulation **102**(17): 2045-50.

Messent, M., K. Sullivan, et al. (1992). "Adult respiratory distress syndrome following cardiopulmonary bypass: incidence and prediction." Anaesthesia **47**(3): 267-8.

Michelson, A. D. (2013). Platelets, Academic Press.

Miller, B. E. and J. H. Levy (1997). "The inflammatory response to cardiopulmonary bypass." Journal of cardiothoracic and vascular anesthesia **11**(3): 355-366.

Millward, H. and B. Bellhouse (1996a). "Scale-up of the vortex wave microfilter using the power ratio." Journal of membrane science **111**(1): 105-113.

Millward, H. R., B. J. Bellhouse, et al. (1995a). "Enhancement of plasma filtration using the concept of the vortex wave." Journal of membrane science **100**(2): 121-129.

Millward, H. R., B. J. Bellhouse, et al. (1995b). "Screw-thread flow promoters: an experimental study of ultrafiltration and microfiltration performance." Journal of membrane science **106**(3): 269-279.

Minntech Therapeutic Technologies (2014). Hemocor HPH Hemoconcentrators, Available at: http://www.medivators.com/sites/default/files/minntech/images/products/50090235F_Hemocor.pdf.

Minton, A. P. (2000). "Effects of excluded surface area and adsorbate clustering on surface adsorption of proteins: I. Equilibrium models." Biophysical chemistry **86**(2): 239-247.

Minton, A. P. (2001). "Effects of excluded surface area and adsorbate clustering on surface adsorption of proteins. II. Kinetic models." Biophysical journal **80**(4): 1641-1648.

Miyahara, T., K. Tsuchiya, et al. (1988). "Wake properties of a single gas bubble in three-dimensional liquid-solid fluidized bed." International journal of multiphase flow **14**(6): 749-763.

Miyaji, K., T. Miyamoto, et al. (2008). "Miniaturized cardiopulmonary bypass system in neonates and small infants." Interactive cardiovascular and thoracic surgery **7**(1): 75-8.

Miyamoto, T., S. i. Takahashi, et al. (1989). "Tissue biocompatibility of cellulose and its derivatives." Journal of biomedical materials research **23**(1): 125-133.

Mockel, D., E. Staude, et al. (1999). "Static protein adsorption, ultrafiltration behavior and cleanability of hydrophilized polysulfone membranes." Journal of membrane science **158**(1): 63-75.

Mohandas, N. and P. G. Gallagher (2008). "Red cell membrane: past, present, and future." Blood **112**(10): 3939-48.

Mohanty, K. and M. K. Purkait (2011). Membrane technologies and applications, CRC Press.

Mongero, L. B. and J. R. Beck (2008). On bypass: advanced perfusion techniques, Springer Science & Business Media.

Monroe, D. M., M. Hoffman, et al. (2002). "Platelets and thrombin generation." Arteriosclerosis, thrombosis, and vascular biology **22**(9): 1381-1389.

Monroe, J. M., D. E. True, et al. (1981). "Surface roughness and edge geometries in hemolysis with rotating disk flow." Journal of biomedical materials research **15**(6): 923-939.

Moore, J. N., E. A. Mahaffey, et al. (1987). "Heparin-induced agglutination of erythrocytes in horses." American journal of veterinary research **48**(1): 68-71.

Moore, R. A. and G. W. Laub (2000). Hemofiltration, Dialysis, and Blood Salvage Techniques During Cardiopulmonary Bypass. Cardiopulmonary Bypass: Principles and Practice. Glenn P. Gravlee, Richard F. Davis, Mark Kurusz and J. R. Utley, Lippincott Williams & Wilkins.

Morgan, S. H., M. A. Mansell, et al. (1985). "Fluid Removal by Hemofiltration in Diuretic Resistant Cardiac-Failure." British Heart Journal **54**(2): 218-219.

Moulin, P., J. C. Rouch, et al. (1996). "Mass transfer improvement by secondary flows: Dean vortices in coiled tubular membranes." Journal of membrane science **114**(2): 235-244.

Mueller, J. and R. H. Davis (1996). "Protein fouling of surface-modified polymeric microfiltration membranes." Journal of Membrane Science **116**(1): 47-60.

Myung, R. J., P. M. Kirshbom, et al. (2003). "Modified ultrafiltration may not improve neurologic outcome following deep hypothermic circulatory arrest." Eur J Cardiothorac Surg **24**(2): 243-8.

Naik, S. K. and M. J. Elliott (1993). "Ultrafiltration and paediatric cardiopulmonary bypass." Perfusion **8**(1): 101-112.

Naik, S. K., A. Knight, et al. (1991b). "A prospective randomized study of a modified technique of ultrafiltration during pediatric open-heart surgery." Circulation **84**(5 Suppl): III422-31.

Naik, S. K., A. Knight, et al. (1991a). "A Successful Modification of Ultrafiltration for Cardiopulmonary Bypass in Children." Perfusion (London) **6**(1): 41-50.

Naito, K., K. Mizuguchi, et al. (1994). "The need for standardizing the index of hemolysis." Artificial organs **18**(1): 7-10.

Najarian, S. and B. J. Bellhouse (1996a). "Effect of liquid pulsation on protein fractionation using ultrafiltration processes." Journal of membrane science **114**(2): 245-253.

Najarian, S. and B. J. Bellhouse (1996b). "Enhanced microfiltration of bovine blood using a tubular membrane with a screw-threaded insert and oscillatory flow." Journal of membrane science **112**(2): 249-261.

Nakahara, T. and F. Yoshida (1986). "Mechanical effects on rates of hemolysis." J Biomed Mater Res **20**(3): 363-74.

Nakamura, Y., M. Masuda, et al. (1990). "Comparative-Study of Cell Saver and Ultrafiltration Nontransfusion in Cardiac-Surgery." Annals of Thoracic Surgery **49**(6): 973-978.

Nath, K. (2008). Membrane Separation Processes, PHI Learning.

Nath, N., J. Hyun, et al. (2004). "Surface engineering strategies for control of protein and cell interactions." Surface Science **570**(1): 98-110.

