BIOCOMPATIBLE CIRCUITS:

INFLAMMATION AND SOLUBLE

ADHESION MOLECULES AFTER

CARDIOPULMONARY BYPASS

Thesis Submitted to

The College of Graduate Studies

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

College of Medicine

University of Saskatchewan

Saskatoon, Saskatchewan

Jo-Anne E. Marcoux

PERMISSION TO USE

The libraries of the University of Saskatchewan may make this thesis available for inspection and may be copied (in whole or in part) with permission from supervisors, Dr Qing Meng and Dr John Krahn. In their absence, permission can be obtained from the Dean of the College of Medicine. Copying, publication or use of this thesis for financial gain without written permission shall not be allowed. Should this thesis be used for scholarly use, due recognition is to be made to the University of Saskatchewan and me.

All requests for use of this thesis (in whole or in part) should be addressed to:

Dean's office

College of Medicine

University of Saskatchewan

Saskatoon, Saskatchewan

Canada

S7N 5E5

ABSTRACT

In the modern era, the most common post-operative complications following cardiopulmonary bypass (CPB) are neurocognitive deficits (NCD) and atrial fibrillation (AF). Both morbidities have been linked to inflammation resulting from surgery, anesthesia and CPB. Microemboli, inadequate oxygen delivery and the inflammatory response consequent to blood contacting artificial components of the CPB circuit have all been linked to postoperative NCD and to a lesser extent post-operative AF.

The artificial components of the CPB circuit consist of stainless steel, polyvinylchloride (PVC), polycarbonate and other carbon-based plastics. In order to attenuate the negative sequelae of blood-circuit contact related inflammatory response, industry developed the biocompatible circuit (BCC) coating for the disposable CPB circuits. Four such coatings were studied and compared to an uncoated control group in a total of 101 patients undergoing routine CPB-assisted cardiac surgical procedures. Soluble adhesion molecule (SAM) activation was studied at different time points and common clinical outcomes such as white blood cell activation, serum renal function parameters urea and creatinine, postoperative bleeding, transfusion requirements, intensive care and hospital length of stay, CPB pump volume balances, changes in weight, postoperative serum lactate and glucose and the development of AF postoperatively, were compared. Additionally, postoperative neurocognitive testing was performed using a simple bedside neurocognitive test called the antisaccadic eye movement test. The patients in all groups were tested for comparison preoperatively and 72 hr postoperatively.

Results: The mandate of BCC coating development and manufacture is to attenuate the well-documented and demonstrated inflammatory response consequent to the contact of blood with artificial CPB surfaces. The studied BCCs significantly decreased platelet transfusions in females. In addition, the BCCs decreased the concentrations of 2 SAMs when measured 6 hours after surgery and CPB. The difference in SAM expression seen between the coated and uncoated

groups at 6 hr was no longer apparent at 72 hr. Very little difference was noted between the four BCC groups.

Patients who developed AF postoperatively seemed predisposed to do so as the serum levels of soluble vascular cell adhesion molecule was significantly higher at baseline and remained so at 6 and 72 hr.

The decreased incidence of platelet transfusions in females, resulting from BCC use, is a highly significant finding within this higher-risk group of patients. As most platelet transfusions occur soon after the patient is disconnected from CPB, the short-term decrease in SAM activation can be linked to this improved clinical finding.

The studied BCC coatings have achieved limited success in their intended mandate to attenuate inflammatory response in terms of improved clinical and laboratory desired outcomes.

ACKNOWLEDGEMENTS

As a relatively recent resident of Saskatoon I would like to thank the people of this lively, vibrant city. In the ten years that I have resided here, I have always felt that I was welcome and warmly included into the folds of the community. This warm welcome has been most strongly felt when surrounded by my colleagues in the operating rooms of the Royal University Hospital. A warm thank you is extended to the perfusionists; Mark Rosin, Glenn Smith, Erick McNair and Victor Uppal. Without your help this study and this thesis would not have been possible. Many thanks go to the cardiac surgeons Dr. Greg Dalshaug, Dr. Taras Mycyk and Dr. Dorothy Thomson. Thank you for allowing your patients to be part of my study and entrusting your patients into my care. Thank you to the nurses of theatre nine. You are all angels and your assistance was tireless. Thank you to the anesthetic assistants. Procuring patient samples was so much more efficient when you were around.

There are many who deserve to be acknowledged outside the operating room. Thank you to Laura Wiwchar at Medical Records. Your assistance and dedication towards all involved in research is invaluable. To the chemistry and hematology technicians for the many additional hr of work spent analyzing my samples thank you. To Heather Neufeld, whose worth is more than her weight in gold, I cannot imagine how I could have learned to run the enzyme immunoassays and accompanying software without her. For the many thankless ways she helped me out, I am truly grateful.

Sincere appreciations go to Dr. John Krahn and Dr. Qing Meng. I know I only tapped a small source of your vast knowledge and experience. You were invaluable resources and despite your busy schedules always seemed to have time for my project. To all the members of my research advisory committee Dr. Paul Lee, Dr. Taras Mycyk and chair Dr. Barry Ziola, thank you for all your suggestions and assistance. A special mention must go to Dr. Taras Mycyk whose excellent editorial skills, advice, humor and sense of fun were always well timed and greatly appreciated.

My sincere appreciation goes to Angie Zoerb. You saved the day more than once and I am very grateful.

Thank you to Tracey Carr. I cannot express how appreciative I was for the day you offered your statistical expertise. You took the time out of your own very busy schedule on too many occasions to count, even coming to my rescue from Zagreb, Croatia. Thank you!

Many thanks to the RUH Foundation for their research grant and the medical companies whose financial assistance and eagerness to participate demonstrates a trust and confidence in their product and enthusiasm for evidence based medicine.

To my parents, my sister and her family; your love, support and encouragement could always be felt a province away. I love you all and wish we all lived in the same city! Lastly, my final words of thanks go to my partner Carolee. Thank you for listening, thank you for your suggestions, thank you for all that you bring to our world. You are my rainbow at the end of each day.

TABLE OF CONTENTS

PERMISSION TO USE ..... **i**

ABSTRACT ..... **ii**

ACKNOWLEDGEMENTS ..... **iv**

TABLE OF CONTENTS ..... **vi**

LIST OF TABLES ..... **x**

LIST OF ABBREVIATIONS ..... **xi**

1 LITERATURE REVIEW **1**

1.1 CPB Circuit 1

1.2 CPB and Inflammatory Response 3

1.2.1 Cellular Inflammatory Components 6

1.2.1.1 Erythrocytes 6

1.2.1.2 Neutrophils/Leukocytes 7

1.2.1.3 Neutrophils and Reperfusion Injury 8

1.2.1.4 Platelets 9

1.2.1.5 Endothelial Cells 11

1.2.1.6 SAMs 11

1.2.1.6.1 sVCAM-1 12

1.2.1.6.2 sICAM-1 13

1.2.1.6.3 sE-selectin 14

1.2.1.6.4 sL-selectin 14

1.2.2 Humoral Inflammatory Components 15

1.2.2.1 Complement 15

1.2.2.2 CPB and Coagulation 16

1.3 CPB and Organ Dysfunction 18

1.3.1 CPB and Renal Function....18
1.3.2 CPB and the Brain....19
1.4 CPB and Diabetes....20
1.5 CPB and Gender....21
1.6 BCC....21
1.6.1 Phosphorylcholine....23
1.6.2 Bioline™....23
1.6.3 PMEA....24
1.6.4 Trillium™....24
2 OBJECTIVES....26
2.1 Comparison of Clinical and Laboratory Outcomes Between Diabetics Treated with an Uncoated Circuit or a BCC....26
2.2 Comparison of BCC Effect on Females....26
2.3 Comparison of BCCs on the Release of SAMs....26
2.4 Comparison of BCC Effect in Attenuating Renal Dysfunction and Consequent Volume Retention....26
2.5 Comparison of BCC Effect on Bleeding and Transfusions....26
2.6 Comparison of BCC Effect on AF....26
2.7 Comparison of BCC Effect on Neurocognitive Outcome, and ICU and Hospital Length of Stay....27
3 MATERIALS AND METHODS....28
3.1. Materials....28
3.1.1 Patient Population....28
3.1.2 Exclusion Criteria....28
3.1.3 Anesthesia....28
3.1.4 CPB and Surgery....29
3.1.5 BCC....29
3.1.5.1 Control....29

3.1.5.2 Phosphorylcholine 30
3.1.5.3 Bioline™ 30
3.1.5.4 PMEA 30
3.1.5.5 Trillium™ 30
3.1.6 Sample Collection 30
3.1.7 Demographics and Laboratory Data Acquisition 30
3.2. Methods 31
3.2.1 Biochemical Analyses 31
3.2.1.1 Enzyme-Linked Immunosorbent Assay (EIA) 31
3.2.1.2 Serum Glucose, Lactate, Urea and Creatinine 31
3.2.2 Statistical Analysis 31
4 RESULTS AND DISCUSSION 33
4.1 Results, Patient Characteristics/Demographics 33
4.1.1 Diabetics 33
4.1.2 Sex-Based Variations 35
4.2 CPB Data and Postoperative Resource Utilization Categorized by Group 36
4.3 CPB and SAM 38
4.4 Mean Serum SAM Levels in all Groups 39
4.4.1 sVCAM-1 Expression 39
4.4.2 sICAM-1 Expression 39
4.4.3 sE-selectin Expression 40
4.4.4 sL-selectin Expression 40
4.5 Effect of BCC on Common Clinical Parameters 41
4.5.1 Changes from Baseline to 72 hr in Serum Creatinine, Urea, Lactate and Glucose 41
4.5.2 Bleeding and Transfusion Requirements 42
4.5.3 Effect of BCC on AF, Changes in Weight, Pump Balances and WBC Counts 43

4.5.4 Neurocognitive Outcomes and Intensive Care and Hospital Length of Stay .44
4.6 Discussion 45
4.6.1 Comparison of BCC Effect on Diabetic Patients.....45
4.6.2 Comparison of BCC Effect on Females 46
4.6.3 SAM Expression due to CPB.....47
4.6.4 Comparison BCC Effect on Release of Soluble Adhesion Molecules 48
4.6.5 Comparison of BCC Effect on Renal Function and Volume Retention 48
4.6.6 Comparison of BCC Effect on Bleeding and Transfusion Requirements 49
4.6.7 Comparison of BCC Effect on Preventing AF 49
4.6.8 Comparison of BCC Effect on NCD 50
4.6.9 Comparison of BCC Effect on ICU and Hospital Length of Stay.....50
5 LIMITATIONS OF THIS STUDY 51
6 CONCLUSION 52
7 REFERENCES.....53

LIST OF TABLES

Table 4.1 Study patient demographics and baseline preoperative conditions 34

Table 4.2 Study outcome differences between diabetics and non-diabetics..... 35

Table 4.3 Significant diabetic outcome differences between treatment groups 35

Table 4.4 Study outcome differences between genders..... 36

Table 4.5 CPB data and postoperative resource utilization categorized by group 37

Table 4.6 Serum SAM values in ng/mL 38

Table 4.7 sVCAM-1 expression in patients with postoperative AF 38

Table 4.8 Serum sVCAM-1 concentrations..... 39

Table 4.9 Serum sICAM-1 concentrations 40

Table 4.10 Serum sE-selectin concentrations 40

Table 4.11 Serum sL-selectin concentrations 41

Table 4.12 Effect of BCC on chemistry and renal function parameters..... 42

Table 4.13 Bleeding and transfusion requirements 43

Table 4.14 Patient changes in WBC count, weight, pump balance and incidence of AF 44

Table 4.15 Patient neurocognitive outcomes, ventilator times, and ICU and hospital length of stay 45

LIST OF ABBREVIATIONS

	Full name
AA	**Amino acids**
ACT	**Activated clotting time**
AF	**Atrial fibrillation**
ANOVA	**Analysis of variance**
ASEMT	**Antisaccadic eye mouvement test**
BCC	**Biocompatible circuit**
BMI	**Body mass index**
C	**Complement**
CABG	**Coronary artery bypass grafting**
CD	**Cluster differentiation**
CHD	**Coronary heart disease**
CPB	**Cardiopulmonary bypass**
CRP	**C-reactive protein**
CTD	**Chest tube drainage**
ECC	**Extra corporeal circulation**
EIA	**Enzyme immunoassay**
HLM	**Heart-lung machine**
Ig	**Immunoglobulin**
IgSF	**Immunoglobulin super family**

ICU	**Intensive care unit**
IL	**Interleukin**
LT	**Leukotriene**
NCD	**Neurocognitive deficits**
PMEA	**Polymethoxyethyl acrylate**
PMN	**Polymorphonuclear leukocyte**
PVC	**Polyvinyl chloride**
RBC	**Red blood cell**
ROS	**Reactive oxygen species**
SAM	**Soluble adhesion molecule**
SD	**Standard deviation**
sE-selectin	**Soluble endothelial selectin**
sICAM-1	**Soluble intercellular adhesion molecule 1**
sL-selectin	**Soluble leukocyte selectin**
sP-selectin	**Soluble platelet selectin**
sVCAM-1	**Soluble vascular cell adhesion molecule 1**
TA	**Tranexamic acid**
TNF	**Tissue necrosis factor**
UFFP	**Units of fresh frozen plasma transfused**
UPlatelets	**Units of platelets transfused**
URBC	**Units of red blood cells transfused**

vWF	**von Willibrand's factor**
WBC	**White blood cell**

1 LITERATURE REVIEW

Many significant improvements have been achieved since the first successful combined clinical application of cardiopulmonary bypass (CPB) and cardiac surgery in 1953 (Gibbon, 1954). Major technical, surgical and perfusion innovations have contributed to excellent current outcomes compared to the high incidence of morbidity and mortality incurred when the two interventions were in their infancy.

CPB was initially developed to facilitate correction of congenital cardiac anomalies by providing a still, bloodless operative field (Gravlee *et al*. 2008). Surgical correction on a beating, fibrillating or inflow occluded heart was technically challenging in terms of time limitation, surgical exposure and surgical accuracy. Arresting the heart, while facilitating surgery, warrants adjunctive circulatory assist devices to provide for the metabolic demands of the body, thereby preventing ischemic injury (Gravlee *et al*. 2008). Extra corporeal circulation (ECC) in the form of CPB, powered by a heart-lung machine (HLM), was developed to maintain the systemic circulation long enough to allow for a controlled surgical correction. Chemical cardioplegic solutions, delivered by a secondary pump on the HLM, were later developed to arrest the heart while providing for its metabolic needs. Clinical experiments demonstrated enhanced cardiac muscle preservation during CPB when the heart was kept both, hypothermic and arrested (Gravlee *et al*. 2008). Decreased lactic acid formation meant the heart could easily resume regular contractions when needed. As CPB technology developed further and patient outcomes improved, usage of CPB expanded to include coronary artery bypass grafting (CABG), valve repairs, valve replacements and complex aortic reconstructions (Gravlee *et al*. 2008).

1.1 CPB Circuit

Definitive repair of congenital intra-cardiac malformations before 1953 was impossible. Development of the HLM is regarded as one of the most important medical inventions of the twentieth century (Gravlee *et al*. 2008). Surgical cardiac correction of congenital and acquired cardiac pathology once thought to be unattainable is now undertaken 2,000 times worldwide every 24 hr (Gravlee *et al*. 2008). Aptly named, the HLM takes over the function of the heart and the lungs while these organs are temporarily isolated from the systemic circulation. During this time, the HLM supports all cardiopulmonary supported metabolic demands of the body.

The first successful attempt at cardiac surgery was the closure of an atrial septal defect in a 5-year old girl. This was done under direct visualization using inflow occlusion and total body hypothermia (Lewis, 1953). This technique worked very well for simple repairs involving the atrium and pulmonic or aortic stenosis, but reinforced the need for a more comprehensive perfusion machine for more complex lesions (Swan *et al.* 1953).

The first few attempts at using a HLM resulted in death (Gibbon, 1968). Death was due to an erroneous preoperative diagnosis in one instance and a massive air embolism in the other. The

perception at the time was that high systemic flows were needed to support the patient. This belief and the crude technology employed to oxygenate the blood lead to the fatal massive air embolus.

Re-examination of previous animal studies lead to a reassessment of how much systemic flow was necessary to maintain basal metabolic demands (Hemmingway, 1913; Patterson, 1914). This new way of thinking brought hope to many working in the cardiac surgical field, as not only did the lower flows add an element of safety to the procedure, lower flows also meant there was less blood obstructing the surgical field (Helmsworth *et al.* 1953; Gibbon *et al.* 1954). Unfortunately, the necessary cannulation needed to connect the patient's circulation to the HLM got in the way of the walnut sized pediatric hearts they were working on. This problem was alleviated by cross-circulation technique, which permitted the cannulas to be placed out of the operative field (Cohen *et al.* 1953). The successful use of the cross-circulation technique used in dogs (whereby one dog's circulation supported the other dog having the surgical correction) lead to the first of many successful procedures on children using the child's parent, effectively, as the pump (Lillehei *et al.* 1955).

In 1955, DeWall and Lellihei began using a disposal bubble oxygenator, which they had developed in their laboratory. As their successes mounted they began adapting the bubble oxygenator to meet the greater physiological demands of adult patients. The bubble oxygenator was eventually replaced by the safer and less disruptive microporous hollow fiber membrane oxygenator (Haworth, 2003).

A further technical advance arrived with the proof that with induced hypothermia, hemodilution was permissible and in fact advantageous (Zuhdi *et al.* 1961). Not only did this effectively decrease the amount of blood needed for surgery (and its associated risks of immunologic sequelae and transmitted infection) but it also improved the quality of perfusion by decreasing the viscosity of blood (Zuhdi *et al.* 1961). When the patient's circulatory volume is joined together with the CPB circuit, the patient's blood volume is diluted with the crystalloid/colloid volume necessary to prime all the components and tubing. The initiation of CPB inevitably results in hemodilution of all cellular and non-cellular elements of the patient's blood.

During routine CPB-assisted cardiac surgical procedures, blood leaves the body from the right atrium through the venous cannula. Blood flows within the tubing, assisted by gravity drainage to a venous reservoir. Blood is then entrained from the venous reservoir and propelled by a mechanical roller pump (or a centrifugal pump) to the heat exchanger/oxygenator, arterial filter and lastly to the arterial cannula inserted into the ascending aorta. Artificial CPB circuit components are made up of various compounds which include polyvinyl chloride (PVC), polyester, silicon, stainless steel, polycarbonate, nylon, Anti-foam A© (used as a defoamer for blood) and di-(2-ethylhexyl)phthalate, a plasticizer used to give flexibility to PVC tubing.