Ndinisa, N. V., A. G. Fane, et al. (2006a). "Fouling control in a submerged flat sheet membrane system: part I bubbling and hydrodynamic effects." Separation science and technology **41**(7): 1383-1409.

Ndinisa, N. V., A. G. Fane, et al. (2006b). "Fouling control in a submerged flat sheet membrane system: Part II Two phase flow characterization and CFD simulations." Separation science and technology **41**(7): 1411-1445.

Nederlof, B., B. Mathieu, et al. (1984). "[Development of membranes for dialysis]." Biomed Tech (Berl) **29**(6): 131-41.

Neote, K., W. Darbonne, et al. (1993). "Identification of a promiscuous inflammatory peptide receptor on the surface of red blood cells." J Biol Chem **268**(17): 12247-9.

Nesbitt, W. S., E. Westein, et al. (2009). "A shear gradient-dependent platelet aggregation mechanism drives thrombus formation." Nat Med **15**(6): 665-73.

Neuhof, C., O. Walter, et al. (2003). "Bradykinin and histamine generation with generalized enhancement of microvascular permeability in neonates, infants, and children undergoing cardiopulmonary bypass surgery." Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies **4**(3): 299-304.

Nevaril, C. G., J. D. Hellums, et al. (1969). "Physical effects in red blood cell trauma." AIChE Journal **15**(5): 707-711.

Nilsson, L., P. O. Persson, et al. (2007). Cleaner Production: Technologies and Tools for Resource Efficient Production, Baltic University Press.

Ninomiya, M., K. Miyaji, et al. (2003). "Influence of PMEA-coated bypass circuits on perioperative inflammatory response." Annals of Thoracic Surgery **75**(3): 913-917.

Nittrouer, C. A., J. A. Austin, et al. (2009). Continental Margin Sedimentation: From Sediment Transport to Sequence Stratigraphy (Special Publication 37 of the IAS), Wiley.

Nobel, P. S. (2009). Physicochemical and Environmental Plant Physiology, Elsevier Science.

Norde, W. (1986). "Adsorption of proteins from solution at the solid-liquid interface." Advances in colloid and interface science **25**: 267-340.

Norde, W. (1996). Driving forces for protein adsorption at solid surfaces. Macromolecular Symposia, Wiley Online Library.

O'Brien, J. R. (1990). "Shear-induced platelet aggregation." Lancet **335**(8691): 711-3.

Obel, I. W. P., P. Marchand, et al. (1967). "Biochemical changes associated with the use of haemodilution with 5% dextrose in water and mannitol for open-heart surgery." Thorax **22**(2): 180-187.

Ognier, S., C. Wisniewski, et al. (2004). "Membrane bioreactor fouling in sub-critical filtration conditions: a local critical flux concept." Journal of Membrane Science **229**(1): 171-177.

Olds, S. B., M. L. London, et al. (2000). Maternal-Newborn Nursing: A Family and Community-Based Approach, Prentice Hall Health.

Olsen, D. B. (2000). "The history of continuous-flow blood pumps." Artif Organs **24**(6): 401-4.

Omidian, H., S. A. Hashemi, et al. (1998). "A model for the swelling of superabsorbent polymers." Polymer **39**(26): 6697-6704.

Omidian, H., K. Park, et al. (2007). "Recent developments in superporous hydrogels." Journal of pharmacy and pharmacology **59**(3): 317-327.

Onoe, M., T. Magara, et al. (2001). "Modified ultrafiltration removes serum interleukin-8 in adult cardiac surgery." Perfusion-Uk **16**(1): 37-42.

Ootaki, Y., M. Yamaguchi, et al. (2002). "Effects of modified ultrafiltration on coagulation factors in pediatric cardiac surgery." Surgery Today **32**(3): 203-206.

Pabby, A. K., S. S. H. Rizvi, et al. (2008). Handbook of membrane separations: chemical, pharmaceutical, food, and biotechnological applications, CRC press.

Paganini, E. P. (1986). Acute Continuous Renal Replacement Therapy, Springer US.

Pall Corporation (2014). Recommended Products for Crossflow Filtration, Available at: http://www.pall.com/main/graphic-arts/recommended-products-for-crossflow-filtration.page.

Pall Life Sciences (2003). Biodyne Membrane Specifications Laboratory Catalogue, Available at: http://www.pall.com/pdfs/Laboratory/03.0754_TransferMembLoRes.pdf: 744.

Pang, C. M., P. Hong, et al. (2005). "Biofilm formation characteristics of bacterial isolates retrieved from a reverse osmosis membrane." Environmental science & technology **39**(19): 7541-7550.

Pankowsky, D. A., N. P. Ziats, et al. (1990). "Morphologic characteristics of adsorbed human plasma proteins on vascular grafts and biomaterials." Journal of vascular surgery **11**(4): 599-606.

Paparella, D., T. M. Yau, et al. (2002). "Cardiopulmonary bypass induced inflammation: pathophysiology and treatment. An update." Eur J Cardiothorac Surg **21**(2): 232-44.

Park, Y. G. (2002). "Effect of ozonation for reducing membrane-fouling in the UF membrane." Desalination **147**(1): 43-48.

Parvatiyar, M. G. (1996). "Interaction of dispersed phase with concentration polarization." Journal of membrane science **115**(2): 121-127.

Parvatiyar, M. G. and R. Govind (1995). "Effect of dispersed phase on reducing concentration polarization." Journal of membrane science **105**(3): 187-201.

Patrick, C. W., A. G. Mikos, et al. (1998). Frontiers in tissue engineering, Elsevier.

Pearl, J. M., P. B. Manning, et al. (1999). "Effect of modified ultrafiltration on plasma thromboxane B2, leukotriene B4, and endothelin-1 in infants undergoing cardiopulmonary bypass." Annals of Thoracic Surgery **68**(4): 1369-1375.

Pearson, H. A. (2003). Blood and Blood-Forming Tissues. Rudolph's Pediatrics. C. D. Rudolph and A. M. Rudolph. New York, McGraw-Gill**:** 1521.

Pedrizzetti, G. and K. Perktold (2003). Cardiovascular Fluid Mechanics, Springer.