Under normal physiological circumstances, intravascular blood cells and proteins are only in contact with the endothelial lining of the vasculature. Upon immediate contact with the artificial surface of the disposable CPB circuit, a systemic inflammatory reaction of varying intensity dependent upon the patient and the CPB circuit occurs. Hemostasis, cellular metabolism and virtually all organ functions are affected (Day *et al.* 2005; De Mendonça-Filho, 2006; Braun *et al.* 2007; Vercaemst, 2008; Gravlee *et al.* 2008).

1.2 CPB and Inflammatory Response

The inflammatory/immune response generally protects organ systems against a host of noxious stimuli. Surgical wounds up-regulate the inflammatory response in order to promote necessary healing and recovery. However, the inflammatory response to CPB compounds the natural response from surgery. It is this excessive response, which can be harmful (Rubens *el al.* 2004; Suleiman *et al.* 2008; Gravlee, 2008). It has been demonstrated that removing the stimulus from CPB (off-pump cardiac surgery, *i.e.,* without CPB) results in a reduced inflammatory response in terms of significantly decreased cytokine levels with an associated decrease in the stress response (Suleiman *et al.* 2008). The majority of the stress response however is related to surgical trauma (Suleiman *et al.* 2008). Meta-analysis has yielded conflicting results. Raja and Dreyfus (2006) demonstrated decreased postoperative bleeding in patients undergoing off-pump CABG surgery. Parolari *et al.* (2009) and Seabra *et al.* (2010) suggest that when comparing short-term clinical outcomes between the two modalities, no statistical difference exists. Indeed, a meta-analysis reviewing long term results by Takagi *et al.* (2010) showed that incomplete coronary revascularization, which can result from CABG surgery off-pump, resulted in an increased risk of late mortality (more than one year after surgery). The solution then may be to continue to use CPB for most cardiac surgeries, but to continue to find ways to decrease the inflammatory response to the artificial environment introduced by the described CPB circuits.

Two physiological protective immune response systems have been identified: innate and acquired. The innate system (nonspecific immunity) is made up of a host of phagocytic and endothelial cells (Mayer, 2007). Phagocytic cells function to engulf and ingest pathogens and cellular debris. Phagocytic cells can be blood-borne (monocytes and neutrophils) or in tissue (dendritic cells and mast cells). Neutrophils will be discussed in section **1.2.1.2.** Monocytes in circulation are attracted to sites of inflammation through a process called chemotaxis (Bosco *et al.* 2008). Triggers of chemotaxis include damaged cells, pathogens and cytokines. Monocytes arriving at sites where inflammation is occuring, can differentiate and extravasate into tissue to become exudate-macrophages, exudate-resident macrophages and peroxidase-negative macrophages (Naito, 1993). These monocyte-derived macrophages are short-lived and non-proliferating (Naito, 1993) whereas tissue, or resident macrophages can survive up to several months and can proliferate and self-regulate as needed. Macrophage characteristics depend upon the environment where they are found (Naito, 1993) and macrophage colony stimulating factors exist to activate genes important for macrophage differentiation (Naito, 1993). Resident

macrophages are named according to their location. Examples are dust cells, kupffer cells, osteoclasts and microglia found in the lung, liver, bone, and neural tissue, respectively (Liles and Van Voorhis, 1995). Monocytes can differentiate into dentritic cells found in external tissues such as the skin and the nose. Along with dendritic cells (Svensson *et al.* 1997), macrophages play a crucial role in initiating immune response. Their presentation of bits of antigen to other phagocytic cells such as helper T cells, results in the production of antibodies. (Bosco *et al.* 862008). The antibodies quickly recognize the antigen upon second introduction allowing for easier engulfment by macrophages (Naito, 1993). Monocytes and macrophages produce monokines (a type of cytokine), following cell activation or damage. Examples of these are IL-1, IL-6, IL-8, IL-10, tissue necrosis factor (TNF) and interferons. All are important in regulating the immune response and inflammation (Wan *et al.* 1997). Natural killer cells are lymphocytes designed to destroy viruses and tumors by the granular proteins they release which cause apoptosis (Mayer, 2007). Innate immunity does not require prior exposure to be sensitized and activated. Soluble factors cytokines, complement, chemokines and acute phase proteins are always in circulation, prepared to protect their host when needed (Wan *et al.* 1997). The combination of surgical trauma, CPB and reperfusion injury of the heart and lungs following CPB results in the up-regulation and release of pro-inflammatory cytokines and stress-related hormones. These contribute to postoperative organ dysfunction by activating endothelial cell-leukocyte adhesion and by promoting vascular inflammation (Franke *et al.* 2005). Stefano and Bilfinger, (1993) examining the effects of CPB using computer-assisted microscopic imaging measured a 48% increase in granulocyte and macrophage activation characterized by conformational changes and locomotion from pre CPB baseline cells. This group was able to conclude that though these cells exhibited heightened activation, they were later unable to respond to chemotactic agents post CPB. This is evidence leukocyte antibody production is impaired temporarily post CPB.

Acquired immunity has memory based upon prior sensitization. Once the body generates an immune response to a specific antigen, acquired immunity becomes more efficient at destroying the known antigen upon next encounter. Vaccinations work in this manner. Antibodies and T and B-lymphocytes are the principle components of acquired immunity (Mayer, 2007). CPB-generated inflammatory response, not being antigen-specific, cannot be attenuated or enhanced by repeated encounters. The ultimate goal remains to manufacture an immunologically inert surface for all blood contacting components of the CPB circuit. Such is the goal of biocompatible circuit (BCC) technology.

BCCs were developed to minimize the inflammatory response occurring when blood comes into contact with the artificial surface. CPB is overwhelmingly noxious and analogous to turning the body inside out. The entire circulatory system is suddenly and intensely exposed to the unnatural environment and milieu of the CPB circuit. Negative manifestations of the inflammatory response to CPB can involve all organ systems (Day *et al.* 2005; De Mendonça-Filho, 2006; Braun *et al.* 2007; Vercaemst, 2008) and although the intensity of individual response cannot be predicted, the magnitude of risk increases the longer a patient is supported by CPB (Kansy *et al.* 2010). Additional variable risk factors associated with CPB include

preoperative patient conditions (co-morbidities) and the amount of shed blood and microemboli returned to the circulation. Additionally, individual responses to heparin anticoagulation and protamine reversal vary. Results of logistic regression by Ferraris *et al.* 1996 linked the preoperative co-morbid state with postoperative morbidities due to the exacerbated inflammatory response from CPB (Ferraris *et al.* 1996).

Design of the CPB circuit plays a role in the intensity of the inflammatory response. Well-designed circuits should not include any abrupt changes in diameter, direction or pressure as these can result in high shear stress and additional platelet activation and adhesion (Spijker *et al.* 2003). CPB circuits should be as short as possible to minimize the blood/artificial surface interaction. Ideally the surface in contact with blood should be as non-reactive as the vascular endothelial lining. The components and tubing of the CPB circuit are PVC and other plastic composites. As these are reactive and not biological surfaces, BCCs have been developed to alleviate this. Certain developed BCC are heparin-based, which present a less thombogenic surface, while others incorporate natural endothelial proteins or inert molecules within their matrix in order to appear less foreign to circulating blood.

Postoperative cerebral dysfunction was noted early on at the advent of CPB-assisted cardiac surgery and wide-ranging research has been conducted to understand and prevent this unfortunate occurrence (Hodges *et al.* 1958). It was noted that blood in contact with air (a consequence of the bubble oxygenators) resulted in air emboli formation, hemolysis and subsequent exacerbation of the inflammatory response to CPB (Hodges *et al.* 1958). The air/blood interface was greatly reduced with the advent of membrane oxygenators (Gravlee *et al.* 2008). Additionally, air/blood interface can be minimized by decreasing the speed of the roller pump controlling the vent and cardiotomy suction thus preventing excessive amounts of air from being drawn in with the blood. Use of pump suction for scavenging shed blood should be discouraged. Excessive negative pressures (Hirose *et al.* 1964; Wielogorske *et al.* 1975; Gregoretti, 1996) and co-aspiration of air (Wright and Sanderson, 1979; de Jong *et al.* 1980; Gregoretti, 1996) attributed to cardiotomy suction contribute to hemolysis. The large raw surface area of the cardiac surgical wound, a primary site of thrombin generation, contains inflammatory cytokines, high concentrations of complement (C) 5a (a product of activated complement) and emulsified fat (Brooker *et al.* 1998; Wendel *et al.* 1999). Blood lost from the surgical wound often collects in the pericardial well and its contact with pericardium also contributes to hemolysis (Pierangeli *et al.* 2001). Consequently, blood which collects in the pleura should not be aspirated and returned to the CPB circuit but rather sent to an autologous transfusion device for processing before return to the patient. Alternatively, in times when bleeding is insignificant, this small amount of shed blood can be discarded. Universal practice of removing shed blood from the surgical field, where possible, reduces the inflammatory response by preventing thrombin, RBC castes and fat emboli from returning to the systemic circulation (Kincaid *et al.* 2000; Pierangeli *et al.* 2001; Aldea *et al.* 2002). In a study by Skrabal *et al.* (2006), patients who had pericardial suction blood returned to the systemic circulation had significantly higher levels of C-reactive protein (CRP), PMN-Elastase, Interleukin (IL) -6, plasma free hemoglobin and lactate dehydrogenase. De Somer *et al.* (2002) found that aspirated wound blood contaminated

by tissue contact showed a significant increase in plasma free hemoglobin and platelet activation resulting in a 30% increase in postoperative blood loss. Edmunds *et al.* (1978) and de Jong *et al.* (1980) found a correlation between the amount of cardiotomy suction used and the fall in circulating platelets.

One of the most common postoperative complications of CPB is atrial fibrillation (AF). The direct link between AF, inflammation and hemodilution was first proposed upon realization that there was an increased incidence of AF two to three days post CPB (Bruins *et al.* 1997). This observed increased incidence of AF was associated with peak serum levels of CRP (Bruins *et al.* 1997). Atrial biopsies showed inflammatory infiltrates suggesting oxidative damage and myocarditis (Frustaci *et al.* 1997; Mihm *et al.* 2001). Meta-analyses by Reston *et al.* (2003) and Athanasiou *et al.* (2003) comparing patients who underwent coronary revascularization surgery either with CPB or without (*i.e.,* on-pump or off-pump), showed statistically significant reduction in AF among patients having undergone off-pump surgery. Meta-analyses by Ranucci *et al.* (2009) reviewing BCCs involving 36 randomized controlled trials and 4360 patients showed a decreased incidence of AF when BCC circuits were used.

In summary, there are several logical strategies to decrease the inflammatory response to the commonly used uncoated CPB circuit; minimal use of cardiotomy suction and vent suction; intelligent circuit design (whereby the blood flow path through the CPB circuit is as gentle to cellular components as possible) and finally BCCs. Medicinal anti-inflammatory prophylaxis techniques exist which include preoperative steroids, antifibrinolytics and non-steroidal anti-inflammatory drug administration, to name a few. These prophylactic therapeutic strategies are beyond the scope of this paper.

1.2.1 Cellular Inflammatory Components

1.2.1.1 Erythrocytes

The erythrocyte or red blood cell (RBC) (diameter 7 μm; number $5x10^6/\mu l$), are supple and flexible, easily slipping through the smallest capillaries. Negative pressures generated from the vent and pump sucker, positive pressures generated by the roller pump and pressure excursions from blood flowing through the oxygenator and arterial filter exposes RBCs to considerable shear forces (Hirayama *et al.* 1985). These shear forces which result in mechanical or ionic pump damage, diminish the inherent deformability of the RBC. Hemolysis can result (Schmid-Schönbein *et al.* 1979). Once the RBC membrane is altered, it is susceptible to attack by activated complement (Westaby, 1983). Adenosine diphosphate stores in the RBC are released into the plasma during cell lysis and may inadvertently alter platelet function resulting in decreased ability of the platelet to participate in hemostasis (Vercaemst, 2008). Tissue function may be affected when hemoglobin is no longer encased within the RBC membrane due to increases in plasma oncotic pressure and viscosity (Vercaemst, 2008). Accumulated RBC

membrane fragments (lipid ghosts) clog microcirculation and are important contributors of organ dysfunction (Yedgar *et al.* 2002; Vercaemst, 2008).

Severe hemolysis results in a hypercoagulable state (Egeberg, 1962). In a study by van Beers *et al.* (2009), RBC-derived microparticles, correlating with markers of hemolysis, also strongly correlated with plasma markers of fibrinolysis (D-dimer) and coagulation activation (prothrombin fragment 1+2). Increased levels of these coagulation activation parameters and plasminogen activator inhibitor during CPB correlate with postoperative organ dysfunction demonstrated as decreased left-ventricular stroke work index, decreased PaO_2/FIO_2 ratio and increased serum creatinine levels (Dixon *et al.* 2005).

1.2.1.2 Neutrophils/Leukocytes

Leukocyte recruitment and activation within minutes of a noxious stimulus, coupled with their inherent cytotoxic capabilities, are central to an organism's ability to fight off infection. Neutrophils are attracted to a site of infection by cytokines. These are expressed by activated endothelium, mast cells and macrophages. Neutrophils also express their own cytokines, which in turn recruit and amplify the reaction of other inflammatory cells (Mayer, 2007). Pus mostly made up of neutrophils bodies, is the evidence of death and destruction from the battles waged by neutrophils having migrated into the tissues to engulf invading pathogens.

Neutrophils (12-15 μm) along with basophils and eosonophils, are frequently referred to as polymorphonuclear leukocytes, due to their multilobed nuclei. Neutrophils are generally the first of the inflammatory cells to arrive at a site of injury or infection (Cohen, 2002). They are efficient phagocytes of pathogens such as bacteria and fungi when coated with antibodies or complement. Their up-regulation by activated endothelium and sL-selectin is the main cause of systemic inflammatory response after CBP (Gravlee *et al*. 2008). Neutrophils, primarily stimulated by IL-8, interferon gamma and C5a in a process called chemotaxis, can also be activated by heparin, histamine, thromboxane A2, IL-1β, TNF and C5b-9 (Menasche *et al.* 2003; Warren *et al.* 2007). Neutrophil activation and adhesion can result in capillary blockage with localized ischemia (Anderson *et al.* 1987). Neutrophils once activated, can release their cytotoxic granular contents into the endothelium where direct cellular damage can result (Haga *et al.* 1993). Neutrophil activation during CPB is evident by the loss of L-selectin, upregulation of complement receptor CD11b/CD18 (also known as macrophage-1 antigen), increased production of reactive oxygen species (ROS) and increased levels of neutrophil granular contents such as elastase (Haga *et al.* 1993; Gadaleta *et al.* 1994).

Three types of neutrophil granules exist (Faurschou *et al.* 2003). Azurophil, or primary granules, function primarily within the cell and contain various proteases such as elastase, cathepsin G and proteinase (Faurschou *et al.* 2003). Azurophils, contain antimicrobial agents such as myeloperoxidase, lysozymes, defensins and bactericidal permeability-increasing protein (Faurschou *et al.* 2003). The enzyme myeloperoxidase converts hydrogen peroxide into

hypochlorous acid a potent cytotoxic agent. Azurophils also contain acid hydrolases cathepsin B, cathepsin D, β-D-glucuronidase, α-mannosidase and phospholipase A_2.

Specific or secondary granules contain cell surface molecule receptors for C3b and chemoattractants. Specific granules initiate inflammation and contain soluble mediators C5 component and macrophage chemotactic agents. Tertiary granules carry lysosomes as do primary and secondary granules. Lysosomes carry enzymes needed to digest all used up organic matter from phagocytosis, endocytosis and autophagy (Faurschou *et al.* 2003).

Ongoing efforts to study and categorize the inflammatory response during CPB continue. Many researchers continue to compare on and off pump results in an effort to isolate the immune response to CPB alone. Work by Lin *et al.* on heat-shock protein 70kDa showed increased inflammatory response within leukocytes of patients undergoing CPB-assisted CABG compared to off-pump CABG (Lin *et al.* 2010).

Work by Despotis *et al.* (1997) has shown positive correlation between increasing white blood cell (WBC) counts during CPB and patients most likely to bleed postoperatively. These increased numbers of WBC's may be actively participating in fibrinolysis and clot instability by degrading fibrin with mediators elastase and cathepsin G. Alternatively, these increased WBC numbers may be simply signaling an exaggerated inflammatory response (Kolev *et al.* 2003). WBC counts were followed in the present study to determine if any of the BCC would be capable of attenuating their proliferation.

Preventing neutrophil activation and adhesion during CPB by manner of removal of neutrophils by filtration (Whitaker *et al.* 2001), drug related down regulation (Chung *et al.* 2005) and/or BCC may result in decreased postoperative organ dysfunction and subsequently, an improvement in clinical outcomes.

1.2.1.3 Neutrophils and Reperfusion Injury

Reperfusion injury refers to the inflammatory response occurring when circulation returns to previously ischemic tissue beds. Ischemic injury occurs when perfusion is impaired. Ischemic cells will deplete their adenosine triphosphate stores by the action of hypoxanthine (Haga *et al*. 1993). This initial cellular ischemia coupled with a lactic acidosis, altered cellular homeostasis and loss of cellular membrane ion gradients is amplified when blood flow is restored upon reperfusion (Gravlee *et al*. 2008). Phagocytes (neutrophils, monocytes and macrophages) increase their oxygen consumption, and manufacture leukotrienes (LT) (signaling molecules) and ROS (Haga *et al.* 1993; Gadaleta *et al.* 1994). Many fatty LT molecules exist such as LTA_4, LTB_4, LTD_4, LTE_4, and LTF_4 (Stables *et al.* 2010). Synthesis of LT occurs in the lipoxygenase pathway where there are produced from arachidonic acid in conjunction with 5-lipoxygenase. Cellular LT production enhances the reactivity of its host or that of neighboring cells thereby regulating the immune response. When these cells are activated, arachidonic acid, through a

series of enzymatic reactions and spontaneous reductions, converts to powerful chemoattractants (Stables *et al.* 2010). Chemoattractants (or conversely chemorepellents) are organic or inorganic substances, which attract or repel motile cells (Sallusto and Mackay, 2004). Effects of chemoattractants can be cell specific such as a ligand target and/or can be concentration dependent. Examples of chemoattractant receptors are CCR3, CCR4, CCR8 and CRTH2 (Romagnani, 2002).