Peppas, N. A., Y. Huang, et al. (2000). "Physicochemical foundations and structural design of hydrogels in medicine and biology." Annu Rev Biomed Eng **2**: 9-29.

Peppas, N. A., R. M. Ottenbrite, et al. (2010). Biomedical Applications of Hydrogels Handbook, Springer.

Perez-Vela, J. L., E. Ruiz-Alonso, et al. (2008). "ICU outcomes in adult cardiac surgery patients in relation to ultrafiltration type." Perfusion-Uk **23**(2): 79-87.

Perktold, K., M. Hofer, et al. (1998). "Validated computation of physiologic flow in a realistic coronary artery branch." J Biomech **31**(3): 217-28.

Perthel, M., L. El-Ayoubi, et al. (2007b). "Clinical advantages of using mini-bypass systems in terms of blood product use, postoperative bleeding and air entrainment: an in vivo clinical perspective (vol 31, pg 1070, 2007)." European Journal of Cardio-Thoracic Surgery **32**(6): 952-952.

Perthel, M., A. Klingbeil, et al. (2007a). "Reduction in blood product usage associated with routine use of mini bypass systems in extracorporeal circulation." Perfusion-Uk **22**(1): 9-14.

Peterson, M. A. (1992). "Linear response of the human erythrocyte to mechanical stress." Physical Review A **45**(6): 4116.

Pillitteri, A. (2010). Maternal & child health nursing: care of the childbearing & childrearing family, Lippincott Williams & Wilkins.

Politis, A. K., G. P. Stavropoulos, et al. (2008). "Numerical modelling of simulated blood flow in idealized composite arterial coronary grafts: transient flow." J Biomech **41**(1): 25-39.

Portela, F., R. Espanol, et al. (1999). "[Combined perioperative ultrafiltration in pediatric cardiac surgery. The preliminary results]." Rev Esp Cardiol **52**(12): 1075-82.

Pradhan, M., S. Vigneswaran, et al. (2012). "Combined effect of air and mechanical scouring of membranes for fouling reduction in submerged membrane reactor." Desalination **288**: 58-65.

Prieske, H., A. Drews, et al. (2008). "Prediction of the circulation velocity in a membrane bioreactor." Desalination **231**(1): 219-226.

Prins, M., M. W. Van Leeuwen, et al. (2009). "Stability and reproducibility of ADVIA 120-measured red blood cell and platelet parameters in dogs, cats, and horses, and the use of reticulocyte haemoglobin content (CH (R)) in the diagnosis of iron deficiency." Tijdschrift voor diergeneeskunde(134): 272-8.

Prior, F. G., T. Gourlay, et al. (1995). "Pulse reverse osmosis: a new theory in the maintenance of fluid balance." Perfusion **10**(3): 159-70.

Prior, F. G., V. Morecroft, et al. (1999). "Oedema, Starling and pulse reverse osmosis: towards a possible biochemical marker for oedema." Int J Artif Organs **22**(3): 138-44.

Prior, F. G., V. Morecroft, et al. (1996). "The therapeutic significance of pulse reverse osmosis." Int J Artif Organs **19**(8): 487-92.

Psoch, C. and S. Schiewer (2005). "Critical flux aspect of air sparging and backflushing on membrane bioreactors." Desalination **175**(1): 61-71.

Puri, R. N. and R. W. Colman (1997). "ADP-induced platelet activation." Crit Rev Biochem Mol Biol **32**(6): 437-502.

Rabe, M., D. Verdes, et al. (2009). "Surface-induced spreading phenomenon of protein clusters." Soft Matter **5**(5): 1039-1047.

Rabe, M., D. Verdes, et al. (2011). "Understanding protein adsorption phenomena at solid surfaces." Advances in colloid and interface science **162**(1): 87-106.

Raja, S. G., S. Yousufuddin, et al. (2006). "Impact of modified ultrafiltration on morbidity after pediatric cardiac surgery." Asian Cardiovasc Thorac Ann **14**(4): 341-50.

Rampling, M. W., H. J. Meiselman, et al. (2004). "Influence of cell-specific factors on red blood cell aggregation." Biorheology **41**(2): 91-112.

Ramsden, J. J. (1995). "Puzzles and paradoxes in protein adsorption." Chem. Soc. Rev. **24**(1): 73-78.

Rand, P. W., E. Lacombe, et al. (1964). "Viscosity of Normal Human Blood under Normothermic and Hypothermic Conditions." J Appl Physiol **19**: 117-22.

Ratkovich, N., C. C. V. Chan, et al. (2009). "Experimental study and CFD modelling of a two-phase slug flow for an airlift tubular membrane." Chemical Engineering Science **64**(16): 3576-3584.

Razzak, J. A. and A. L. Kellermann (2002). "Emergency medical care in developing countries: is it worthwhile?" Bulletin of the World Health Organization **80**(11): 900-905.

Redkar, S. G. and R. H. Davis (1995). "Cross-flow microfiltration with high-frequency reverse filtration." AIChE journal **41**(3): 501-508.

Ricci, Z., A. Polito, et al. (2013). "Assessment of modified ultrafiltration hemodynamic impact by pressure recording analytical method during pediatric cardiac surgery." Pediatr Crit Care Med **14**(4): 390-5.

Riemersma, R. A., R. Logan, et al. (1982). "Effect of heparin on plasma free fatty acid concentrations after acute myocardial infarction." British heart journal **48**(2): 134-139.

Rios, G. M., H. Rakotoarisoa, et al. (1987). "Basic transport mechanisms of ultrafiltration in the presence of fluidized particles." Journal of membrane science **34**(2): 331-343.

Rivera, E. S., T. R. Kimball, et al. (1998). "Effect of veno-venous ultrafiltration on myocardial performance immediately after cardiac surgery in children - A

prospective randomized study." Journal of the American College of Cardiology **32**(3): 766-772.

Rockel, A., J. Hertel, et al. (1986). "Permeability and secondary membrane formation of a high flux polysulfone hemofilter." Kidney international **30**(3): 429-432.

Rodak, B. F., G. A. Fritsma, et al. (2013). Hematology: clinical principles and applications, Elsevier Health Sciences.