This catalytic mechanism not only occurs in phagocytes, but also in mast cells, eosinophils and basophils. One of the functions of LT is to trigger smooth muscle contraction and their production is usually coupled with the production of histamine (Nelson and Cox, 2008). LT chemotactic effect on neutrophils is to recruit the necessary cells to regulate the inflammatory response, which frequently results in increased vascular permeability (Dahlen *et al.* 1981).

ROS are a natural byproduct of metabolism. ROS participate in cell signaling leading to activation and transcription of genes relevant for cell growth and differentiation (Novo *et al.* 2008). The effect of ROS on cells and tissues during periods of stress however, known as oxidative stress, can be quite damaging. Over a long period of time ROS can cause oxidative damage to DNA, proteins and lipids resulting in cancer, cardiovascular disease, type 2 diabetes, rheumatoid arthritis and neurodegenerative diseases (Novo *et al.* 2008). ROS are responsible for tissue injury because they oxidize or chlorinate molecular proteins and membrane lipids. ROS migration across cell membranes can lead to cell damage and apoptosis (Gadaleta *et al.* 1994). Major ROS include the superoxide anion (O_2-), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) (catalyzed by myeloperoxidase), hydroxyl radicals (OH-) and oxidation of other cellular molecules leading to new free radical species. Enzymes such as superoxide dismutase, lactoperoxidase, glutathione peroxidase and, importantly, uric acid exist to defend organisms from ROS through reaction with unpaired valence shell electrons (Gravlee *et al*. 2008; Novo *et al.* 2008). Inadequate tissue perfusion during CPB consequent to low systemic flows or linear flow situations (as opposed to normal pulsatile flow) can lead to tissue hypoxia and reperfusion injury. Additionally insufficient myocardial perfusion during the period of time where the aorta is crossclamped amplifies the resultant reperfusion injury occurring when normal blood flow to the coronary arteries is restored.

1.2.1.4 Platelets

Platelets are anucleate fragments of megakaryocytes possessing highly organized structures and glycogen energy stores (Cecil, 1993). At only 2-3 μm in diameter, the short-lived platelet plays a pivotal role in hemostasis. Clot formation could not occur without sufficient numbers of working platelets. Counts below 100,000/μl may result in an increased risk of bleeding (Cecil, 1993). Platelet activation occurs due to binding of its membrane receptor GPIb to von Willebrand's factor (vWF). vWf is a large glycoprotein produced in subendothelial connective tissue and platelet alpha granules (Sadler, 1998). vWf and platelets present in plasma have high affinity to exposed collagen vWF, and tissue factor beneath the endothelial surface when there is

a vessel injury (Sadler, 1998). Collagen can anchor platelets at their receptor GPIa/IIa at one locus while simultaneously binding to GPVI at a second locus. In this manner, platelet binding and activation can occur simultaneously (Jaffe, 1994). Upon platelet activation, a physical transformation arises which exposes the previously covered receptor GPIIb/IIIa. Receptor GPIIb/IIIa can now bind with vWf multimers (one basic vWF monomer is 2050 amino acids (AA) and the platelet adheres firmly to subendothelial collagen (Sadler, 1998). Activated platelets, once bound to the vessel wall, attract more of the same and stimulate vessel constriction. vWf, fibrinogen, tissue factor and C3a are potent stimulators of platelets aggregation (Wan *et al.* 1997; Sadler, 1998). These actions are crucial to arresting blood loss consequent to vascular damage.

Not only are platelets critical for hemostasis but they also migrate to sites of injury and participate in the inflammatory response by secreting proinflammatory cytokines (TNF, cluster differentiation (CD) 40L and interleukin 1β) and chemokines monocyte chemotactic protein 1, CCL5 or RANTES and kCXCL4 or platelet factor 4) (Wagner *et al.* 2003). Activated platelets induce expression of monocyte chemotactic protein 1, and soluble intercellular adhesion molecule-1 (sICAM-1) on endothelium (Gawaz, 1998). This action prompts activation of endothelial nuclear factor-κB and nuclear factor-κB-regulated genes. NF-κB, induced by TNF, is a direct modulator of hypoxia inducible factors (van Uden *et al.* 2008). Hypoxia inducible factor promotes glycolysis and angiogenesis (Wong *et al.* 2008). Consequently, activated platelets promote the early onset of inflammation in atherogenesis.

Other pro-inflammatory factors released from platelets include serotonin, bradykinin, prostaglandins, prostacycline, thromboxane and histamine, all functioning to increase cell proliferation and migration to the injured area to support healing (Stadelmann *et al.* 1998). Adhered platelets also support leukocyte rolling, adhesion and transmigration through the endothelium and into the affected tissue (Sadler, 1998).

The pump sucker is a surgical convenience associated only with CPB-assisted cardiac surgery. Systemic heparinization delays shed blood from clotting. Consequently, this blood can be aspirated from around the wound, returned to the HLM, filtered by the cardiotomy reservoir and returned to the circulation. Blood contacting injured vessels or the CPB circuit causes platelets to aggregate together. Platelets will also aggregrate with fibrin, monocytes and neutrophils (Gorbet *et al.* 2004; Edmunds *et al.* 2006). Once again falling platelets counts are often consequent to long CPB times and excessive use of the pump sucker (de Jong *et al.*1980; Kansy *et al.* 2010).

Platelet aggregation and sequestration can occur within the currently employed membrane oxygenators leading to oxygenator failure. This rare but serious complication requires stopping CPB and immediately replacing the defective component for another. Platelet sequestration interrupts the blood path within the oxygenator leading to large changes in pressure, excessive shear stress, hemolysis and possibly compromised gas exchange (Myers *et al.* 2003). Postoperative platelet transfusions may be required.

Excessive pressure gradations became an unwanted complication when albumin was removed from CPB priming solutions (Gu *et al.* 2006). Vendors have maintained that the push for biocompatible coatings came as a response to the high changes in pressure occurring when albumin was removed from the prime (B. Klanke, personal communication, May, 2009). To its credit, BCC have almost completely eliminated this unwelcome complication (Marcoux, 2009).

The unavoidable activation of platelets throughout CPB results in compromised hemostasis postoperatively. CPB-assisted cardiac surgery should be completed as quickly and efficiently as possible as duration of CPB correlates with the extent of platelet destruction and postoperative bleeding (Wahba *et al.* 1996; Greilich *et al.* 2002).

1.2.1.5 Endothelial Cells

Endothelial cells form a single layer of simple squamous epithelium within the vasculature. Endothelial cells regulate the movement of fluid into and out of circulation while providing a non-thrombogenic surface for blood to come in contact with. Endothelial cells are also involved in the entrapment of leukocytes via expression of surface proteins and soluble adhesion molecules (SAM) whenever a vessel is injured (Laffey *et al.* 2002; Colman *et al.* 2006). Hypoxia, surgical manipulation and the artificial CPB circuit activate endothelial cells (Gravlee *et al*. 2008). Circulating thrombin, proinflammatory cytokines, C5a, and oxidants become stimulated and in turn activate the SAMs soluble endothelial-selectin (sE-selectin), sICAM-1, and soluble vascular cell adhesion molecule 1 (sVCAM-1). SAMs function to capture circulating leukocytes and assist their transport through the endothelium into the interstitial space.

Endothelial cells produce many of the protein factors and molecules critical to the balance of the homeostatic thrombotic status. These molecules include adhesive proteins such as the integrins, cadherins and selectins, vWf, protein S, tissue factor pathway inhibitor, tissue plasminogen activator, urokinase plasminogen activator, plasminogen activator inhibitor and heparan sulphate (Colman *et al.* 2006). Endothelial cells also produce many vasoconstrictive and vasodilatory molecules such as nitric oxide, histamine, norepinephrine and bradykinin (Colman *et al.* 2006**).** Linear flow and embolic generation resulting from CPB change homeostasis activating endothelial cells. The resulting thrombin formation, fibrinolysis and acute inflammation, increases endothelial cell permeability and leukocyte migration into the interstitial space (Colman *et al.* 2006). Clinically this is manifested by tissue edema.

1.2.1.6 SAMs

Soluble adhesion molecules, briefly discussed under heading **1.2.1.5 Endothelial cells**, play an integral role in the immune response. Elevated levels found in circulation are evidence of a heightened inflammatory response. Molecules are known as sVCAM-1, sICAM-1, sE-selectin, soluble leukocyte selectin (sL-selectin) and soluble platelet selectin (sP-selectin). sICAM-1 and

sVCAM-1 are members of the immunoglobulin superfamily (IgSF). The selectin family E, L and P, are so named because they include lectin adhesion molecules in their structure (Alexander, 1994). Lectins are proteins that bind sugar polymers (Parham, 2005). The identifying letter before selectin refer to where these SAM can be found: endothelium, leukocytes and platelets, respectively. Cytokines such as TNF and IL-1 are responsible for excitation of most SAMs. SAMs can only be detected in soluble form in circulation. Elevated levels have been linked to dyslipidemia, atherosclerosis (Hackman, 1996) connective tissue disorders and some cancers (Blann, 1999). Long-term prospective study results from meta-analyses undertaken by Danesh *et al.* (1998) and Ross *et al.* (1999) have determined a link between elevated mononuclear cells, CRP and coronary heart disease (CHD). Additionally, there is a strong association between CHD and elevated serum levels of sICAM-1 and sE-selectin (Hwang, 1997; Ridker, 1998). Cell adhesion molecules sICAM-1 and sVCAM-1 are found in higher concentrations in individuals with atherosclerotic vessel walls compared to healthy endothelial tissue (Poston, 1992; Davies, 1993). sICAM, sVCAM-1 and sE-selectin have been found in elevated levels in obese, hypertensive and diabetic children. SAMs and endothelial activation appear related to the earliest stages of atherosclerosis (Glowinska *et al.* 2005). Elevated levels of sICAM-1, sVCAM-1 and sL-selectin have been found in patients with obstructive sleep apnea syndrome (Ohga *et al.* 1999).

Just as elevated levels of SAMs have been found in patients with atherosclorosis, they have also been associated in patients with chronic AF (Blann, 1999; Roldán, 2003). Upregulation of monocyte adhesion receptor CD11b, and increased levels of circulating monocytes and neutrophils, are also associated with an increased incidence of postoperative AF (Fontes *et al.* 2005). As the BCCs boast anti-inflammatory properties, examining SAM expression and their association with AF beg comparison among the tested circuits.

Multiple studies have compared the effects of BCC on SAM release (Schreurs, 1998; Andresen, 2002; Alex, 2003; Bical, 2006). Studies have also compared SAM expression differences between on-pump and off-pump cardiac surgery. To date there are no studies comparing the release patterns of sVCAM-1, sICAM-1, sE-selectin and sL-selectin with respect to the four BCCs included in this study.

1.2.1.6.1 sVCAM-1

VCAM-1 is also known as CD106. CD refers to a human gene and the protein it encodes (Carlson *et al.* 1988). At approximately 100-110 kDaltons, VCAM-1 is a transmembrane glycoprotein with seven C-2 type immunoglobulin domains (Cybulsky *et al.* 1991). All IgSF proteins possess variable domains named after the immunoglobulin (Ig) molecules. They are categorized according to size and function (Barclay, 2003). The seven C-2 type Ig domains mentioned above, refer to variability and number of beta strands forming the sandwich like structure characteristics of Ig domain (Harpaz *et al.* 1994). VCAM-1 is composed of 715 AA. Most of this molecule (677 AA) is normally found in the extracellular space. Twenty-two AA are

found in the transmembrane segment. Nineteen AA are found in the tail sitting in the cytoplasm of its host cell (Imhof *et al.* 1995). Multiple N-linked glycosylation sites sit in the extracellular region where every C2-Ig domain is capped by a disulphide bridge (Imhof *et al.* 1995). Soluble VCAM-1 has been identified in blood, culture supernates and cerebrospinal fluid (Pigott *et al.* 1992). Neurons, fibroblasts, macrophages, endothelial cells and smooth muscle cells have all been found to express VCAM-1 (Imhof *et al.* 1995).

VCAM-1 plays an important role in attaching lymphocytes, eosinophils, basophils and monocytes to the endothelial cells (Imhof *et al.* 1995). The soluble ectodomain of VCAM-1 (sVCAM-1) is proteolytically released from the endothelial cell surface into circulation in response to proinflammatory cytokines (Morisaki *et al.* 1995), (Singh *et al.* 2005). Cytokines actively involved in proteolysis of VCAM are TNF, IL-1α and IL-1β, IL-4, and IL-13 (Morisaki *et al.* 1995), (Singh *et al.* 2005). Heightened VCAM-1 activation can last up to 24 hr and elevated VCAM-1 shedding levels can be observed within 8 hr of cytokine stimulation (Singh *et al.* 2005). Cytokine stimulation increases cell surface expression of VCAM-1, which makes it more approachable by proteases (Singh *et al.* 2005).

VCAM-1 binds to two types of integrins: $\alpha4\beta1$ (VLA-4) and $\alpha4\beta7$ (LPAM-1). Both are expressed on all leukocytes except neutrophils (Koisumi *et al.* 1995; Sudhoff *et al.* 1996). The above integrins are also known as VCAM-1 ligands. "VCAM-1 to VCAM-1" ligand interactions are thought to be the stimulus required for leukocyte extravasation (Imhof *et al.* 1995). It is also thought that VCAM-1 (and ICAM-1) regulates osteoclastogenesis via cell-to cell contact mechanism (Fujii *et al.* 2003) and the induction of sickle cell adherence to vascular endothelial cells during hypoxemia (Klings *et al.* 2008).

Elevated sVCAM-1 levels have been found in the serum of patients with rheumatoid arthritis (Croft *et al.* 1999) systemic lupus erythematosus and multiple sclerosis (Baraczka, 2001). Elevated levels found in the cerebrospinal fluid of patients with intracerebral bleeds have been associated with poor clinical outcomes (Kraus, 2002). Elevated levels have also been found in myelomonocytic leukemia (Sudhoff *et al.* 1996) and bronchial asthma (Koisumi *et al.* 1995). The intensity of sVCAM-1 expression correlates with the magnitude of the inflammatory response (Croft *et al.* 1999). Normal circulating levels of sVCAM-1 are below 1012 ng/mL (Papayianni *et al.* 2002).

1.2.1.6.2 sICAM-1

ICAM-1 (also known as CD54) is a 90 kDalton cell-surface glycoprotein active in regulating leukocyte migration and activation (Yang *et al.* 2005). Found almost everywhere in the body, ICAM-1 is activated at sites of inflammation by cytokines (IL-1 and TNF) on macrophages, lymphocytes, endothelial and epithelial cells (Yang *et al.* 2005). ICAM-1 mediates the adhesion and paracellular migration of leukocytes expressing lymphocyte function-associated antigen 1 and membrane attack complex (Marlin *et al.* 1987; Riet *et al.* 1993). This important adhesion

molecule promotes angiogenesis, T cell proliferation and cytokine release while prolonging antigen release by dentritic cells. sICAM production is due to proteolytic cleavage (Budnik *et al.* 1996).

Found in urine, bronchoalveolar lavage fluid, cerebrospinal fluid and serum, elevated levels of sICAM indicate vascular endothelial cell activation or damage and are associated with cardiovascular disease, hypertension, type II diabetes (Morisaki *et al.* 1997) sepsis (Nakae *et al*. 1996) and obesity (Nelson *et al.* 2007). Normal levels of sICAM are <206 ng/mL (Sano, 1994; Wu *et al.* 2003; Alexandrakis *et al.* 2004).

1.2.1.6.3 sE-selectin

E-selectin is a 115 kDalton transmembrane glycoprotein. Also known as endothelial leukocyte adhesion molecule-1 or CD62E, E-selectin is only present on endothelial cell membranes. E-selectin is transiently expressed 6 hours after activation by inflammatory cytokines (IL-1β or TNF), or endotoxins (Lidington *et al.* 2002). Along with L and P-selectin, it contributes to leukocyte adhesion onto endothelium. Maximum expression occurs six hr after stimulation after which E-selectin is released into the vascular space. Finding elevated levels of sE-selectin in the blood is a sign of endothelial activation (Gearing *et al.* 1993).

At the site of inflammation, E-selectin mediates the rolling attachment of leukocytes (neutrophils, monocytes and memory T-cells) to the endothelium, a crucial step in extravasation of the leukocyte (Banks *et al.* 1993; Cummins *et al.* 1997). sE-selectin, perpetually present in the blood of healthy individuals arises from proteolytic cleavage of the surface expressed molecule. Certain pathological conditions such as sepsis, systemic inflammatory response syndrome (SIRS) and hemodynamic compromise, result in elevated circulating levels (Cummins *et al.* 1997). E-selectin has proinflammatory and angiogenic properties as well (Sezer *et al.* 2000). Normal serum levels of sE-selectin are below 29 ng/mL (Alexandrakis *et al.* 2004). Higher than normal levels of sE-selectin result in enhanced microvascular permeability and tissue edema. If the edema is excessive and remains unchecked, tissue ischemia and necrosis are possible consequences (Visser, 2006).

1.2.1.6.4 sL-selectin

L-selectin has been given many names since its discovery: CD62L, leukocyte adhesion molecule-1, leukocyte endothelial cell adhesion molecule, DREG, MEL-14 antigen, TQ1, Leu-8 and lymph node homing receptor (Cotran, 1998). This single-chain transmembrane glycoprotein is expressed by granulocytes, lymphocytes, and monocytes (Cotran, 1998). Two types of L-selectin have been identified; one is found on lymphocytes and has a relative mass of 74 kDaltons and the other is found on neutrophils and is 90-110 kDaltons (Cotran, 1998). L-selectin

is composed of an amino-terminal calcium dependent lectin section (Cotran, 1998). Along with its large transmembrane section, L-selectin has a short section extending into the cytoplasm (Ivetic *et al.* 2004). In areas of acute or chronic inflammation, these SAMs aid extravasation of neutrophils and leukocytes into the surrounding tissues and lymph nodes (Ivetic *et al.* 2004). L-selectin in concert with P-selectin mediates the initial contact with circulating leukocytes which starts them rolling along the endothelial surface. A secondary, stronger reaction involving L-selectin and E-selectin leads to the extravasation through the vessel endothelial wall into the inflamed lymphoid tissues (Cotran, 1998; Ivetic *et al.* 2004; Parham, 2005).