Rodgers, V. G. J. and R. E. Sparks (1992). "Effect of transmembrane pressure pulsing on concentration polarization." Journal of membrane science **68**(1): 149-168.

Rodriguez, R. A., M. Ruel, et al. (2005). "High flow rates during modified ultrafiltration decrease cerebral blood flow velocity and venous oxygen saturation in infants." Ann Thorac Surg **80**(1): 22-8.

Romagnoli, A., J. Hacker, et al. (1976). "External Haemoconcentration After Deliberate Haemodilution." Annual Meeting of the American Society of Anesthesiologists, Park Ridge 269-269.

Rosenfeld, A. J. and S. M. Dial (2011). Clinical pathology for the veterinary team, John Wiley & Sons.

Rosengart, T. K., W. DeBois, et al. (1998). "Retrograde autologous priming for cardiopulmonary bypass: A safe and effective means of decreasing hemodilution and transfusion requirements." Journal of Thoracic and Cardiovascular Surgery **115**(2): 426-438.

Rosse, W. F. and J. P. Adams (1980). "The variability of hemolysis in the cold agglutinin syndrome." Blood **56**(3): 409-16.

Rupprecht, H. J., H. Darius, et al. (1998). "Comparison of antiplatelet effects of aspirin, ticlopidine, or their combination after stent implantation." Circulation **97**(11): 1046-52.

Saatvedt, K., H. Lindberg, et al. (1996). "Ultrafiltration after cardiopulmonary bypass in children: effects on hemodynamics, cytokines and complement." Cardiovasc Res **31**(4): 596-602.

Sablani, S. S., A. K. Datta, et al. (2006). Handbook of food and bioprocess modeling techniques, CRC Press.

Sadler, T. W. (2011). Langman's medical embryology, Lippincott Williams & Wilkins.

Sakota, D., R. Sakamoto, et al. (2008). "Mechanical damage of red blood cells by rotary blood pumps: selective destruction of aged red blood cells and subhemolytic trauma." Artif Organs **32**(10): 785-91.

Saksena, S. and A. L. Zydney (1994). "Effect of solution pH and ionic strength on the separation of albumin from immunoglobulins (IgG) by selective filtration." Biotechnol Bioeng **43**(10): 960-8.

Sarkar, B., S. DasGupta, et al. (2009). "Electric field enhanced fractionation of protein mixture using ultrafiltration." Journal of Membrane Science **341**(1): 11-20.

Sata, T. (2004). Ion exchange membranes: preparation, characterization, modification and application, Royal Society of chemistry.

Schaller, J., S. Gerber, et al. (2008). Human Blood Plasma Proteins: Structure and Function, Wiley.

Schetz, M. (1994). "Removal of cytokines in septic patients using continuous veno-venous hemodiafiltration." Crit Care Med **22**(4): 715-6; author reply 719-21.

Schlunzen, L., J. Pedersen, et al. (1998). "Modified ultrafiltration in paediatric cardiac surgery." Perfusion **13**(2): 105-9.

Schmid-Schonbein, H., R. E. Wells, et al. (1971). "Fluid drop-like behaviour of erythrocytes--disturbance in pathology and its quantification." Biorheology **7**(4): 227-34.

Schooneman, F., J. F. Stoltz, et al. (1986). Plasmapheresis in normal donors: technical and biological study of two plasma filtration systems. Progress in Artificial Organs-1985, ISAO Press Cleveland, OH**:** 925.

Schrier, R. W. (2010). Renal and electrolyte disorders, Lippincott Williams & Wilkins.

Secomb, T. W., A. R. Pries, et al. (1986). "Hematocrit fluctuations within capillary tubes and estimation of Fahraeus effect." International journal of microcirculation, clinical and experimental/sponsored by the European Society for Microcirculation **5**(4): 335-345.

Segers, P. A., J. F. Heida, et al. (2001). "Clinical evaluation of nine hollow-fibre membrane oxygenators." Perfusion **16**(2): 95-106.

Seghaye, M. C., R. G. Grabitz, et al. (1996). "Inflammatory reaction and capillary leak syndrome related to cardiopulmonary bypass in neonates undergoing cardiac operations." Journal of Thoracic and Cardiovascular Surgery **112**(3): 687-697.

Seifter, J., D. Sloane, et al. (2005). Concepts in Medical Physiology, Lippincott Williams & Wilkins.

Sekiyama, K. D., M. Yoshiba, et al. (1994). "Circulating proinflammatory cytokines (IL-1B, TNF-a, and IL-6) and IL-1 receptor antagonist (IL-1Ra) in fulminant hepatic failure and acute hepatitis." Clinical & Experimental Immunology **98**(1): 71-77.

Selgrade, B. P. and G. A. Truskey (2012). "Computational fluid dynamics analysis to determine shear stresses and rates in a centrifugal left ventricular assist device." Artif Organs **36**(4): E89-96.

Semrad, M., M. Krajcek, et al. (2015). Cardiovascular Surgery, Karolinum.

Seneviratne, M. (2007). A Practical Approach to Water Conservation for Commercial and Industrial Facilities, Elsevier Science.

Shapiro, S. I. and M. C. Williams (1970). "Hemolysis in simple shear flows." AIChE Journal **16**(4): 575-580.

Shen, M., I. Garcia, et al. (2004). "Effects of adsorbed proteins and surface chemistry on foreign body giant cell formation, tumor necrosis factor alpha release and procoagulant activity of monocytes." Journal of Biomedical Materials Research Part A **70**(4): 533-541.

Shin'oka, T., D. Shum-Tim, et al. (1996). "Higher hematocrit improves cerebral outcome after deep hypothermic circulatory arrest." J Thorac Cardiovasc Surg **112**(6): 1610-20; discussion 1620-1.

Si-Hassen, D., A. Ould-Dris, et al. (1996). "Optimisation of an intermittent cross-flow filtration process of mineral

suspensions." Journal of Membrane Science **118**(2): 185-198.

Siembida, B., P. Cornel, et al. (2010). "Effect of mechanical cleaning with granular material on the permeability of submerged membranes in the MBR process." Water research **44**(14): 4037-4046.

Sigma-Aldrich. (2014). "Thermal Transition of Homopolymers." General Polymer Science, 2014, from https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Aldrich/General_Information/thermal_transitions_of_homopolymers.pdf.

Singh, A. K., M. R. De Leval, et al. (1976). "Pulmonary artery banding for truncus arteriosus in the first year of life." Circulation **54**(6 SUP.3): 17-19.

Skaryak, L. A., P. M. Kirshbom, et al. (1995). "Modified Ultrafiltration Improves Cerebral Metabolic Recovery after Circulatory Arrest." Journal of Thoracic and Cardiovascular Surgery **109**(4): 744-752.

Slack, S. M. and T. A. Horbett (1995). "The Vroman effect-A critical review." Proteins at interfaces II **602**: 112-128.

Slaughter, M. S., F. D. Pagani, et al. (2013). "HeartWare ventricular assist system for bridge to transplant: combined results of the bridge to transplant and continued access protocol trial." The Journal of Heart and Lung Transplantation **32**(7): 675-683.

Slaughter, M. S., M. A. Sobieski, et al. (2009). "HeartWare miniature axial-flow ventricular assist device: design and initial feasibility test." Texas Heart Institute Journal **36**(1): 12.

Solem, J. O., L. Tengborn, et al. (1987). "Cell saver versus hemofilter for concentration of oxygenator blood after cardiopulmonary bypass." Thorac Cardiovasc Surg **35**(1): 42-7.

Solomon, B. A., F. Castino, et al. (1978). "Continuous flow membrane filtration of plasma from whole blood." ASAIO Journal **24**(1): 21-26.

Somasundaran, P. (2006). Encyclopedia of surface and colloid science, CRC Press.

Song, L. (1998). "Flux decline in crossflow microfiltration and ultrafiltration: mechanisms and modeling of membrane fouling." Journal of membrane science **139**(2): 183-200.

Sonntag, J., I. Dahnert, et al. (1998). "Complement and contact activation during cardiovascular operations in infants." Ann Thorac Surg **65**(2): 525-31.

Sorin Group USA (2009). Sorin DHF.02 Pediatric Hemoconcentrator, Available at: http://www.mybloodfirst.com/downloads/CP_Dideco_DHF02_DS(4).pdf.

Sowemimo-Coker, S. O. (2002). "Red blood cell hemolysis during processing." Transfus Med Rev **16**(1): 46-60.

Stark, J., M. De Leval, et al. (1980). "Open heart surgery in the first year of life. Current state and future trends." Advances in Cardiology **27**: 243-253.

Stark, J., M. R. de Leval, et al. (1974). "Corrective surgery of transposition of the great arteries in the first year of life. Results in 63 infants." Journal of Thoracic and Cardiovascular Surgery **67**(5): 673-681.

Stark, J., D. Gandhi, et al. (1978). "Surgical treatment of persistent truncus arteriosus in the first year of life." Heart **40**(11): 1280-1287.

Starling, E. H. (1896). "On the Absorption of Fluids from the Connective Tissue Spaces." The Journal of physiology **19**(4): 312-26.

Staub, N. C. (1978). "Pulmonary edema: physiologic approaches to management." Chest **74**(5): 559-64.

Steffen, P., C. Verdier, et al. (2013). "Quantification of depletion-induced adhesion of red blood cells." Phys Rev Lett **110**(1): 018102.

Steneker, I., R. N. I. Pietersz, et al. (1995). "Leukocyte filtration mechanisms. Factors influencing the removal of infectious agents from red cell concentrates." Immunological investigations **24**(1-2): 87-93.

Stepner, T. A., C. S. Vassilieff, et al. (1985). "Cell-plasma interactions during membrane plasmapheresis." Clin. Hemorheol **5**: 11-19.

Stoney, W. S. (2009). "Evolution of cardiopulmonary bypass." Circulation **119**(21): 2844-2853.

Su, B., C. Zhao, et al. (2011). Polyethersulfone hollow fiber membranes for hemodialysis, Intech.

Sultana, N. (2012). Biodegradable Polymer-Based Scaffolds for Bone Tissue Engineering, Springer Berlin Heidelberg.

Sur, H. W. and Z. Cui (2001). "Experimental study on the enhancement of yeast microfiltration with gas sparging." Journal of chemical technology and biotechnology **76**(5): 477-484.

Sutera, S. P. (1977). "Flow-induced trauma to blood cells." Circ Res **41**(1): 2-8.

Sutera, S. P., P. A. Croce, et al. (1972). "Hemolysis and subhemolytic alterations of human RBC induced by turbulent shear flow." Trans Am Soc Artif Intern Organs **18**(0): 335-41, 347.

Szaba, F. M. and S. T. Smiley (2002). "Roles for thrombin and fibrin (ogen) in cytokine/chemokine production and macrophage adhesion in vivo." Blood **99**(3): 1053-1059.

Tamari, Y., R. L. Nelson, et al. (1984). "Effects of the hemoconcentrator on blood." J Extra-Corpor Technol **16**(3): 89-94.

Tanaka, Y. (2007). Ion Exchange Membranes: Fundamentals and Applications, Elsevier Science.

Tang, L. and J. W. Eaton (1993b). "Fibrin (ogen) mediates acute inflammatory responses to biomaterials." The Journal of experimental medicine **178**(6): 2147-2156.

Tang, L. and J. W. Eaton (1995). "Inflammatory responses to biomaterials." American journal of clinical pathology **103**(4): 466-471.

Tang, L. and J. W. Eaton (1999b). "Natural responses to unnatural materials: A molecular mechanism for foreign body reactions." Molecular Medicine **5**(6): 351.

Tang, L., A. H. Lucas, et al. (1993a). "Inflammatory responses to implanted polymeric biomaterials: role of surface-adsorbed immunoglobulin G." Journal of Laboratory and Clinical Medicine **122**(3): 292-300.

Tang, L., M.-S. Sheu, et al. (1999a). "Anti-inflammatory properties of triblock siloxane copolymer-blended materials." Biomaterials **20**(15): 1365-1370.