Leukocytes cannot participate in inflammatory response unless they are activated. Treatment of neutrophils with phorbal esters and chemotactic factors results in down-regulation of L-selectin surface expression followed by shedding of sL-selectin from the neutrophil (Jutila *et al.* 1989). The corresponding increase in the now measurable soluble version of L-selectin inhibits neutrophil migration to areas of inflammation (Jutila *et al.* 1989; Kishimoto *et al.* 1989). In other words, circulating sL-selectin in high enough concentrations is thought to inhibit the binding of neutrophils and lymphocytes to the endothelium. When L-selectin is shed off the neutrophil, the neutrophils cannot deform and become activated. The same occurs in regards to L-selectin and lymphocytes (Jung *et al.* 1990). Ergo, shedding of L-selectin limits inflammation of leukocytes (Hafezi-Moghadam *et al.* 2001). The average plasma levels in healthy adults ranges between 1-6 μg/mL (Tu *et al.* 2002). High and low concentrations of sL-selectin have been found in healthy and unhealthy subjects suggesting that sL-selectin must be present to a certain degree for leukocyte extravasation to occur (Smalley *et al.* 2005). Leukocyte attachment to cytokine-activated endothelium is completely inhibited at sL-selectin concentrations of 8-15 μg/mL (Schleiffenbaum *et al.* 1992).

1.2.2 Humoral Inflammatory Components

1.2.2.1 Complement

More than 30 proteins make up the primary recognition system and effector immune response of the complement activation system (Paul, 1999). Complement functions include controlling inflammation, opsonization of antigenic particles/molecules and pathogen membrane disruption (Paul, 1999). Individual elements of complement react with each other whereby an enzyme created by one reaction can in turn go on to stimulate another. In this manner, a small initial stimulus can be amplified to bring forth destruction of an invading pathogen. The complement system has three different pathways (classical, alternative and lectin). The classical system is upregulated by antibody/antigen binding on cell surfaces. The alternative system is upregulated either as a product of the classical system or by surfaces presented by viruses, bacteria and the CPB circuit. The lectin system though similar to the classical system, is activated by mannose-binding lectin, and ficolins attaching to pathogen surfaces (Gravlee *et al*. 2008). All three pathways terminate in a similar fashion by forming the membrane attack complex. The membrane attack complex can lyse through the membrane of the offending invader allowing an influx of ions and water into the cell, potentially leading to cell lysis.

During CPB and for a short time thereafter, complement activation occurs mainly by the classical and alternative pathways (Levy *et al.* 2003). Blood contact with the artificial surface is the primary stimulus for alternative pathway stimulation leading to the formation of anaphylatoxins C3a and C5a (Wan *et al.* 1997; Gorbet *et al.* 2004). These stimulate the release of histamine from mast cells and basophils increasing vascular permeability. C3a and C5a also stimulate the release of oxygen free radicals and lysosomal enzymes from WBC. Protamine neutralization of heparin post CPB can activate the classical pathway leading to an associated rise in C4a and a further rise in C3a. (Wan *et al.* 1997). Histamine release, and generalized inflammation follow (Miller *et al.* 1997). Consequently, organ dysfunction after CPB is primarily due to complement and neutrophil interaction (Edmunds *et al.* 2006; Edmunds *et al.* 2008).

1.2.2.2 CPB and Coagulation

Endothelial cells provide a nonthrombogenic surface. The delicate balance existing between procoagulants and anticoagulants is disrupted when blood contacts the ECC (Nieuwland *et al.* 1997). CPB intensifies thrombosis and would therefore not be possible without large systemic anticoagulant doses of heparin. Heparin is readily isolated from bovine lung and porcine intestinal mucosa (Rodriguez *et al.* 1979). Although not the ideal anticoagulant because it does not completely prevent thrombin formation, it is easily reversed with protamine sulphate and it is inexpensive to manufacture. Heparin greatly enhances the affinity of antithrombin III for thrombin (Rosenberg, 1989). Once bonded to antithrombin III, thrombin is unavailable to cleave fibrinogen into fibrin. Long unfractionated strands of heparin inhibit thrombin and, to a lesser extent, factors Xa, IXa, XIa, XIIa kallikrein and plasma (Pixley *et al.* 1985). Inactivated by heparin, thrombin can no longer activate factors VIII and V, which serve to accelerate the rate of fibrin formation (Hill-Eubanks *et al.* 1989).

Thrombin generation can also occur when platelets, monocytes, neutrophils and endothelial cells are activated by the enzymatic responses to CPB (Nieuwland *et al.* 1997). Continuous formation of thrombin throughout CPB despite relatively high doses of systemic heparin administration results in a consumptive coagulopathy (Gravlee *et al*. 2008). The longer a patient is supported by ECC the more coagulation proteins are consumed in an endless anti-thrombotic/prothrombotic cycle. Bursts of thrombin and fibrinogen generation occur when initiating CPB and again upon reperfusion of the previously ischemic myocardium (Chandler and Velan, 2003). Fibrin and thrombin generation return to baseline levels 2 hours after the conclusion of CPB (Chandler and Velan, 2003). Coagulation and inflammation are intricately tied together as one pathway feeds back to the other and vice versa (Ott, 2005).

Two pathways of the coagulation cascade lead to fibrin formation: the contact activation pathway (intrinsic) and the tissue factor pathway (extrinsic). Of the two pathways, tissue factor (also known as FIII, tissue thromboplastin and CD142) appears most important because individuals deficient in contact activation proteins who undergo CPB do not experience

excessive postoperative bleeding (Burman *et al.* 1994). Exposure of blood to tissue factor, only present when the endothelium is breached, results in an immediate response by circulating platelets and plasma coagulation proteins (Furie *et al.* 2005). Tissue factor immediately binds with readily available factor VII. Tissue factor and factor (F) VII complex activates FIX and FX. Activated FX and its co-factor activated FV form a prothrombinase complex serving to activate prothrombin to thrombin. Thrombin then goes on to catalyze FV to activated FV, FVIII to activated FVIII and platelets (Colman *et al.* 2006).

The contact activation pathway involves high-molecular-weight kininogen, prekallikrein, FXII and FXI. The contact activation pathway and the tissue pathway once activated, come together to form the common pathway resulting in the cross-linked fibrin clot.

Three reversible alterations in coagulation typically occur during the clinical application of CPB: systemic heparinization, hypothermia and hemodilution. Heparin, though completely necessary for anticoagulation, has an unfortunate secondary antiplatelet effect by binding to vWf at platelet receptor GPIb. As well, inadequate protamine sulphate administration following CPB can result in continuing heparin anticoagulation interfering with hemostasis. Additionally, heparin bound to protein can become unbound following CPB and can contribute to postoperative bleeding. This occurrence, known as heparin rebound, is a frequent postoperative complication observed in the intensive care unit after the patient's arrival from the operating room.

Hypothermia has been shown to decrease the speed of enzymatic reactions. For every 10°C decrease in temperature, a concurrent 50% decrease in metabolic rate occurs (Kirkland and Barratt-Boyes, 1993). Consequently, enzymatic activity driving the soluble coagulation cascade is adversely affected (Wolberg *et al.* 2004). Above 33°C activity of the clotting factors is minimally reduced, however, below 33°C platelet adhesion and aggregation are significantly impaired (Watts *et al.* 1998). Thankfully, hypothermic impairment of coagulation and platelet activity can be reversed with adequate warming of the patient to 37°C. Inadequate warming or inadvertent cooling once the patient is separated from bypass frequently occurs as the chest cavity may be exposed to the cooler temperatures typical of most operating rooms, contributing to postoperative hemorrhage.

The necessary combination of the CPB circuit prime (crystalloid and colloid) with a patient's circulation results in the hemodilution of patient's cellular blood components. The degree of hemodilution is dependant upon the priming volume of the circuit, size and volume status of the patient, and degree of preoperative anemia. Average human blood volume is equivalent to 5 liters. The volume required to prime the CPB circuit at our institution is 1.8 liters. Therefore the resultant hemodilution is 36%. Davidson *et al.* (2003) measured coagulation factors changes induced by hemodilution and CPB. They concluded that FVIII concentration was barely affected whereas, FII and FX decreased by more than 50% (Davidson *et al.* 2003). Thrombin potential (amount of thrombin able to be formed in plasma measured in nmol/L) was also decreased by 50%. This study suggests that, the consequence of simultaneous multiple factor deficiencies

result in greater decreases in thrombin generation as compared to what would occur if only one factor out of the many was deficient (Gravlee *et al*. 2008).

The longer a patient is supported by a HLM the greater the risk of post-operative coagulopathy. After more than 3 hr of time spent on a HLM (time dependant on many clinical factors), a demonstrable decrease in the patient's ability to make effective clot occurs (Kansy, 2010) in spite of adequate rewarming and heparin reversal.

1.3 CPB and Organ Dysfunction

Postoperative organ dysfunction is uncommon and usually temporary after routine CPB-assisted cardiac surgery (Gravlee *et al*. 2008). Organs most commonly affected are the kidneys and the brain (Gravlee *et al*. 2008). There is a positive correlation between microemboli production and postoperative organ dysfunction (Westaby, 1987). Production of microemboli during CPB whether due to air, fibrin emboli, macroaggregates of platelets, leukocytes, fat globules, foreign material or RBC fragments, are directly proportional to the amount of time a patient is supported by the HLM (Blauth, 1995; Hessel, 2003). Particulate emboli aspirated from the wound by the cardiotomy suckers, are the primary source of microemboli formation (Brooker, 1998; Hessel, 2003). Aspiration of shed blood mixed with air will generate microbubbles (Blauth, 1995) while surgical manipulation of the atheromatous aorta may generate solid emboli (Blauth, 1995). Most postoperative organ dysfunction is likely due to a combination of factors involving microemboli and the previously mentioned inflammatory response.

1.3.1 CPB and Renal Function

Postoperative renal failure, defined as an increase of serum creatinine by 25% from baseline (Brown *et al.* 2006), is uncommon (1%) following routine cardiac surgery in patients with normal, preoperative kidney function. This undesired side effect has been linked to CPB, hemolysis, inadequate perfusion, microemboli and inflammation (Liu *et al.* 2000). In the presence of preoperative kidney dysfunction, the risk of postoperative renal failure increases to 20% (Liu *et al.* 2000). Creatinine present in the blood as a by-product of muscle breakdown is mostly filtered without absorption. Therefore any increase in circulating creatinine is indicative of a decrease in glomerular filtration rate (Stevens *et al.* 2006).

Glomerular filtration rate is largely dependent upon blood pressure despite a certain amount of existing autoregulation. Increased blood pressure can result in a significant increase in urine output while a mean arterial pressure as low as 50 mmHg will have the opposite result (Gravlee *et al*. 2008).

Perioperative risk factors associated with postoperative renal dysfunction include congestive heart failure, diabetes mellitus, previous coronary artery bypass grafting, elevated preoperative creatinine, CPB times longer than 3 hr, low cardiac output, hypovolemia, excessive

hemodilution, age greater than 70, and renal emboli (Mangano *et al.* 1998). Thirty day mortality for patients requiring dialysis following cardiac surgery and CPB is increased to 63% in comparison to an accepted mortality of 0.9% for elective patients not afflicted with postoperative renal dysfunction or other significant co-morbidities (Mangano *et al.* 1998).

Neutrophil activation as a component of systemic inflammatory response syndrome has been implicated as a cause of perioperative renal dysfunction (Rinder *et al.* 2003; Stallwood, 2004). Significant hemolysis can lead to glomerular dysfunction and damage from the RBC casts (Yedgar *et al.* 2002). Certain intra-cardiac surgical procedures such as valve repairs or replacements can result in excessive use of the pump sucker for return of shed blood into the systemic circulation. Hemoglobinuria is due to fragmented RBC filtration through the glomerulus. This phenomenon is manifested in a reddish tinged urine production.

1.3.2 CPB and the Brain

Major surgery with or without CPB can result in a wide spectrum of neurologic injury, especially in the elderly population (Moller *et al*. 1998). Risks are proportionally greater for cardiac surgical patients because many have preexisting risk factors for stroke and cognitive impairment (Gravlee *et al*. 2008). These risk factors include advanced age, aortic atherosclerotic disease, carotid stenosis, previous/recent myocardial infarction, atrial arrhythmias, hypertension, diabetes and previous neurological events (Newman *et al.* 1995). Technological and surgical advancements have reduced the risk of stroke to 3-8% but the incidence of neurocognitive deficits (NCD) at hospital discharge (10-80%) and at 3 months and 6 months are higher (5-20%) (Newman *et al.* 2001). All 261 patients in the above mentioned study by Newman *et al.* (2001) were free of any history of cerebrovascular disease, renal disease and active liver disease prior to undergoing cardiac surgery.

Microemboli and macroemboli are introduced to all patients at some point during CPB and cardiac surgery (Stump *et al.* 1996). As previously described, they originate from aortic atheromas, cellular debris (leukocyte and platelet-fibrin aggregates) and air microbubbles generated by the HLM and surgical field (Pugsley *et al.* 1994). Most overt strokes are associated with surgical/physical manipulation of the aorta (Pugsley *et al.* 1994). Widespread cerebral microembolizations are likely the primary mechanism of NCD while the ensuing inflammatory process likely exacerbates the magnitude of injury (Beamer *et al.* 1995; McKeating *et al.* 1998; Pantoni *et al.* 1998).

Hypertension and diabetes are independent risk factors for postoperative brain injury in cardiac surgery. The mechanism of injury in this group is likely the result of regional cerebral hypoperfusion (Oliveira *et al.* 2008) and previously mentioned microembolization. Degenerative vascular changes means that blood flow is critically dependent on perfusion pressure rather than vascular autoregulation. As the brain has minimal tolerance for ischemic episodes, any decreases in cerebral circulation may have a profound effect (Sowers *et al.* 1995).

Locally, inflammation of endothelial cells lining cerebral vessels occurs because of microvascular occlusion due to embolic blockade or as a result of leukocyte-endothelial-platelet binding (del Zoppo, 1997). Either way, there is a definitive interaction between inflammation, ischemia and the vascular endothelium. Leukocyte degranulation leads to free radical, hydrogen peroxide and proteolytic enzyme release further impairing vascular integrity (del Zoppo, 1997). Exposure of the blood to the CPB surface may amplify what would otherwise have been a harmless localized reaction.

1.4 CPB and Diabetes

Diabetes is a major risk factor for cardiovascular disease (Kannel *et al.* 1979). Diabetic patients are usually hypertensive and frequently have a history of myocardial infarction by the time they arrive in the operating room for CABG (Gravlee *et al*. 2008). Due to the nature of their disease, coronary vessel diameter is typically less than in non-diabetic patients (Cariou *et al.* 2000). Preoperatively, diabetics have a greater likelihood of having triple vessel disease and a lower left ventricular ejection fraction (Jones *et al.* 1996). Diabetes mellitus is an independent risk factor for postoperative renal complications and this risk triples if renal function is already compromised preoperatively (creatinine > 140 μmol/l) (Mangano *et al.* 1998). Postoperative complications involving the kidneys have been linked to inflammation due to CPB, nephrotoxic medications, embolization, hemoglobinemia and inadequate renal perfusion (Mangano *et al.* 1998).

Post CABG risk of cognitive impairment in diabetics is significantly elevated compared to non-diabetics. Vigilant insulin treatment of hyperglycemia perioperatively appears to attenuate impairment (Kadoi *et al.* 2005). Prolonged periods of low venous O_2 saturation during CPB, hypertension and high levels of HbA1c correlate with cognitive impairment (Kadoi *et al.* 2005).

The risk of postoperative mediastinitis is 2-4% in diabetics compared to 1-2% in non-diabetics, while mortality is tripled for diabetics should an infection of the mediastinum occur (Zerr *et al.* 1997). Tight glycemic control in diabetics postoperatively can reduce mediastinal infection risk to 1.5% (Furnary *et al.* 2003). Decreased endothelial cell proliferation equals decreased wound healing and increased endothelial cell expression of sVCAM-1 in diabetics in a hyperglycemic state (Birnbaum *et al.* 2006).

Diabetics are known to be prothrombotic (Dalal *et al.* 2002). This translates into the potential of premature closure of surgical bypass grafts (Lytle *et al.* 1985). Diabetics are generally not given antifibrinolytics because the administration of these drugs can precipitate premature graft closure above and beyond the risk associated with the prothrombotic state of their disease.

The added postoperative risks to diabetics undergoing cardiac surgery and CPB suggest that BCC might be especially useful in attenuating the heightened inflammatory response experienced by these patients (Marcoux *et al.* 2009).

1.5 CPB and Gender

Females tend to be smaller and carry a lower circulating RBC volume compared to their male counterparts. Females therefore experience a slightly higher incidence of postoperative morbidity and mortality (Athanasiou *et al.* 2002; Puskas *et al.* 2008). The added perioperative risks to females undergoing CPB-assisted surgery suggest that BCC might be especially useful in decreasing morbidity and mortality in this high-risk group.

1.6 BCC

The ideal BCC should be as safe and functional as endothelial lining. The endothelial lining is difficult to reproduce however due to its complicated nature. Many biocompatible circuits have been used clinically worldwide throughout the years. Four of the BCC available in Canada were included in this study. After three decades of research, a non-reactive, non-thrombogenic BCC has yet to be manufactured. Nonetheless, in order for a BCC to be clinically practical, it must be durable, clinically and biologically inert, cost effective, easy to make and easy to sterilize. Additionally, there should be documented pre-market evidence describing an improvement in outcomes relative to uncoated circuits.

Upon contact, plasma proteins are adsorbed onto the foreign extracorporeal surface completely covering the inner surface of the CPB circuit (Horbett, 1993). A subset of plasma proteins (mostly fibrinogen) is irreversibly bound to the circuit. Concentration of these proteins, once adsorbed to the CPB surface, is two to three times greater than what normally circulates throughout the vasculature (Horbett, 1993). Surface activity of the artificial surface will determine the composition of the bound monolayer of plasma proteins. Fibrinogen is selectively adsorbed and its concentration along with other plasma proteins adsorbed can change dependent upon many factors. These include duration of CPB, temperature, flow and hematological status of the patient, *i.e.,* platelet count (Horbett, 1993). Though it is known that a hydrophilic artificial surface for blood contact is generally effective in decreasing platelet adhesion and thrombus formation (Kim, 1996) development of new biocompatible surfaces remains, by and large, by trial and error because of the lack of sufficient knowledge regarding blood response to artificial surfaces.

The two methods developed to coat the inner surface of the BCC are surface bound heparin and surface-modifying additives. Commonly used for coating BCC, heparin can be bound to the PVC surface by either ionic or covalent bonding. Bonding of heparin molecules to the CPB component does not prevent it from having a bioactive surface (Wendel *et al.* 1999). Clinical

trials comparing the two types of heparin coatings had not proven the superiority of one type of bond over the other (Gorman *et al.* 1996; te Velthuis *et al.* 1997) until meta-analysis by Mahoney, (1998), which showed improved outcomes attained by covalently bonded heparin-coated BCC.

Heparin-bonded circuits have demonstrated variably attenuated complement activation (Lappegard, 2005), but without obvious improvement in clinical outcomes (Videm *et al.* 1999). Small concentrations of surface-modifying additives are added to the bulk biomaterial to reduce reactivity within their environment (Frechet, 1994). Synthetic polymers such as Trillium™ (Medtronic Inc.) (Baksaas *et al.* 1999), hyaluronan (Gunaydin, 2005), and poly-2-methoxyethylacrylate (PMEA) (Terumo Med Inc.) (Ueyama, 2004) can be used to encapsulate and control reactive sites on the surface of the tubing (Frechet, 1994).

In general, BCCs have demonstrated decreased fibrinogen deposition, decreased complement activation, decreased platelet activation and decreased inflammatory response compared to uncoated CPB circuits (Gunaydin, 2004; De Vroege *et al.* 2005; Zimmermann *et al.* 2007). A recent meta-analysis by Ranucci *et al*, (2009), unavailable when this study was conducted, concluded that patients undergoing CPB with a BCC had fewer RBC transfusions, lower rates of AF and spent a shorter time in intensive care unit (ICU) postoperatively. Seventy-eight percent of the BCC circuits included in their meta-analysis had heparin-based coatings, unlike our study where heparin and non-heparin based circuits were equally divided. Additionally some studies in the meta-analysis used full dose heparin in order to maintain activated clotting time (ACT) > 480s whereas others relied on lower circulating heparin levels (ACT < 300s). All patients in the present study received full dose heparin necessary to maintain ACT > 480s equivalent to a heparin dose in the range of 400-500 units/kg.

To date, duplicating the endothelial lining, and/or all properties therein, remains beyond reach. Genetic engineering is likely the only manner with which a true biomaterial could be manufactured (Edmunds, 1995). To date, this goal remains commercially and practically elusive.

The present study compared four BCCs and their abilities to attenuate the inflammatory response and improve clinical outcomes. Furthermore, it also compared the effectiveness of circuit design, *i.e.,* how effectively blood activation and hemolysis was minimized. Oxygenators have the greatest effect on the blood because they have the largest surface area within the CPB circuit. The surface area for blood: gas transfer of modern oxygenators designed to oxygenate and ventilate adults, ranges from 1.45-4.0 m^2 (Gravlee *et al*. 2008). Coated oxygenators included in this study had surface areas ranging from 1.8-2.5 m^2 where as the surface area for blood: gas transfer within the natural lungs is reported to be approximately 70 m^2 (Hasleton, 1972). It is truly remarkable that the small surface area in commercial oxygenators can provide sufficient gas exchange capabilities to support an adult. One need only remember that patients undergoing CPB are anesthetized and cooled, and oxygen delivery can be anywhere from 21-100%.

Gas transfer is limited by the factors governed by the laws of diffusion. These include partial pressure differences driving the gases, surface area available for diffusion, thickness of the membrane and the amount of time the gas is in contact with a surface (dwell time). Solubility of the gas related to its molecular weight is also an important factor. Solubility need not be considered here however because oxygen, carbon dioxide and nitrogen are the only gases used consistently during CPB. Decreasing the distance gas needs to travel across artificial membranes, increasing the surface area for diffusion while minimizing priming volumes and increases in shear stress are all factors which come into play when oxygenator design is considered. These considerations are important because the greater the change in pressure (from high to low) when blood is in transit through the oxygenator (or any component of the CPB circuit), the greater the amount of blood damage (hemolysis and platelet activation) induced (Kawahito *et al.* 2001). Additionally most modern day oxygenators are coupled with a heat exchanger. Their efficiency at cooling and warming patients intraoperatively need also be considered.

All oxygenators used in this study were made of porous, hollow, polypropylene fibers encapsulated in a polycarbonate shell. At the start of CPB, direct blood to gas contact occurs through these one-micron pores until a monolayer of plasma proteins is laid down. Poor oxygenator design and inadequate quality control has in the past lead to excessive platelet adhesion, high-pressures and oxygenator failure (Groom, 2002).

1.6.1 Phosphorylcholine

Phosphorylcholine-coated surfaces are coated with a derivative of the naturally occurring phosphatidylcholine (a constituent of the lipid bilayer of the cell membrane) (Whelan *et al.* 2000). This BCC is designed to mimic the inert phospholipid nature of the endothelial wall (Gunaydin *et al.* 2004; Rubens, 2002).

In an animal model, phosphorylcholine-coated stents implanted in the porcine coronary arteries did not produce an adverse inflammatory response throughout the study period of 12 weeks post implantation (Whelan *et al.* 2000). Schulze *et al.* (2009) demonstrated improved platelet counts and reduced levels of TNF and IL-6 in their phosphorylcholine group when compared to an uncoated circuit group. Nevertheless, Draaisma *et al.* (2006) found no difference in clinical outcomes or complement activation when comparing 13 neonates and infants in a phosphorylcholine-coated group *vs* 15 infants in an uncoated group undergoing CPB-assisted cardiac surgical procedures.

1.6.2 Bioline™

Bioline™ (Maquet Dynamed Inc)-coated surfaces (used clinically since 1992) are composed of polypeptides and high molecular weight heparin (Wendel *et al.* 1999). The heparin molecules attached via ionic interactions and covalent bonds to polypeptides, are adsorbed onto the CPB

components (Wendel *et al.* 1999). The Bioline™-coating is similar in design to the Trillium™ coating (Jordan *et al.* 2007). A study by Seeburger *et al.* (2005) comparing Bioline™-coated circuits to Safeline (Maquet Dynamed Inc) recombinant albumin-coated circuits, demonstrated a greater increase in leukocyte gene expression in the Bioline™ circuits. A study by Remadi *et al.* (2004), using a miniaturized Bioline™-coated circuit *vs* an uncoated control group, found decreased postoperative CRP levels and decreased troponin I levels in a group of adults undergoing aortic valve replacement. De Vroege *et al.* (2005) found decreased pulmonary dysfunction and decreased levels of C3b/c and elastase-alpha (1)-antitrypsin complex when comparing 26 Bioline™-coated circuits *vs* 25 uncoated circuits in patients undergoing CABG. Harig *et al.* (1999) found suppression of IL-6 combined with up-regulation of IL-10 in Bioline™-coated circuits when compared to an uncoated control group.

1.6.3 PMEA

Non-heparin-based PMEA has an exterior hydrophilic layer in contact with the blood and an inner hydrophobic polyethylene layer adherent to the CPB component. The hydrophilic water layer is designed to be nonreactive with similarly negatively charged cellular components in the blood. Contact activation is thus inhibited (Ikuta, 2004; Ueyama, 2004). A study by Suzuki *et al.* (2008) demonstrated decreased elevation of thrombin-antithrombin complex and neutrophil elastase levels in the PMEA coated group (n=6) when compared to a small uncoated group of pediatric patients (n=5) undergoing ventricular septal defect repair. In a larger study by Skrabal *et al.* (2006), 19 patients undergoing CPB-assisted cardiac surgical procedures were randomly assigned to the PMEA-coated group and 20 patients were assigned to an uncoated group. No significant difference was found in assayed inflammatory parameters between groups. An in vitro study comparing PMEA-coated oxygenators *vs* a heparin-coated and an uncoated oxygenator demonstrated similar attenuated increases of platelet activation in the PMEA and heparin-coated groups compared to control (Zimmermann, 2004).

1.6.4 Trillium™

Trillium™ surface-modifying additives (Medtronic Inc. Minneapolis, MN) are hydrophilic water-soluble synthetic polymers (plastic) bound to the components of the CPB circuit. Trillium™ coating is composed of sulphate/sulphonate groups, polyethylene chains and heparin (Gunaydin *et al.* 2004). Incorporating heparin as part of the coating is thought to partially inhibit activation of thrombin (Jordan *et al.* 2007). Polyethylene oxide is used as a spacer group. Its hydrophilicity and dynamic motion is thought to inhibit platelet interaction (Jordan *et al.* 2007). A clinical study by Baksaas *et al.* (1999) concluded that the Trillium™-coated CPB circuit caused less granulocyte and platelet activation as well as platelet loss compared to an uncoated circuit group. Tevaearai *et al.* (1999) compared a reduced heparin protocol (ACT= 180 sec) for 6 hr of CPB in calves to full heparinization (ACT > 480 sec) with an uncoated circuit. The Trillium™-coated group (n = 3) had an attenuated fall in circulating platelets and lower plasma-free hemoglobin levels. At the end of the procedure, there was significantly less fibrin clot found

in the dissected CPB circuit and fewer clot emboli found in the kidneys of the experimental animals upon autopsy.

2 OBJECTIVES

2.1 Comparison of Clinical and Laboratory Outcomes Between Diabetics Treated with an Uncoated Circuit or a BCC

Experiments and data analysis were conducted to assess whether diabetic patients who undergo CPB with a BCC have better outcomes than diabetic patients who undergo CPB with an uncoated circuit.

2.2 Comparison of BCC Effect on Females

Experiments and data analysis were conducted to assess whether female patients who undergo CPB with a BCC have better outcomes than female patients who undergo CPB with an uncoated circuit.

2.3 Comparison of BCCs on the Release of SAMs

Experiments were conducted to assess whether SAM analysis is consistent with published laboratory analysis to date.

2.4 Comparison of BCC Effect in Attenuating Renal Dysfunction and Consequent Volume Retention

Data analysis was conducted to assess whether patients who undergo CPB with a BCC have preserved renal function and diminished volume retention compared to patients who undergo CPB with an uncoated circuit.

2.5 Comparison of BCC Effect on Bleeding and Transfusions

Data analysis was conducted to assess whether patients who undergo CPB with a BCC have less bleeding postoperatively and fewer transfusions compared to patients who undergo CPB with an uncoated circuit.

2.6 Comparison of BCC Effect on AF

Data analysis was conducted to assess whether patients who undergo CPB with a BCC experience postoperative AF less frequently than patients who undergo CPB with an uncoated circuit.

2.7 Comparison of BCC Effect on Neurocognitive Outcome, and ICU and Hospital Length of Stay

Experiments and data analysis were conducted to assess whether patients who undergo CPB with a BCC have preserved neurocognitive outcome, and decreased ICU and hospital length of stay compared to patients who undergo CPB with an uncoated circuit.

3 MATERIALS AND METHODS

3.1. Materials

3.1.1 Patient Population

Ethics approval was obtained from the Biomedical Ethics Board of the University of Saskatchewan (EC # 2006-248) and the Saskatoon Health Region. Written informed consent was procured from all 101 consecutive patients undergoing coronary artery bypass grafting and/or valve surgery. Patients were enlisted into this prospective cohort study between July 2007 and April 2008. A preoperative antisaccadic eye movement test (ASEMT) was administered when patients were enlisted in the study. Each test was graded on 20 attempts only after the patient had clear understanding of the required visual response. A follow-up test was conducted on the morning of the 3rd postoperative day. This neurocognitive test is well described by Currie *et al.* (1991).

The sample size of 101 patients was determined sufficient to detect an observed effect size of 0.5 (medium effect) for statistical analysis using a one-tailed test with an observed power of 0.802 (Soper, 2010).

3.1.2 Exclusion Criteria

SAMs are markers of inflammation and BCCs are designed to attenuate the inflammatory response. As a result, patients admitted as acutely emergent or afflicted with a chronic inflammatory disorder were excluded. Examples of these include: rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, Wegener's granulomatosis, sarcoidosis, osteoarthritis, debilitating chronic obstructive pulmonary disease, ulcerative colitis, psoriasis, and patients afflicted with multiple sclerosis. Patients who had suffered a recent stroke, seizures or afflicted with a neurodegenerative disease (Alzheimer's or Parkinson's) were also excluded.

3.1.3 Anesthesia

Upon entering the operating room, patients were pre-medicated with oxygen and 1-2 mg intra-venous of ativan. Intra-venous induction medications consisted of midazolam, sufentanil and propofol. Isoflurane and a sufentanil infusion were used for the maintenance of anesthesia while on bypass. Fifteen mL of whole blood was drawn from the arterial line and divided into a blood gas syringe, 3 vacuum tubes, ACT and heparin dose response cartridges. Heparin dose response cartridges were used to determine patient sensitivity to heparin and to calculate the pre-bypass loading dose. Upon initiation of CPB, patients received the diuretic furosemide, the sedative midazolam and the muscle relaxant rocuronium. Before disconnection from CPB, patients received magnesium sulphate, calcium gluconate, dopamine and/or epinephrine.

3.1.4 CPB and Surgery

All efforts were made in this study to have the shortest CPB circuits possible in order to minimize the inflammatory response to the artificial surface. Additionally differences in set up of all BCC circuits and the control group were minimized to allow for accurate circuit comparisons.

The S3 or the S5 HLMs (Stockart, Germany) were used for all patients. All CPB circuits had open venous reservoirs and uncoated cardioplegia systems. Priming volume was consistent for the five CPB circuits and consisted of a combination of crystalloid and colloid; 50-300 mL normosol; 500 mL pentastarch 10%; 2.5 mL/kg of mannitol 20%; 50 mL of sodium bicarbonate and 10,000 IU of heparin. A pre-bypass filter effective to 0.2 μm was always used for priming of the CPB circuit. Every effort to minimize the use of cardiotomy suction was done. A cell saver was in use throughout the entire procedure minimizing shed blood return through the pump suckers back to the patient. The cell saver was used to remove and process shed blood from the wound before return to the patient. The volume of blood collected to the cell saver and returned to each patient was not significantly different between groups.

CPB was initiated once the ACT reached 480 sec and the circulating heparin concentration within the patient reached 3mg/kg of body weight (300 IU/kg). Patients were cooled to 32-33 ºC and supported using a non-pulsatile roller-pump. A cardiac index of 1.8-2.4 L/min/m^2 was maintained. Patients were transfused if the hemoglobin level fell below 75-80 mg/L. All efforts were made to maintain mean arterial blood pressure within 60-80 mmHg.

Shortly after initiation of CPB, a crossclamp was applied to the aorta and the heart was arrested with warm blood and KCl (20-30 meq/L). Cardioplegia was cooled upon cardiac quiescence and maintenance blood and KCL (5-10meq/L) was given intermittently. Once the coronary bypasses were completed and the valve(s) was repaired or replaced, de-airing and a final dose of warm KCL-free blood was given to the heart. Upon spontaneous return of the heart's own cardiac rhythm and adequate systemic rewarming to 36.5 ºC the patient was weaned from CPB.

Once all the cannulas were removed from the patient, a calculated protamine dose was given to reverse all circulating heparin. The remaining hemodiluted contents of the CPB circuit following CPB was processed by the cell saver and the resulting concentrated volume of RBC's suspended in saline was infused into the patient.

3.1.5 BCC

3.1.5.1 Control

The control circuit consisted of uncoated Dideco PVC tubing (Dideco, Mirandola, Modena, Italy), a Trillium™-coated Affinity oxygenator (Medtronic, Minneapolis, Minnesota), an uncoated 20 μm arterial line filter and an uncoated venous reservoir (Medtronic, Minneapolis,

Minnesota). The static priming volume of the Affinity oxygenator is 270 mL while the membrane surface area is 2.5 m^2.

3.1.5.2 Phosphorylcholine

The Phosphorylcholine-coated BCC (Sorin/Dideco, Mirandola, Modena, Italy) consisted of Sorin/Dideco disposables, PVC tubing, a 20 µm arterial filter and a venous reservoir. The static priming volume of the Primox oxygenator is 250 mL while the membrane surface area is 1.87 m^2.

3.1.5.3 Bioline™

The Bioline™-coated BCC (Maquet-Dynamed, Germany) consisted of Maquet-Dynamed disposables, PVC tubing, a 40 µm arterial filter (a 20 µm arterial filter was not available) and a venous reservoir. The static priming volume of the Quadrox oxygenator is 250 mL while the membrane surface area is 1.8 m^2.

3.1.5.4 PMEA

The PMEA-coated BCC circuit (Terumo, NJ, USA) consisted of Terumo disposables, PVC tubing, a 20 µm arterial filter and a venous reservoir. The static priming volume of the Capiox RX25 oxygenator is 250 mL while the membrane surface area is 2.5 m^2.

3.1.5.5 Trillium™

The Trillium™-coated BCC consisted of Medtronic disposables, PVC tubing, a 20 µm arterial filter and a venous reservoir. The static priming volume of the Affinity oxygenator is 270 mL while the membrane surface area is 2.5 m^2.

3.1.6 Sample Collection

Three blood samples were collected and transferred into vacutainer tubes at three selected intervals. The first was a preoperative control sample taken in the operating room. The second sample was taken six hr postoperatively from the arterial line when the patient was in ICU. The final samples were collected via venipuncture on the ward 72 hr postoperatively. All samples were labeled, refrigerated at 4 ºC and left to clot until centrifugation within 4 hr. Samples were centrifuged at 3000 rotations per min for 15 min. Serum was then transferred into seven labeled, cryogenic, Eppendorf tubes and stored at -80 ºC until analysis.

3.1.7 Demographics and Laboratory Data Acquisition

Patient demographics are categorized and tabled according to circuit group. The following data was included for comparison: weight; body mass index (BMI); age; smoking history;

preoperative medications and cardiac risk factors. Preoperative laboratory results were summarized. Parameters included for comparison were; white blood cell counts; serum urea and creatinine; baseline ASEMT results and percentage of patients with existing chronic AF per group.

3.2. Methods

3.2.1 Biochemical Analyses

3.2.1.1 Enzyme-Linked Immunosorbent Assay (EIA)

To evaluate inflammatory response, serum levels of the following SAMs sVCAM-1, sICAM-1, sE-selectin and sL-selectin were quantified using commercial EIA kits (R&D Systems, Minneapolis, MN). All tests were run according to manufacturer's instructions. Upper limit of normal for Quantikine human sVCAM-1, Quantikine human sICAM-1/CD54, human sE-selectin/CD62E and human sL-selectin/CD62Ls was 200 ng/mL, 50 ng/mL, 8 ng/mL and 856 ng/mL, respectively. Sensitivity for sVCAM-1, sICAM-1, sE-selectin and sL-selectin was 0.3 ng/mL, 0.096 ng/mL, 0.009 ng/mL and 0.6 ng/mL, respectively. Coefficient of variance for intra-assay and inter-assay precision for sVCAM-1, sICAM-1, sE-selectin and sL-selectin ranged between 5.0-8.8%, 5.0-6.8%, 6.6-8.7% and 3.6-7.8%, respectively.