Tang, L., T. P. Ugarova, et al. (1996). "Molecular determinants of acute inflammatory responses to biomaterials." Journal of Clinical Investigation **97**(5): 1329.

Tang, L., Y. Wu, et al. (1998). "Fibrinogen adsorption and host tissue responses to plasma functionalized surfaces." Journal of biomedical materials research **42**(1): 156-163.

Tassani, P., J. A. Richter, et al. (1999). "Influence of combined zero-balanced and modified ultrafiltration on the systemic inflammatory response during coronary artery bypass grafting." Journal of Cardiothoracic and Vascular Anesthesia **13**(3): 285-291.

Tempe, D. K. and S. Virmani (2002). "Coagulation abnormalities in patients with cyanotic congenital heart disease." J Cardiothorac Vasc Anesth **16**(6): 752-65.

Thomas, D. G. (1973). "Forced convection mass transfer in hyperfiltration at high fluxes." Industrial & Engineering Chemistry Fundamentals **12**(4): 396-405.

Thompson, L. D., D. B. McElhinney, et al. (2001). "A prospective randomized study comparing volume-standardized modified and conventional ultrafiltration in pediatric cardiac surgery." Journal of Thoracic and Cardiovascular Surgery **122**(2): 220-228.

Thurston, G. B. (1972). "Viscoelasticity of human blood." Biophys J **12**(9): 1205-17.

Tiranuntakul, M. (2011). Evaluation of fouling in a pilot scale membrane bioreactor, James Cook University.

Tonz, M., T. Mihaljevic, et al. (1995). "Acute lung injury during cardiopulmonary bypass. Are the neutrophils responsible?" Chest **108**(6): 1551-6.

Torina, A. G., O. Petrucci, et al. (2010). "The effects of modified ultrafiltration on pulmonary function and transfusion requirements in patients underwent coronary artery bypass graft surgery." Rev Bras Cir Cardiovasc **25**(1): 59-65.

Tracey, E. M. and R. H. Davis (1994). "Protein fouling of track-etched polycarbonate microfiltration membranes." Journal of Colloid and Interface Science **167**(1): 104-116.

Tripathi (2011). Concise Textbook of Physiology for Dental Students, Elsevier (A Divisionof Reed Elsevier India Pvt. Limited).

Trujillo, A. P. and H. V. Thurman (2002). Essentials of Oceanography, Pearson College Division.

Tsubota, K., S. Wada, et al. (2006). "Particle method for computer simulation of red blood cell motion in blood flow." Comput Methods Programs Biomed **83**(2): 139-46.

Tsuchiya, K. and L.-S. Fan (1988). "Near-wake structure of a single gas bubble in a two-dimensional liquid-solid fluidized bed: vortex shedding and wake size variation." Chemical engineering science **43**(5): 1167-1181.

Turgeon, M. L. (2005). Clinical hematology: theory and procedures, Lippincott Williams & Wilkins.

UCDavis (2001). Clinical Diagnostic Laboratory CBC Reference Intervals. UCDavis Veterinary Medicine Veterinary Medical Teaching Hospital, Available at: http://www.vetmed.ucdavis.edu/vmth/local_resources/pdfs/lab_pdfs/UC_Davis_VMTH_Hematology_Reference_Intervals.pdf.

Ueda, T., K. Hata, et al. (1997). "Effects of aeration on suction pressure in a submerged membrane bioreactor." Water Research **31**(3): 489-494.

Ueyama, K., K. Nishimura, et al. (2004). "PMEA coating of pump circuit and oxygenator may attenuate the early systemic inflammatory response in cardiopulmonary bypass surgery." Asaio Journal **50**(4): 369-372.

Ugarova, T. P., A. Z. Budzynski, et al. (1993). "Conformational changes in fibrinogen elicited by its interaction with platelet membrane glycoprotein GPIIb-IIIa." Journal of Biological Chemistry **268**(28): 21080-21087.

Ungerleider, R. M. (2005). "Optimizing response of the neonate and infant to cardiopulmonary bypass." Cardiology in the young **15 Suppl 1**: 142-8.

Utoh, J. and H. Harasaki (1992). "Damage to erythrocytes from long-term heat stress." Clin Sci (Lond) **82**(1): 9-11.

Valette, P., M. Thomas, et al. (1999). "Adsorption of low molecular weight proteins to hemodialysis membranes: experimental results and simulations." Biomaterials **20**(17): 1621-1634.

van Eijndhoven, R. H., S. Saksena, et al. (1995). "Protein fractionation using electrostatic interactions in membranel filtration." Biotechnol Bioeng **48**(4): 406-14.

Van Oss, C. J. (2006). Interfacial forces in aqueous media, CRC press.

Vendruscolo, M. and C. M. Dobson (2007). "Chemical biology: More charges against aggregation." Nature **449**(7162): 555-555.

Vertrees, R. A., J. Auvil, et al. (1982). " A technique of hemoconcentration." Journal of Extracorporeal Technololgy **14**: 431-44.

Viswanath, D. S., T. Ghosh, et al. (2007). Viscosity of Liquids: Theory, Estimation, Experiment, and Data, Springer Netherlands.

Vitale, F., L. Turchetti, et al. (2011). "Modeling of Mechanical Blood Damage: a Discussion of Current Approaches and Alternative Proposals." CHEMICAL ENGINEERING **24**.

Vladkova, T. G. (2010). "Surface engineered polymeric biomaterials with improved biocontact properties." International Journal of polymer science **2010**.

Vroman, L. and A. L. Adams (1969). "Findings with the recording ellipsometer suggesting rapid exchange of specific plasma proteins at liquid/solid interfaces." Surface Science **16**: 438-446.

Vroman, L., A. L. Adams, et al. (1980). "Interaction of high molecular weight kininogen, factor XII, and fibrinogen in." Blood **55**(1).

Vroman, L., A. L. Adams, et al. (1971). Interactions among human blood proteins at interfaces. Federation proceedings.

Vrouwenvelder, J. S., J. A. M. Van Paassen, et al. (2009). "A critical flux to avoid biofouling of spiral wound nanofiltration and reverse osmosis membranes: fact or fiction?" Journal of Membrane Science **326**(1): 36-44.