In order to attain a linear standard curve, each EIA kit required specific sample dilution. Testing started at full strength and ended at the following dilutions for each kit: sVCAM-1, 20-fold; sICAM-1, 20-fold; sE-selectin, 10-fold; sL-selectin, 100-fold.

The EIA Colormetric readings were done on a Biotek plate reader; model EL808 with standard filter wheels (405 nm, 450 nm, 490 nm, 630 nm). The spectrophotometer uses Biotek Gen5 Microplate Data Collection and Analysis software (Winooski, VT).

3.2.1.2 Serum Glucose, Lactate, Urea and Creatinine

Serum lactate levels were analyzed on a Radiometer Flex 815 analyzer (Radiometer, Canada). Glucose, urea and creatinine levels were determined on a Cobas 4000 series analyzer (Roche Diagnostics Canada, Laval, Quebec). Analytical detection limits of lactate, glucose, urea and creatinine values respectively are; 0.0-30 mmol/L; 0.0-41.6 mmol/L; 0.5-40 mmol/L and 5.0-2700 μmol/L. Blood was collected via an arterial line and contained within BD Vacutainer tubes containing plasma separator lithium heparin until analysis.

3.2.2 Statistical Analysis

Descriptive statistics were run for all independent outcome variables. In order to better understand the data, initial observations included mean, standard deviation (SD), minimum result, maximum result and standard error. Upon presentation of results, only mean, standard deviation and P values are shown where applicable. One-way analysis of variance (ANOVA) for

all 101 patients in each group was undertaken to ensure comparability of base line demographic variables. One-way ANOVA's were done to ascertain mean SAM differences between groups related to preoperative medications Aprotinin and Tranexamic Acid (TA), postoperative AF, permanent AF and smokers. As there were three categories for smoking (non, x-smoker, current smoker), ANOVA was rerun excluding x-smokers. The importance of gender and diabetes was ascertained using a one-way ANOVA. Chi-square was executed for categorical data. Correlations were run comparing all independent (circuit groups and demographics) and dependent outcome variables to ascertain and confirm existing relationships. Dependent group outcomes were compared using ANOVA and paired t-test comparisons. Paired two-tailed t-tests were performed to compare results of all laboratory parameters at the three different time intervals. A *P* value of less than 0.05 was considered significant.

4 RESULTS AND DISCUSSION

4.1 Results, Patient Characteristics/Demographics

Demographics were collected and compiled before surgery. Postoperative data collection continued until the patient was discharged from hospital. Demographics and clinical characteristics of the patients are summarized in Table 4.1.

ANOVA was used to expose any differences between the groups. The five different groups were classified as independent variables or factors while the patient demographics were classified as the dependent variables when running the analysis. Other than antifibrinolytic administration, there were no significant differences. Further post-hoc analysis was not necessary.

Aprotinin and TA, both antifibrinolytics, where unequally administered and dosage was not standardized according to patient weight. The groups Bioline™ and PMEA did not receive Aprotinin as this drug was removed from market after the study began. Patients in the Bioline™ and PMEA BCC groups were therefore administered TA. Though they are not identical drugs, they were both given to achieve the same goal, which is to decrease postoperative hemorrhage. Patients with a history of diabetes did not receive antifibrinolytics.

4.1.1 Diabetics

To determine if there was enhanced SAM expression in diabetic patients, a one-way ANOVA was run using diabetic patients as the predictor. The only SAM trending towards significance was sVCAM-1 at 6 hours ($P = 0.067$). Expression in the diabetic group was more elevated. Otherwise, there was no difference in SAM expression between diabetic and non-diabetic patients.

Laboratory results were significantly elevated in diabetic patients compared to non-diabetics for serum creatinine at baseline ($P = 0.013$), serum creatinine at six hr ($P = 0.019$), serum creatinine at 72 hr ($P = 0.001$), baseline serum urea ($P < 0.001$) and serum urea at 72 hr ($P = 0.011$) as is demonstrated in Table 4.2. Scrutiny of the phosphorylcholine BCC group in which there were 8 diabetic and 12 non-diabetic patients revealed significantly elevated preoperative serum creatinine levels in the diabetic patients. Serum creatinine levels remained significantly elevated at the 72nd postoperative hr. Significantly different laboratory results in all groups did not translate into worse postoperative outcomes in regards to ICU or hospital length of stay. Neurologic outcomes were similar between diabetics and non-diabetics as well. Comparison between the diabetic patients treated with a BCC and diabetic patients treated with the control circuit yielded several interesting findings despite the fact that the analysis was not sufficiently powered. Results in Table 4.3 show decreased transfusions of RBCs and platelets, coupled with a decrease in postoperative chest tube drainage (CTD) in the BCC group.

Table 4.1 Study patient demographics and baseline preoperative conditions

	Control N = 18	Phosph N = 20	Bioline™ N = 21	PMEA N = 21	Trillium™ N = 21	*P* value
Sex (% Female)	50	20	24	24	19	0.185
Diabetics (%)	17	40	29	10	24	0.201
Age (years)	67± 9.6	68 ± 9.2	67 ± 12.4	67± 8.9	69 ± 9.7	0.966
Smoke (%)	5	20	22	10	10	0.357
Weight	83± 16.8	88 ± 15.4	82 ± 16.5	88 ± 11.3	86 ± 9.4	0.569
BMI	30± 5.2	30 ± 4.5	28 ± 4.7	30± 4.4	30 ± 5.0	0.945
Aprotinin (%)	28	25	0	0	38	0.001
TA (%)	33	25	29	38	19	0.714
WBC $x10^9$/L	6.8± 1.4	7.1± 1.5	6.6 ± 1.9	7.0± 2.4	6.6 ± 1.8	0.857
Chronic AF (%)	0	20	10	10	10	0.380
Serum Creatinine	97± 17.3	102 ± 23	94 ± 16.1	92 ± 13.8	100 ± 23	0.463
Serum Urea	5.8± 1.8	5.4 ± 1.9	5.5 ± 1.6	5.0± 1.2	6.1 ± 2.5	0.354
ASEMT-1 (%)	88	94	92	95	94	0.185

Mean ± SD where applicable. Phosph phosphorylcholine; Weight kg; BMI body mass index; WBC, white blood cell count; TA Tranexamic Acid; Creatinine μmol/L; Urea mmol/L; ASEMT-1, antisaccadic eye movement test refers to preoperative cognitive neurological assessment scores

Table 4.2 Study outcome differences between diabetics and non-diabetics

	Diabetics	Non-Diabetics	*P* value
Females (N)	18	9	0.172
Males (N)	15	59	0.172
RBC (units)	1.71 ± 2.01	0.79 ± 1.3	0.045
Baseline Serum Creatinine	105.54 ± 28.15	94.51 ± 14.59	0.013
6 hr Serum Creatinine	111.21 ± 28.88	98.82 ± 19.69	0.019
72 hr Serum Creatinine	115.67 ± 32.29	98.18 ± 18.08	0.001
Baseline Urea	6.80 ± 2.58	5.18 ± 1.48	0.000
72 hr Urea	7.27 ± 3.74	5.57 ± 2.28	0.011

Mean ± SD; Creatinine µmol/L; Urea, mmol/L

Table 4.3 Significant diabetic outcome differences between treatment groups

	BCC group N = 21	Control Group N = 3	*P* Value
RBC (units)	1.38 ± 1.94	4.0 ± 0.00	0.031
Platelets (units)	0.48 ± 1.50	5.0 ± 8.66	0.022
CTD (mL)	748.81 ± 396.83	1551.67 ± 1611.48	0.046
Ventilation (hr)	8.57 ± 4.01	14.00 ± 6.23	0.053

Mean ± SD

4.1.2 Sex-Based Variations

When all 101 patients were evaluated for sex-based outcome variations, several significant differences were found as shown in Table 4.4. In comparison to their male counterparts, females were transfused more units of RBC ($P = 0.009$), had a higher postoperative glucose ($P = 0.014$), a lower serum creatinine at 6 hr ($P = 0.011$), a lower urea at 6 hr ($P = 0.044$), spent more time on a ventilator ($P < 0.001$) and more time in ICU ($P = 0.022$). In order to ascertain the importance of the identical number of females in the control group (females = 9, males = 9), one-way ANOVA using sex as the predictor was conducted looking at all postoperative outcomes. A lower preoperative baseline serum creatinine was the only significantly different sex-based observation in the control group. When the females in the control group were compared to the females in the BCC group, L-selectin at 6 hr was lower in the BCC group (P = 0.020) demonstrating decreased activation of WBC. Importantly, females in the BCC group also received fewer platelet transfusions (p = 0.039).

Table 4.4 Study outcome differences between genders

Variable	Male (n=74)	Female (n=27)	*P* value
BMI	29.32	31.7	0.023
RBC (units)	0.77 ± 1.44	1.67 ± 1.67	0.009
6 hr Glucose	8.98 ± 2.15	10.23 ± 2.56	0.014
6 hr Creatinine	105.22 ± 21.84	92.30 ± 22.65	0.011
6 hr Urea	5.82 ± 1.75	5.00 ± 1.88	0.044
Ventilation Time (hr)	7.56 ± 3.4	14.44 ± 13.39	0.000
ICU Time (hr)	23.69 ± 4.54	28.70 ± 17.03	0.022

Mean ± SD; Creatinine µmol/L; Urea µmol/L; Glucose mmol/L

4.2 CPB Data and Postoperative Resource Utilization Categorized by Group

Patient CPB data and postoperative results categorized by BCC groups are shown in Table 4.5. One-way ANOVA was used to expose any differences between groups. The five different groups were classified as independent variables or factors, while CPB data and resource utilization were classified as dependent variables when running analysis. No significant differences were found. Of note, three of the five groups (control, Phosph and Trillium) were administered the antifibrinolytic aprotinin. Two of the three groups receiving aprotinin showed significantly reduced CTD postoperatively; ($P = 0.03$) for the phosphorylcholine group and ($P =$

0.043) for the Trillium™ group, respectively. All groups including Bioline and PMEA received TA. TA was not as effective at decreasing postoperative hemorrhage.

Table 4.5 CPB data and postoperative resource utilization categorized by group

N	Control 18	Phosph 20	Bioline™ 21	PMEA 21	Trillium™ 21	*P* value
Bypass (min)	100 ± 21.2	100 ± 20.1	110 ± 20.8	101 ± 25.9	97 ± 21.7	0.413
Crossclamp (min)	80 ± 19.1	79 ± 18.5	88 ± 19.9	82 ± 22.9	77 ± 18.8	0.449
Surgery (% CABG)	67%	70%	81%	86%	76%	0.539
CTD (mL)	789 ± 733	756 ± 464	848 ± 430	1004 ± 729	899 ± 627	0.718
RBC (units)	1.4 ± 1.7	0.8 ± 1.5	1.3 ± 1.7	0.7 ± 1.2	1.0 ± 1.6	0.535
FFP (units)	0.1 ± 0.5	0.3 ± 1.3	0.29 ± 0.96	0	0	0.550
Platelets (units)	1.4 ± 3.8	1.0 ± 2.1	0.71 ± 1.8	0.24 ± 1.1	1.0 ± 2.6	0.643
Ventilation Time (hr)	10.4 ± 4.9	8.1 ± 4.1	8.6 ± 3.7	12.7 ± 15.7	7.2 ± 2.8	0.198
ICU Time (hr)	24.9 ± 6.6	23.7 ± 4.7	24.7 ± 6.3	28.8 ± 18.5	23.0 ± 3.8	0.365
Hospital Length of Stay (days)	5.6 ± 2.1	5.9 ± 3.9	5.9 ± 3.2	5.9 ± 1.9	5.1 ± 1.1	0.875
Pump balance (mL)	784 ± 782	432 ± 466	719 ± 497	454 ± 485	765 ± 989	0.267

Mean ± SD where applicable. Phosph phosphorylcholine; CTD Chest tube drainage, cumulative to 18 hr. Test for homogeneity of variance significant ($P < 0.05$) for UFFP and UPlatelets

4.3 CPB and SAM

Table 4.6 shows mean serum SAM results and SD. All but sE-selectin baseline compared to 6 hr postoperatively showed significant changes in a two-paired sample t-test of SAM levels.

Table 4.6 Serum SAM values in ng/mL

N = 101	Baseline Pre-op	6 hr post-op	72 hr post-op
sVCAM-1	721 ± 308	799 ± 356**	1036 ± 377*††§§*
sICAM-1	251 ± 76	226 ± 78**	308 ± 120 *††§§*
sE-selectin	37 ± 14	39 ± 16	34 ± 14 *††§§*
sL-selectin	668 ± 223	507 ± 187**	547 ± 199 *††§*

Mean ± SD

** 6 hr compared to baseline ($P < 0.01$);

†† 72 hr compared to baseline ($P < 0.01$);

§ 72 hr compared to 6 hr ($P < 0.05$);

§§ 72 hr compared to 6 hr ($P < 0.01$).

Significantly elevated levels of sVCAM-1 ($P = 0.013$) were found at baseline in patients who went on to develop AF postoperatively. These levels were still significantly elevated at 6 ($P = 0.033$) and 72 hr ($P = 0.002$). sVCAM-1 expression in patients with AF is demonstrated in Table 4.7. One patient with AF had exceptionally high sVCAM-1 levels at all time intervals and was removed from the analysis as an outlier.

Table 4.7 sVCAM-1 expression in patients with postoperative AF

N = 100	AF N = 32	No AF N = 68	*P* Value
Baseline sVCAM-1	784 ± 261	661 ± 207	0.013
6 hr sVCAM-1	845 ± 303	718 ± 255	0.033
72 hr sVCAM-1	1116 ± 319	919 ± 256	0.002

Mean ± SD in ng/mL; AF atrial fibrillation; No AF no atrial fibrillation

4.4 Mean Serum SAM Levels in all Groups

4.4.1 sVCAM-1 Expression

sVCAM-1 expression is shown in Table 4.8. Serum levels rose in all groups from baseline through to 72 hr with a significant change from baseline to 72 hr and from 6 hr to 72 hr in all groups.

There was no significant difference in sVCAM-1 expression identified between groups either at baseline or at 6 and 72 hr. When the control group was compared to all the patients in the BCC group, no difference in sVCAM expression was found.

Table 4.8 Serum sVCAM-1 concentrations

sVCAM-1	Control N = 18	Phosph N = 20	Bioline™ N = 21	PMEA N = 21	Trillium™ N = 21
Pre-op baseline	697 ± 214	822 ± 506	654 ± 216	673 ± 239	760 ± 260
6 hr	785 ± 321	949 ± 525	727 ± 274	731 ± 281	809 ± 314
72 hr	1035 ± 335††§§	1177 ± 548††	906 ± 282††§§	988 ± 312††§§	1079 ± 334††§§

Mean group results in ng/mL ± SD; Phosph phosphorylcholine
†† 72 hr compared to pre-op ($P < 0.01$);
§ 72 hr compared to 6 hr ($P < 0.05$);
§§ 72 hr compared to 6 hr ($P < 0.01$).

4.4.2 sICAM-1 Expression

sICAM expression is shown in Table 4.9. Significant differences were observed in all groups between baseline and 72 hr, and between 6 hr and 72 hr. All sICAM values decreased at 6 hr from baseline, whereas values at 72 hr were significantly higher than that at baseline. A significant difference in sICAM expression between baseline and 6 hr was found in the uncoated control group alone.

There was no significant difference in sICAM expression identified between BCC groups. When all patients in the BCC group were compared to the control group in an independent samples t-test however, it was found that sICAM decreased by a greater amount ($P = 0.001$) at 6 hr in the control group compared to the BCC study group.

Table 4.9 Serum sICAM-1 concentrations

sICAM	Control N = 18	Phosph N = 20	Bioline™ N = 21	PMEA N = 21	Trillium™ N = 21
Pre-op baseline	231 ± 87	247 ± 84	272 ± 68	270 ± 62	232 ± 72
6 hr	200 ± 75*	227 ± 62	243 ± 71	231 ± 103	224 ± 75
72 hr	297 ± 153 †§§	284 ± 110 †† §§	326 ± 120 §§	328 ± 93 ††§§	302 ± 130 †§§

Mean group results in ng/mL ± SD; Phosph phosphorylcholine;
* 6 hr compared to pre-op baseline ($P < 0.05$);
† 72 hr compared to pre-op baseline ($P < 0.05$);
†† 72 hr compared to pre-op baseline ($P < 0.01$);
§§ 72 hr compared to 6 hr ($P < 0.01$)

4.4.3 sE-selectin Expression

sE-selectin expression is shown in Table 4.10. Very little difference in sE-selectin expression was found between any time periods other than in Trillium and PMEA. The Trillium group showed a significant change in sE-selectin expression between 6 and 72 hr, and between baseline and 72 hr. A significantly lower value was observed in the PMEA group from baseline to 72 hr. No significant difference was found between control and BCC groups.

Table 4.10 Serum sE-selectin concentrations

sE-selectin	Control N = 18	Phosph N = 20	Bioline™ N = 21	PMEA N = 21	Trillium™ N = 21
Pre-op baseline	40 ± 13	33 ± 13	40 ± 15	38 ± 17	33 ± 12
6 hr	40 ± 15	38 ± 19	43 ± 15	37 ± 17	35 ± 14
72 hr	39 ± 14	31 ± 13	39 ± 12	34 ± 16†	29 ± 12††§§

Mean group results in ng/mL ± SD; Phosph phosphorylcholine
† 72 hr compared to pre-op baseline ($P < 0.05$);
†† 72 hr compared to pre-op baseline ($P < 0.01$);
§§ 72 hr compared to 6 hr ($P < 0.01$)

4.4.4 sL-selectin Expression

sL-selectin results are shown in Table 4.11. Change in serum levels from baseline to 6 hr was significant for phosphorylcholine, Bioline™, Trillium™ and PMEA. This change was not

significant for the control group. Change in expression was significant from 6 hr to 72 hr in the phosphorylcholine and Bioline™ groups. Change in expression was significant in all groups from baseline to 72 hr.

sL-selectin levels were significantly elevated in the Control group when compared to Bioline™ ($P < 0.007$) and Trillium™ ($P < 0.039$) at 6 hr postoperatively. No significant differences between the groups remained at 72nd hr. When the BCC patients were grouped together and compared to the patients in the control group it was found that sL-selectin expression was significantly higher ($P = 0.005$) in the control group at 6 hr. This difference no longer existed at 72 hr.

Table 4.11 Serum sL-selectin concentrations

sL-selectin	Control N = 18	Phosph N = 20	Bioline™ N = 21	PMEA N = 21	Trillium™ N = 21
Pre-op baseline	736 ± 229	751 ± 340	604 ± 170	666 ± 157	594 ± 136
6 hr	635 ± 194$^{\#}$	528 ± 255**	437 ± 137**$^{\#}$	486 ± 122**	471 ± 159**$^{\#}$
72 hr	617 ± 161††	620 ± 303$^{\dagger\dagger\,\S}$	507 ± 183†§	520 ± 155††	594 ± 125††

Mean group results in ng/mL ± SD; Phosph phosphorylcholine
** 6 hr compared to pre-op baseline ($P < 0.01$)
$\dagger$ 72 hr compared to pre-op baseline ($P < 0.05$)
$\dagger\dagger$ 72 hr compared to pre-op baseline ($P < 0.01$)
$\S$ 72 hr compared to 6 hr ($P < 0.05$)
Difference between control and Bioline™ ($P = 0.007$) and control and Trillium™ ($P = 0.039$)

4.5 Effect of BCC on Common Clinical Parameters

4.5.1 Changes from Baseline to 72 hr in Serum Creatinine, Urea, Lactate and Glucose

Measured serum creatinine and urea levels along with postoperative serum lactate and glucose results are shown in Table 4.12. Phosphorylcholine group alone exhibited a significant change in mean serum creatinine levels between baseline and 6 hr. This group also demonstrated a significant change from 6 to 72 hr unlike any other group. Measured serum creatinine levels from baseline to 72 hr were significantly different in the two heparin-coated BCC groups Bioline™ and Trillium™.

Serum urea values were significantly different from baseline to 6 hr and from baseline to 72 hr in the phosphorylcholine and Bioline™ BCC groups.

Trillium™ urea levels at 6 hr postoperatively were significantly higher than Bioline™ (P = 0.044) and PMEA (P = 0.021) with continuing significance between Trillium™ and PMEA (P = 0.038) at 72 hr. Glucose and lactate levels were not different between groups.

Table 4.12 Effect of BCC on chemistry and renal function parameters

Variable	Control	Phosph	Bioline™	PMEA	Trillium™
N	18	20	21	21	21
Pre serum creatinine	97 ± 17	102 ± 23	94 ± 16	92 ± 14	100 ± 23
6 hr serum creatinine	104 ± 27	111 ± 27*	96 ± 23	96 ± 14	102 ± 21
72 hr serum creatinine	103 ± 21	106 ± 23§	103 ± 22†	92 ± 14	109 ± 31†
Pre urea	5.8 ± 1.8	5.4 ±1.9	5.5 ± 1.6	5.0 ± 1.2	6.1 ± 2.5
6 hr urea	5.6 ± 1.6	6.0 ± 1.9**	5.0 ± 1.7**#	4.9 ± 1.4#	6.5 ± 2.0#
72 hr urea	6.0 ± 2.9	6.9 ± 2.9†	5.6 ± 2.1†	4.6 ± 1.4#	7.0 ± 3.7#
6 hr serum lactate	1.9 ± 0.9	1.8 ± 0.9	1.7 ± 0.88	2.0 ± 1.0	1.9 ± 1.2
6 hr glucose	10.1 ± 1.7	8.6 ± 2.3	8.5 ± 2.4	10.4 ± 2.6	9.0 ± 1.9

Mean group results ± SD where applicable. Phosph phosphorylcholine; Creatinine μmol/L, urea mmol/L, lactate mmol/L, glucose mmol/L

* 6 hr compared to pre-op baseline ($P < 0.05$);
** 72 hr compared to pre-op baseline ($P < 0.01$);
† 72 hr compared to pre-op baseline ($P < 0.05$);
§ 72 hr compared to 6 hr ($P < 0.05$);
# Group differences ($P < 0.05$)

4.5.2 Bleeding and Transfusion Requirements

Table 4.13 shows postoperative bleeding and transfusion requirements. This was the blood lost through chest tubes, measured at 18 hr, and units of blood products transfused until hospital discharge. These differences were not statistically different between groups nor were they different when all the BCC groups were compared to the control group.

Table 4.13 Bleeding and transfusion requirements

	Control	Phosph	Bioline™	PMEA	Trillium™	*P* value
N	18	20	21	21	21	------
CTD (mL)	789 ± 733	756 ± 464	848 ± 430	1004 ± 729	899 ± 627	0.718
RBC (units)	1.4 ± 1.7	0.8 ± 1.5	1.3 ± 1.7	0.7 ± 1.2	1.0 ± 1.6	0.535
FFP (units)	0.1 ± 0.5	0.3 ± 1.3	0.29 ± 0.96	0	0	0.550
Platelets (units)	1.4 ± 3.8	1.0 ± 2.1	0.71 ± 1.8	0.24 ± 1.1	1.0 ± 2.6	0.643

Mean ± SD where applicable. Phosph phosphorylcholine; CTD Chest tube drainage, cumulative to 18 hr; RBC units of RBC transfused; FFP units of FFP transfused; Platelets units of platelets transfused. Test for homogeneity of variance significant ($P < 0.05$) for FFP and Platelets

4.5.3 Effect of BCC on AF, Changes in Weight, Pump Balances and WBC Counts

WBC counts were significantly different between all time periods for each group except Bioline™ from 6 hr to 72 hr as shown in Table 4.14. Table 4.14 also shows the significantly different weight changes from baseline to 6 and 72 hr. There were no significant differences in mean pump balances between groups.

Despite what appears to be a large increase in new incidences of AF in the PMEA BCC group (8 new patients), this was not significant ($P = 0.24$).

Table 4.14 Patient changes in WBC count, weight, pump balance and incidence of AF

	Control	Phosph	Bioline™	PMEA	Trillium™
	N = 18	N = 20	N = 21	N = 21	N = 21
Pre WBC	6.8 ± 1.4	7.1 ± 1.5	6.6 ± 1.9	7.0 ± 2.4	6.6 ± 1.8
6 hr WBC	14.5 ± 3.9**	15.5 ± 3.4**	12.2 ± 4.4**	15.5 ± 6.8**	15.9 ± 6.0**
72 hr WBC	11.6 ± 2.9†††§§	12.5 ± 3.4†††§§	11.2 ± 2.8††	12.4 ± 5.1†††§§	12.1 ± 3.7†††§§
Pre Weight	82.9 ± 16.8	88.0 ± 15.4	82.4 ± 17.2	88.3 ± 11.3	85.8 ± 9.4
6 hr Weight	85.2 ± 16.4**	91.3 ± 14.7**	85.9 ± 17.2**	92.7 ± 11.0**	88.2 ± 10.2**
72 hr Weight	83.0 ± 15.9†§§	90.6 ± 15.5††	84.2 ± 17.1†††§§	90.0 ± 11.1§§	87.4 ± 10.0†††§§
Pump balance (mL)	784 ± 782	432 ± 466	719 ± 497	454 ± 485	765 ± 989
AF Chronic N (%)	0 (0)	4 (20)	2 (10)	2 (10)	2 (10)
AF Postop N (%)	3 (17)	3 (19)	3 (24)	8 (42)	6 (32)
AF Discharge N (%)	0 (0)	1 (6)	0 (0)	2 (11)	0 (0)

Mean group results ± SD where applicable; Phosph phosphorylcholine; WBC white blood cells x 10^9/L; Weight kg; AF atrial fibrillation; N number of patients

** 6 hr compared to pre (P<0.01)

† 72 hr compared to pre (P<0.05)

†† 72 hr compared to pre (P<0.01)

§§ 72 hr compared to 6 hr (P<0.01)

4.5.4 Neurocognitive Outcomes and Intensive Care and Hospital Length of Stay

Mean group results of the antisaccadic eye movement test, which were done preoperatively and at 72 hr postoperatively, are shown in Table 4.15. One-way ANOVA was used to expose possible differences between groups. When running statistical analyses, the five groups were classified as independent variables or factors while ventilation, ICU time and hospital length of stay were classified as dependent variables. No significant differences were found. Paired samples t-tests were run for assessment of the ASEMT. Significant differences from baseline

values were seen for PMEA and Trillium™ groups. Total time requiring mechanical ventilation and intensive care, as well as total time spent in hospital was not significantly different between groups.

Table 4.15 Patient neurocognitive outcomes, ventilator times, and ICU and hospital length of stay

Group	Control N = 18	Phosph N = 20	Bioline™ N = 21	PMEA N = 21	Trillium™ N = 21	*P* value
Pre-op ASEMT (%)	88	94	92	94	94	0.185
72 hr ASEMT (%)	93	86	85	81 ($P = 0.029$)	87 ($P = 0.023$)	
Ventilation Time (hr)	10.4 ± 4.9	8.1 ± 4.1	8.6 ± 3.7	12.7 ± 15.7	7.2 ± 2.8	0.198
ICU Time (hr)	24.9 ± 6.6	23.7 ± 4.7	24.7 ± 6.3	28.8 ± 18.5	23.0 ± 3.8	0.365
Hospital Length of Stay (days)	5.6 ± 2.1	5.9 ± 3.9	5.9 ± 3.2	5.9 ± 1.9	5.1 ± 1.1	0.875

Mean ± SD; Phosph phosphorylcholine; ASEMT antisaccadic eye movement test; Phosph phosphorylcholine

4.6 Discussion

4.6.1 Comparison of BCC Effect on Diabetic Patients

Diabetic patients are susceptible to premature graft closure and renal failure due to the nature of their disease. Antifibrinolytic administration was withheld in these patients because of the potential drug-induced amplification of these risks. As the diabetic patients in this study were not administered antifibrinolytics, a relevant comparison of postoperative bleeding and transfusion requirements was possible. Diabetics who underwent CPB with a BCC did bleed less and did receive fewer RBC transfusions perioperatively. Unfortunately, as there were only 3 diabetic patients in the control group *vs* 21 diabetic patients in the BCC group, the results from these analyses cannot be considered conclusive because of insufficient power. This comparison in a well-designed study merits future consideration.

Markers of inflammation were higher in diabetics preoperatively as demonstrated by increased sVCAM-1 serum levels. Postoperatively, WBC counts were elevated and serum urea and creatinine levels were significantly higher than in non-diabetics. At best, one would hope to see no difference in the above stated laboratory results between diabetics and non-diabetic when a BCC is used. This was not the case. ICU and hospital length of stay was not significantly different between any of the groups observed or between the BCC groups and the uncoated control group. Scrutiny of the phosphorylcholine BCC group consisting of eight diabetics and 12 non-diabetics failed to demonstrate important clinical outcome differences. This may have been due to a type II error. Larger study groups may have unmasked important clinical outcome differences as opposed to our observations in a smaller group. The diabetic patients in the BCC groups did not have better outcomes than the diabetic patients in the control group. BCCs may not be effective enough in diminishing inflammatory response to the point of improving important clinical outcomes such as ICU and hospital length of stay, both of which are influenced by a multitude of critical factors beyond coated CPB circuits.

4.6.2 Comparison of BCC Effect on Females

When all 101 patients were included for comparison, our observations yielded the expected sex-based outcome differences, based upon reported studies. The CPB circuit impacted more significantly on females, resulting in higher tranfusion requirements and lower serum creatinine and urea levels, all of which are subsequent to hemodilution incurred during CPB. The mean BMI of the 27 females included in this study was higher than for the 74 males, vividly demonstrating how susceptible females are to the hemodilution effect of CPB. Lower circulating RBC volumes (relative to increased hemoglobin levels in males) predisposes females to hemodilution, transfusions, respiratory problems, and mediastinal infections (Ranucci M, *et al.* 2008). Females in our study did indeed spend significantly more time on a mechanical ventilator and in ICU as compared to males. Higher postoperative glucose levels could be the result of an elevated inflammatory response in females when compared to males. A type II error was likely the reason why no sex-based outcome differences were observed when the 9 females were compared to the 9 males in the control group.

Females in the BCC groups had improvements in outcomes when compared to the females in the control group. The decreased inflammatory response observed, though short lived, was enough to decrease the need for platelet transfusions. Clinical protocols resulting in decreased transfusion requirements should be adopted as standard of practice. The role of the BCC in improving the remaining laboratory and clinical outcomes in females was however limited. Further improvements will likely only be achieved from a multi-factorial effort at diminishing the preoperative anemia associated with females coupled with a smaller CPB circuit.

4.6.3 SAM Expression due to CPB

Results from this study showed that SAM expression changed significantly from baseline to seventy-two hr in all 5 groups except for sE-selectin. Wildhirt *et al.* (2001) and Serrano *et al.* (2010) noted similar significant increases in sICAM expression in their on-pump group from baseline to seventy-two hr compared to their off-pump group in their study. Higher on-pump postoperative sICAM levels did not result in worse clinical outcomes for the patients in those studies. The results of Wei *et al.* (2003) show the same immediate postoperative fall in sICAM levels after on-pump procedures as seen in our study whereas the immediate postoperative sICAM level in off-pump cardiac procedures remained near baseline levels. In both groups sICAM levels had risen in a similar fashion at seventy-two hr to what was observed in our study. Patients who undergo CPB can expect to gain anywhere from 2-4 kg during the perioperative period. This increased weight is due to the added fluid volume from CPB induced hemodilution and the necessary volume requirements due to interstitial fluid shifts (third spacing). Greater cell membrane permeability is consequent to the inflammatory response. Consequently, it would seem that the decreased sICAM levels seen at 6 hr are sensitive to CPB induced hemodilution. Mobilization of this fluid via diuresis in the postoperative period generally returns the patient to their preoperative weight. There was nothing found in the literature to explain why sICAM was sensitive to hemodilution while sVCAM was not. Importantly, results from Kalawski *et al.* (2003) compared sVCAM and sICAM analysis from blood sampled from an arterial line vs blood sampled from the coronary sinus upon reperfusion in patients undergoing CABG. The aim of their study was to assess the effectiveness of cold blood over cold crystalloid cardioplegia in terms of myocardial protection. Measured sICAM levels were elevated 30 min post-reperfusion when measured from the coronary sinus but decreased when the samples were drawn from the arterial line. Further evidence that sICAM is susceptible to hemodilution. sVCAM levels did not differ based on the sampling site. When sampled from the coronary sinus, sICAM and sVCAM increased more significantly in the cold crystalloid group after myocardial reperfusion demonstrating unsurprisingly improved myocardial protection when using whole blood. As the significant drop in sICAM levels in this study was evident in the un-coated control group alone, one could conclude that the BCC were able to attenuate the initial inflammatory response as evidenced by higher 6 hr BCC sICAM levels.

Postoperative sVCAM-1 (Wan *et al.* 2004) and sE-selectin (Matata *et al.* 2000) levels were higher in the on-pump group when compared to off-pump cardiac surgery as well. No postoperative clinical outcome differences between the on and off-pump groups in these studies were observed despite these higher SAM levels. Hambsch *et al.* (2002) stated that sL-selectin levels, similarly significantly reduced postoperatively in the on-pump group compared to our study, were the result of circulating neutrophils post-CPB having significantly reduced adhesiveness and activity. This phenomenon also appears to be sustained for greater periods of time (as much as three days) in the on-pump group when compared to their control group of off-pump patients.

Patient response to surgery and CPB was varied and manifested by the large SD in serum sICAM expression at 72 hr. Despite the fact that all patients presented to the operating room with the typical co-morbidities associated with cardiac disease, patient response to surgery and CPB was observably wide-ranging. Interestingly, the SD seen in the PMEA group was also quite large at 6 hr. Whether or not this is a relevant observation due to the BCC PMEA is difficult to determine.

Expression of sVCAM-1, sICAM, sE-selectin and sL-selectin confirms previously established patterns of expression for patients undergoing CPB (Blume, 1997; Galea, 1998; Wei, 2003; Pasnik, 2004). Not surprisingly, preoperative serum sICAM, sE-selectin and sL-selectin levels in our older cardiac patients were higher than the levels reported for normal healthy controls groups.

4.6.4 Comparison BCC Effect on Release of Soluble Adhesion Molecules

There were few significant differences in SAM release between the BBC and the uncoated control group. sICAM concentrations were significantly different in the control group from baseline to 6 hr compared to non-significant changes in all the BCC groups. Additionally, sL-selectin levels were significantly lower at 6 hr compared to baseline in all the BCC groups unlike the control group where the mean fall from baseline to 6 hr was less pronounced. The lower mean serum levels of sL-selectin measured in the BCC groups at 6 hr compared to the uncoated control group demonstrates the protective effect of the BCC resulting in decreased activation and shedding of this SAM from the leukocyte thereby enabling future leukocyte extravasation into surgically disrupted tissues only where needed.

When looking at group differences, sL-selectin levels at 6 hr were significantly higher in the control group when compared to mean levels in the Bioline™ and Trillium™ BCC. Bioline™ and Trillium™ BCC coatings are both made with heparin. These results may indicate that heparin-based coatings may be more effective in preserving neutrophil adhesiveness. Unfortunately, differences between the BCC groups and the control group were no longer evident at seventy-two hr. These results might indicate that BCC, designed to decrease the inflammatory response consequent to the artificial environment of CPB might indeed be successful at doing so but that this effect is limited.

4.6.5 Comparison of BCC Effect on Renal Function and Volume Retention

BCC group results were no different from the control group in regards to postoperative kidney function parameters such as serum urea and creatinine. A significant difference was found in the phosporylcholine-coated group alone between baseline and 6 hr serum creatinine levels. Very significantly, the pressure drop across the primox oxygenator was excessively higher than in any of the other oxygenators tested. As previously mentioned, high changes in pressure are correlated

with increased levels of hemolysis. Increased levels of hemolysis may have resulted in the augmented short-term decrease in renal function over and above the usual deterioration observed. The CPB circuit, and particularly oxygenator design may be a more important consideration when it comes to postoperative renal function then whether or not the CPB has a BCC.

No significant difference was found in postoperative weight (fluid) gain between the control group and the BCC groups demonstrating, once again, the limited effect the BCCs have in attenuating inflammatory response. Of note the PMEA group had the greatest 6 hr weight gain of all the groups. This is a relevant finding, considering PMEA had significantly low postoperative ASEMT scores and the highest incidence of AF.