Wahlgren, M. and U. Elofsson (1997). "Simple models for adsorption kinetics and their correlation to the adsorption of Î²-lactoglobulin A and B." Journal of colloid and interface science **188**(1): 121-129.

Wakeman, R. J. and C. J. Williams (2002). "Additional techniques to improve microfiltration." Separation and Purification Technology **26**(1): 3-18.

Walpoth, B., S. Geroulanos, et al. (1979). "Reduction of post bypass hemodilution by hemofiltration." Eur Soc Artif Organs **6**: 315-21.

Wang, L. K., J. P. Chen, et al. (2010). Membrane and desalination technologies, Springer.

Wang, M. J., I. S. Chiu, et al. (1996). "Efficacy of ultrafiltration in removing inflammatory mediators during pediatric cardiac operations." Annals of Thoracic Surgery **61**(2): 651-656.

Wang, P., K. L. Tan, et al. (2002). "Plasma-induced immobilization of poly (ethylene glycol) onto poly (vinylidene fluoride) microporous membrane." Journal of Membrane Science **195**(1): 103-114.

Wang, W., H. M. Huang, et al. (1998). "Modified ultrafiltration in paediatric cardiopulmonary bypass." Perfusion-Uk **13**(5): 304-310.

Waters, J. H., B. Williams, et al. (2007). "Modification of suction-induced hemolysis during cell salvage." Anesth Analg **104**(3): 684-7.

Wei, P., K. Zhang, et al. (2013). "CFD modeling of hydrodynamic characteristics of slug bubble flow in a flat sheet membrane bioreactor." Journal of Membrane Science **445**: 15-24.

Wells, R. and H. Schmid-Schonbein (1969). "Red cell deformation and fluidity of concentrated cell suspensions." J Appl Physiol **27**(2): 213-7.

Wenten, I. G. (1995). "Mechanisms and control of fouling in crossflow microfiltration." Filtration & separation **32**(3): 252-253.

Westaby, S. (1983). "Complement and the damaging effects of cardiopulmonary bypass." Thorax **38**(5): 321-325.

Wheeldon, D. R. and D. W. Bethune (1986). "Blood conservation during cardiopulmonary bypass - autologous transfusion, cell saving and haemofiltration." Cardiopulmonary bypass-principles and management. Baltimore: Williams & Wilkins: 289-311.

Wicaksana, F., A. G. Fane, et al. (2006). "Fibre movement induced by bubbling using submerged hollow fibre membranes." Journal of Membrane Science **271**(1): 186-195.

Williams, G. D., C. Ramamoorthy, et al. (1997). "Comparison of the effects of red cell separation and ultrafiltration on heparin concentration during pediatric cardiac surgery." J Cardiothorac Vasc Anesth **11**(7): 840-4.

Wilson, C. J., R. E. Clegg, et al. (2005). "Mediation of biomaterial-cell interactions by adsorbed proteins: a review." Tissue engineering **11**(1-2): 1-18.

Wintrobe, M. M. and J. P. Greer (2009). Wintrobe's Clinical Hematology, Wolters Kluwer Health/Lippincott Williams & Wilkins.

Winzeler, H. B. and G. Belfort (1993). "Enhanced performance for pressure-driven membrane processes: the argument for fluid instabilities." Journal of Membrane Science **80**(1): 35-47.

Wood, M. E. and G. K. Philips (2003). Hematology/oncology secrets, Elsevier Health Sciences.

Xu, L.-C. and C. A. Siedlecki (2007). "Effects of surface wettability and contact time on protein adhesion to biomaterial surfaces." Biomaterials **28**(22): 3273-3283.

Xu, Z.-K., X.-J. Huang, et al. (2009). Surface engineering of polymer membranes, Springer Science & Business Media.

Yamamoto, K.-i., M. Hiwatari, et al. (2005). "Membrane fouling and dialysate flow pattern in an internal filtration-enhancing dialyzer." Journal of Artificial Organs **8**(3): 198-205.

Ye, S. H., J. Watanabe, et al. (2003). "Antifouling blood purification membrane composed of cellulose acetate and phospholipid polymer." Biomaterials **24**(23): 4143-4152.

Yeleswarapu, K. K., J. F. Antaki, et al. (1995). "A mathematical model for shear-induced hemolysis." Artif Organs **19**(7): 576-82.

Yndgaard, S., L. W. Andersen, et al. (2000). "The effect of modified ultrafiltration on the amount of circulating endotoxins in children undergoing cardiopulmonary bypass." Journal of Cardiothoracic and Vascular Anesthesia **14**(4): 399-401.

Yokoyama, K., S. Takabayashi, et al. (2009). "Removal of prostaglandin E2 and increased intraoperative blood pressure during modified ultrafiltration in pediatric cardiac surgery." J Thorac Cardiovasc Surg **137**(3): 730-5.

Yong, A. S. C., G. J. Pennings, et al. (2011). "Intracoronary shear-related up-regulation of platelet P-selectin and platelet-monocyte aggregation despite the use of aspirin and clopidogrel." Blood **117**(1): 11-20.

Yuan, Y. and T. R. Lee (2013). Contact angle and wetting properties. Surface science techniques, Springer**:** 3-34.

Zeman, L. J. and A. L. Zydney (1996). Microfiltration and ultrafiltration: principles and applications, M. Dekker.

Ziegler, W. H. and C. A. Goresky (1971). "Transcapillary Exchange in Working Left Ventricle of Dog." Circulation Research **29**(2): 181-&.

Zurcher, S., D. Wackerlin, et al. (2006). "Biomimetic surface modifications based on the cyanobacterial iron chelator anachelin." Journal of the American Chemical Society **128**(4): 1064-1065.

Zydney, A. L. and C. K. Colton (1987). "Fundamental studies and design analysis for cross-flow membrane plasmapheresis." Artificial Organs: 343-358.

Zydney, A. L., W. M. Saltzman, et al. (1989). "Hydraulic resistance of red cell beds in an unstirred filtration cell." Chemical engineering science **44**(1): 147-159.

Chapter 11

Appendices

11. Appendices

Below is a scaled down copy of the poster presented at *The 2014 Asian Symposium on Healthcare Without Borders* conference held in Hiroshima, Japan 6th - 8th August 2014.

Development of an Appropriate Haemofiltration Technology for use in Paediatric Cardiopulmonary Bypass in Developing Countries

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Introduction

Cardiopulmonary bypass (CPB) is known to cause great changes in a patient's physiological fluid homeostasis which frequently results in accumulation of fluid in the interstitial space, more commonly known as edema (Hirleman 2008).

The specific circumstances explaining why CPB in paediatric patients so often leads to edema are also not completely determined, but it is thought to be the result of a combination of the inflammatory response and capillary leak brought about by exposure of the blood to the artificial surfaces of the CPB circuit (Miyaji 2008), the extended subjection to hypothermic conditions and increased haemodilution (Ungerleider 2005).

If the inflammatory response is allowed to reach a severe level it will result in systematic immune response syndrome, which can entail substantial edema along with serious morbidities such as acute respiratory distress syndrome and organ failure (Hirelman 2008).

Figure 1. Postoperative congenital heart disease infant patient https://oh.org.au/think-our-projects-cardiac/

Modified Ultrafiltration

Modified ultrafiltration (MUF) was first described by Naik, Knight and Elliott of The Hospital for Sick Children, Great Ormond Street, London in 1991 (Naik 1991) and is a technique which has been shown to successfully moderate inflammatory response and haemodilution in paediatric patients for whom edema is a more serious morbidity.

MUF involves removing excess blood plasma/water by postoperatively pumping blood through a haemofilter in a shunt position between the aorta and right atrium.

A number of academic papers (e.g. Grünenfelder 2000, Basiouni 2005) have reported that the benefits of MUF are not only from this fluid removal but also from the removal of inflammatory mediators

MUF also results in reduced requirements for blood transfusions which are expensive, risk transmission of infectious diseases and are unavailable in a timely manner to many living in developing countries.

A New Concept

Current haemofilters require use of a vacuum pump which could pose a clinical risk, are expensive to purchase and maintain, and not designed for use in developing countries.

The University of Strathclyde Bioengineering Department is developing a MUF device which moderates CPB induced inflammatory response and haemodilution but replaces the application of a vacuum with chemically driven extraction of plasma to improve safety and reduce complexity and cost.

Additionally removal of vacuum pump means the device is smaller and lighter than current systems which allows for more easy transportation thus improving access to the technique for those in remote locations and making the technique more suitable for use in disaster relief scenarios.

The device could potentially form part of an array of devices and technologies appropriate for emergency surgery in developing countries.

Device Development

The MUF device being developed uses passive chemical extraction of excess plasma to increase the volumetric cell fraction of the patients blood from post-bypass 'haemodiluted' levels (approximately 20% PCV) to normal pre-bypass levels (approximately 40% PCV) [Figure 2]. This can be achieved in a relatively short time [Figure 3] which, with further improvement, will enable the device to be used during the rewarming phase of CPB.

The extraction occurs across a 1um pore size control membrane to ensure the patient is relieved of the excess plasma whilst retaining erythrocytes, leukocytes and thrombocytes.

Figure 2. Pre-bypass whole blood, pre-MUF diluted blood & post-MUF haemoconcentrated blood respectively, showing the removal of excess plasma by the device.

It is important to return good quality haemoconcentrated blood to the patient. Microscopic inspection of the blood before and after dilution and re-concentration has shown minimal damage to erythrocytes (Figure 4), and additionally, platelet counts have demonstrated minimal loss of platelets.

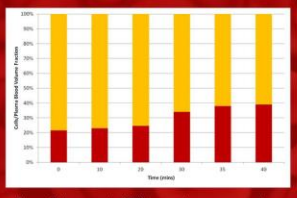

Figure 3. Increase in PCV% over time represented as a fraction of the whole blood volume. The fraction increases from typical post-bypass levels (~20%) back to normal pre-bypass levels (~40%).

The MUF device has been demonstrated to re-concentrate the blood at flow rates and pressures compatible with current CPB procedure standards and directly replaces current haemofilters in the CPB circuit. This means that no adaption or re-training of clinicians or surgeons is required.

The simplicity of the device's design in terms of set-up and operation, coupled with the intention for the device to be adjustable and self-limiting to meet individual patient needs, means that highly skilled staff are not required to manage or monitor the system, nor to adjust plasma extraction rates, whilst ensuring good quality, appropriately haemo-concentrated blood is returned to the patient.

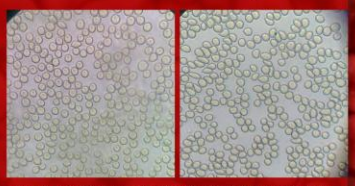

Figure 4. Red blood cells x100 magnification pre-MUF (left) and post-MUF (right). It can be seen that there is minimal damage to the cells meaning the patinet will receive good quality haemoconcentrated blood and the device should not lead to any haemolysis related complications.

References

Hirleman E. Larson DF. *'Cardiopulmonary bypass and edema: physiology and pathophysiology'*. Perfusion 2008 Nov;23(6):pp311-22
Ungerleider RM. *'Optimizing response of the neonate and infant to cardiopulmonary bypass'*. Cardiology in the Young 2005 Feb;15 Suppl 1:pp142-8
Naik SK, Knight A, Elliott MJ. *'A successful modification of ultrafiltration for cardiopulmonary bypass in children'*. Perfusion 1991;6(1):pp41-50
Grünenfelder J, Zünd G, Schoeberlein A, Maly FE, Schurr U, Guntli S, Katja Fischer K & Turina M. *'Modified ultrafiltration lowers adhesion molecule and cytokine levels after cardiopulmonary bypass without clinical relevance in adults'* Eur J of Cardiothorac. 2000;17 pp77-83
Alaa-Basiouni S, Mahmoud, Mohamed S, Burhani, Ali A, Hannet, Ahmad A, Jamjoom, Iskander S, Al-Githmi, Ghassan M, Baslaim, *Effect of Modified Ultrafiltration on Pulmonary Function After Cardiopulmonary Bypass* Chest. 2005; 128(5):3447-3453

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