4.6.6 Comparison of BCC Effect on Bleeding and Transfusion Requirements

Although the need to transfuse cardiac surgical patients has been greatly reduced since the days before permissive hemodilution became an accepted practice, CPB-assisted cardiac surgical patients still frequently require RBC transfusions perioperatively due to anemia. These patients may then go on to require additional platelet and plasma transfusions post-CPB as well. The risks involved with receiving a blood transfusion are well known (Hajjar, 2010) and all measures were taken to reduce this risk.

In this study, 38% of all patients received a RBC transfusion, 3% received plasma and 13% received platelets at some point during their peri-operative phase. No significant transfusion differences between patients in the control group and patients in the BCC groups where found. This is a different finding from that of Ranucci *et al.* (2009), (a systematic review) which concluded that BCCs reduce the risk of RBC transfusions. Unfortunately, the widely varying antifibrinolytic administration in this study may have concealed any benefit the BCCs might have realized.

4.6.7 Comparison of BCC Effect on Preventing AF

Significantly elevated levels of sVCAM-1 were found at baseline in patients who went on to develop AF postoperatively. These levels were still significantly elevated at 6 and 72 hr. These results do not agree with results of Cosgrave *et al.* (2005). Their study of 149 patients compared SAM levels at baseline and at 72 hr between patients who developed AF postoperatively and those that did not. No significant differences were observed between the two groups in their study though preoperative and postoperative sVCAM-1 levels were higher in patients who went on to develop AF. Thirty-seven percent of patients in their study went on to develop new onset AF compared to twenty-three percent in our study. Incidence of postoperative AF was not different in the control group compared to patients in the BCC group, however, forty-two percent of patients in the PMEA group developed postoperative AF. Although this result is not statistically significant, it is clinically and alarmingly relevant. AF contributes to postoperative morbidity and mortality experienced by patients who have undergone CPB-assisted cardiac

surgery. PMEA-coated circuits increasing the inflammatory response and incidence of AF would be the opposite clinical response one would hope for.

4.6.8 Comparison of BCC Effect on NCD

Upon examination it was found that patients in the PMEA and Trillium™ BCC groups had significantly worse ASEMT scores at 72 hr compared to their baseline scores. Though not statistically significant, the increased incidence of AF and in ICU length of stay found in the PMEA group likely contributed to the observed decrease in postoperative ASEMT scores. Nothing could be found to explain the decreased postoperative ASEMT scores in the Trillium™ BCC group. Previously published comprehensive neurological examinations comparing pre and postoperative CPB-assisted cardiac surgical patients, have demonstrated subtle, but consistent short-term postoperative neurocognitive deterioration, which would agree with the results of the patients in this study. By far the most surprising finding of the study might be that ASEMT scores where higher postoperatively compared to preoperatively in the uncoated control group alone. These results might indicate that BCC are not protective at all and may in fact be harmful. Alternatively, a learning curve in researcher/patient communication may also have been responsible for these uncharacteristic findings as this was the first group in which the ASEMT was used in this study.

4.6.9 Comparison of BCC Effect on ICU and Hospital Length of Stay

BCC were unable to improve ICU and hospital length of stay compared to patients who underwent CPB with uncoated circuits. The results from this study are different from the finding of Ranucci *et al.* (2009). Their systematic review of BCC technology involving 36 RCT and 4360 patients showed significantly improved ICU length of stay for patients undergoing CPB-assisted cardiac procedures employing BCC *vs* uncoated circuits. Often, large numbers of patients must be used to detect subtle differences in outcomes. Clinical and laboratory outcome differences may not have translated to improved clinical outcomes such as ICU and hospital length of stay in this study due to the many variables influencing postoperative recovery which were uncontrollable.

Differences between the groups in the present study might have been obvious had the correct outcome parameters been studied. Measuring postoperative usage of cardiac ionotropes and left ventricular contractility, are but two additional parameters, which may have detected clinical differences between the groups.

5 LIMITATIONS OF THIS STUDY

Type II errors may have influenced many of the results in this study. Costs associated with the purchase of EIA kits for serum analysis limited the number of patients included into each group. Larger group sizes would likely have resulted in the same statistically significant findings as demonstrated in the previously published study by Ranucci *et al.* (2009) and Marcoux *et al.* (2009). Marcoux *et al.* (2009) found that when all 181 original patients were included for analyses, more time was spent in ICU by patients in the PMEA group. In the present study, this difference was not significant. Additionally, in the present study, no gender-based outcome differences were observed when the 9 females were compared to the 9 males in the control group unlike the significant differences found when all 101 patients were compared.

Much effort was made to homogenize the perioperative experience for all patients. Even the three cardiac surgeons and four perfusionists were analyzed in order to rule out their contributions towards outcomes comparison results. Being as it may, certain variables could not be accounted for, nor controlled. Antifibrinolytic administration throughout the study was never standardized. Dosing was inconsistent, as was time of administration. Additionally, one of the antifibrinolytics, aprotinin, was removed from market midway through the study due to results from an ongoing systematic review demonstrating a small increase in postoperative renal failure and death associated with its administration (Fraser *et al.* 2008).

Had this study been a blinded, prospective, randomized control trial, rather than a prospective cohort study, the resulting data would have been considered more robust statistically speaking. Randomized control trials are the gold standard in study design. A prospective cohort study was chosen here because it added an element of patient safety. Repeatedly setting up and using the same CPB circuit 36 times in a row before switching to the next manufacturer's BCC meant familiarity with the CPB circuit was possible. Familiarity and routine are elements shown to improve patient safety and outcomes within the busy, critical and at times stressful environment of the cardiac operating room (Kurusz *et al.* 1990). Storage and delivery of enough BCC to allow for randomization would have been extremely difficult, if not impossible, in this local circumstance.

Finally, the present study did not consider the effect of BCC on complement activation. In a study by Fukutomi *et al.* (1996), decreased complement activation was demonstrated when a heparin-coated BCC was compared to an uncoated circuit. Future comparison of complement activation between all the BCC included in the present study may yield significant differences between the groups and the uncoated circuit despite the fact that the clinical outcome parameters measured here were not different.

6 CONCLUSION

The mandate of BCC development is to attenuate the well-documented and repeatedly demonstrated inflammatory response consequent to the contact of blood with artificial CPB surfaces. This broad based inflammatory response is linked to many, concurrent post-CPB morbidities, including NCD, AF, respiratory failure and renal dysfunction of variable degrees. These complications contribute to prolonged ventilation times, ICU admissions and postoperative hospital length of stay.

Four clinically approved and widely used BCC's were studied and compared to an uncoated control group in patients undergoing routine CPB-assisted cardiac surgery at a single institution.

On the basis of the clinical parameters assessed, the lone significant positive clinical outcome parameter detected was the resultant drop in platelet transfusions amongst females in the BCC group. On the basis of the laboratory parameters assessed, the significant difference seen (sICAM and sL-selectin) between the coated and uncoated groups at 6 hr was no longer apparent 72 hr postoperatively. Few differences were noted among the four BCC groups.

In regards to postoperative arrhythmias, patients who developed AF postoperatively seemed predisposed to do so as inflammatory marker sVCAM-1 was significantly higher in these patients at baseline, and remained so at 6 and 72 hr.

In this study, BCC resulted in a modest short-term attenuation of the inflammatory response. Decreased platelet transfusions in females, was, the only detectable improvement in observed clinical outcomes. Despite the flaws in study design associated with antifibrinolytic administration, the results of this study showed the limited beneficial impact of BCC technology.

7 REFERENCES

Aldea GS, Soltow LO, Chandler WL, *et al*. 2002. Limitation of thrombin generation, platelet activation, and inflammation by elimination of cardiotomy suction in patients undergoing coronary artery bypass grafting treated with heparin-bonded circuits. *J Thorac Cardiovasc Surg,* 123(4), 742-55.

Alexander RW. 1994. Inflammation and coronary artery disease. *N Engl J Med,* 331(7), 468-469.

Alexandrakis MG, Passam FH, Sfiridaki K, *et al.* 2004. Clinical significance of circulating endothelial adhesion molecules (sE-selectin and sICAM) in untreated multiple myeloma patients. *Clinica Chimica Acta*, 349(1-2), 39-43.

Alex J, Rao VP, Griffin SC, Cale A, Cowen ME, Guvendik L. 2003. Systemic inflammatory response after cardiopulmonary bypass: the mediators of leukocyte-endothelial adhesion. *Clin Intensiv Care*, 14(3&4), 141-148. "Journal no longer exists"

Anderson LW, Baek L, Degn H, Lehd J, Krasnik M, Rasmussen JP. 1987. Presence of circulating endotoxins during cardiac operations. *J Thorac Cardiovasc Surg,* 93(1), 115-119.

Andreoli TE, Bennett JC, Carpentier CJ, *et al*. 1993. Hematopoiesis. *Cecil Essentials of Medicine.* 3rd ed. Philadelphia, PE: W.B. Saunders Company.

Andresen TK, Svennevig JL, Videm V. 2002. Soluble sVCAM-1 is a very early marker of endothelial cell activation in cardiopulmonary bypass. *Perfusion,* 17(1), 15-21.

Athanasiou T, Al-Ruzzeh S, Stanbridge RD, *et al.* 2003. Is the female gender an independent predictor of outcome after off-pump coronary bypass. *Ann Thorac Surg,* 75, 1153-1160.

Athanasiou T, Aziz O, Mangoush O, *et al*. 2004. Do off-pump techniques reduce the incidence of postoperative atrial fibrillation in elderly patients undergoing coronary artery bypass grafting? *Ann Thorac Surg*, 77, 1567-1574.

Baksaas ST, Videm V, Fosse E, *et al*. 1999. In vitro evaluation of new surface coatings for extracorporeal circulation. *Perfusion*, 114(1), 11-9.

Banks RE, Gearing AJ, Hemingway IK, Norfolk DR, Perren TJ, Selby PJ. 1993. Circulating intercellular adhesion molecule-1 (sICAM-1) E-selectin and vascular cell adhesion molecule-1 (sVCAM-1) in human malignancies. *Br J Cancer*, 68, 122-124.

Baraczka B, Nékám K, Pozsonyi T, Jakab L, Szongoth M, Seszták M. 2001. Concentration of soluble adhesion molecules (sVCAM-1, sICAM-1 and sL-selectin) in the cerebrospinal fluid and

serum of patients with multiple sclerosis and systemic lupus erythematosis with central nervous involvement. *Neuroimmunomodulation*, 9(1), 49-54.

Barclay A. 2003. Membrane proteins with immunoglobulin-like domains--a master superfamily of interaction molecules. *Semin Immunol,* 15(4), 215–23.

Beamer NB, Coull BM, Clark WM, *et al.* 1995. Interleukin-6 and interleukin-1 receptor antagonist in acute stroke. *Ann Neurol,* 37, 800-805.

Bical OM, Fromes Y, Gaillard D, *et al.* 2006. Comparison of the inflammatory response between miniaturized and standard CPB circuits in aortic valve surgery. *Eur J Cardiothorac,* 29, 669-702.

Birnbaum DE, Lehle K. 2006. CPB in high-risk groups: CPB in diabetics. *Perfusion*, 21, 235-238.

Blann AD, Li-Saw-Hee F, Lip GY. 1999. Increased membrane and soluble P-selectin in atrial fibrillation. *Circulation*, 100(17):e86-7.

Blauth CI. 1995. Macroemboli and microemboli during cardiopulmonary bypass. *Ann Thorac Surg,* 59(5), 1300-3.

Blume Ed, Nelson DP, Gauvreau K, *et al.* 1997. Soluble adhesion molecules in enfants and children undergoing cardiopulmonary bypass. *Circulation*, 96(9 Suppl), 352-7.

Bosco MC, Puppo M, Blengio F, *et al*. 2008. Monocytes and dentritic cells in a hypoxic environment: Spotlights on chemotaxis and migration. *Immunobiology*, 213(9-10), 733-49. Epub Sep 21.

Braun JP, Buhner S, Kastrup M, *et al*. 2007. Barrier function of the gut and multiple organ dysfunction after cardiac surgery. *J Int Med Res*, 35(1), 72-83.

Brooker RF, Brown WR, Moody DM, *et al*. 1998. Cardiotomy suction: a major source of brain lipid emboli during cardiopulmonary bypass. *Ann Thorac Surg*, 65(6), 165.

Brown JR, Cochran RP, Lawrence J, *et al*. 2006 Perioperative increases in serum creatinine are predictive of increased 90-Day mortality after coronary artery bypass graft surgery. *Circulation*, 114(1 Suppl), 1409-13

Bruins P, te Velthuis H, Yazdanbaksh AP, *et al*. 1997. Activation of the complement system during and after cardiopulmonary bypass surgery: postsurgery activation involves C-reactive protein and is associated with postoperative arrhythmia. *Circulation,* 96(10), 3542-8.

Budnik A, Grewe M, Gyulfo K, Krutmann J. 1996. Analysis of the production of soluble ICAM-1 molecules by human cells. *Exp Hematol*, 24(2), 352-9.

Burman JF, Chung HI, Lane DA, *et al*. 1994. Role of factor XII in thrombin generation and fibrinolysis during cardiopulmonary bypass. *Lancet*, 344, 1192-1193.

Cariou B, Bonnevie I, Mayaudon H. 2000. Angiographic characteristics of coronary artery disease in diabetic patients compared with matched non-diabetic subjects. *Diabetes Nutr Metab,* 13, 134-41.

Carlson M, Nakamura Y, Payson R, *et al*. 1988. Isolation and mapping of a polymorphic DNA sequence (pMCT108.2) on chromosome 18. *Nucleic Acids Res,* 16(9), 4188.

Chandler WL, Velan T. 2003. Estimating the rate of thrombin and fibrin generation in vivo during cardiopulmonary bypass. *Blood,* 101(11), 4355-62.

Chung A, Wildhirt SM, Wang S, *et al*. 2005. Combined administration of nitric oxide gas and iloprost during cardiopulmonary bypass reduces platelet dysfunction: A pilot clinical study. *J Thorac Cardiovasc Surg*, 129, 782-790.

Cohen M, Lillehei CW. 1953. Autogenous lung oxygenator with total cardiac bypass for intracardiac surgery. *Surg Forum*, 4, 34-40.

Colman RW, Clowes AW, George JN, Colman RW, Clowes AW, George JN. 2006. Overview of thrombosis. *Hemostasis and thrombosis.* 5th ed. Philadelphia: Lippincott Williams & Wilkins

Cosgrave J, Foley JB, McGovern E, *et al*. 2005. Perioperative serum inflammatory response and the development of atrial fibrillation after coronary artery bypass surgery. *Heart,* 91, 1475-1476.

Cotran RS, Mayadas-Norton T. 1998. Endothelial adhesion molecules in health and disease. *Pathol Biol* (Paris), 46(3), 164-70.

Croft D, McIntyre P, Wibulswas A, *et al*. 1999. Sustained elevated levels of sVCAM-1 in cultured fibroblast-like synoviocytes can be achieved by TNF-alpha in combination with either IL-4 or IL-13 through increased mRNA stability. *Am J Pathol*, 154(4), 1149-1158.

Cummings CJ, Sessler CN, Beall LD, Fisher BJ, Best AL, Fowler AA III. 1997. Soluble E-selectin levels in sepsis and critical illness. Correlation with infection and hemodynamic dysfunction. *Am J Respir Crit Care Med*, 156(2 Pt 1), 431-437.

Currie J, Ramsden B, McArthur C, Maruff P. 1991. Validation of a Clinical Antisaccadic Eye Movement Test in the Assessment of Dementia. *Arch Neurol*, 48, 644-648.

Cybulsky MI, Fries JW, Williams AJ, *et al.*1991. Gene structure, chromosomal location, and basis for alternative mRNA splicing of the human VCAM1 gene. *Proc Natl Acad, Sci U*S*A***,** 88**,** 7859-63.

Dahlen SE, Bjork J, Hedqvist P, *et al*. 1981. Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: in vivo effects with relevance to the acute inflammatory response. *Proc Natl Acad Sci U S A*, 78(6), 3887-91.

Dalal PM, Parab PV. 2002. Cerebrovascular disease in type 2 diabetes mellitus. *Neurol India,* 50(4), 380-5.

Danesh J, Collins R, Appleby P, Peto R. 1998. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *JAMA,* 279(18), 1477-82.

Davidson SJ, Burman JF, Phillips SM, *et al.* 2003. Correlation between thrombin potential and bleeding after cardiac surgery in adults. *Blood Coagul Fibrinolysis*, 14(2), 175-179.

Davies MJ, Gordon JL, Gearing AJ. 1993. The expression of the adhesion molecules sICAM-1, sVCAM-1, PECAM, and E-selectin in human atherosclerosis. *J Pathol*, 171(3), 223-9.

Day JR, Taylor KM. 2005. The systemic inflammatory response syndrome and cardiopulmonary bypass. *Int J Surg*, 3(2), 129-40.

De Jong JC, ten Duis HJ, Smit Sibinga CT, *et al*. 1980. Hematologic aspects of cardiotomy suction in cardiac operations. *J Thorac Cardiovasc Surg*, 79(2), 227-236.

De Mendonça-Filho HT, Pereira KC, Fontes M, *et al*. 2006. Circulating inflammatory mediators and organ dysfunction after cardiovascular surgery with cardiopulmonary bypass: a prospective observational study. *Crit Care,* 10(2), R46.

De Somer F, Van Belleghem Y, Caes F, *et al.* 2002. Tissue factor as the main activator of the coagulation system during cardiopulmonary bypass. *J Thorac Cardiovasc Surg,* 123, 951-958.

De Vroege R, van Oeveren W, van Klarenbosch J, Stooker W. 2005. The impact of heparin-coated cardiopulmonary bypass circuits on pulmonary function and the release of inflammatory mediators. *Artif Organs*, 29(6), 490-7.

Del Zoppo GJ. 1997. Microvascular responses to cerebral ischemia/inflammation. *Ann N Y Acad Sci*, 823, 132-147.

Despotis GJ, Levine V, Goodnough LT. 1997. Relationship between leukocyte count and patient risk for excessive blood loss after cardiac surgery. *Crit Care Med*, 25, 1338-1346.

Dixon B, Santamaria J, Campbell D. 2005. Coagulation activation and organ dysfunction following cardiac surgery. *Chest,* 128(1) 229-236.

Draaisma AM, Hazekamp MG, Anes N, *et al*. 2006. Phosphorylcholine coating of bypass systems used for young infants does not attenuate the inflammatory response. *Ann Thorac Surg*, 81(4), 455-9.

Edmunds LH Jr, Saxena NC, Hillyer P*, et al*. 1978. Relationship between platelet count and cardiotomy suction return. *Ann Thorac Surg*, 25, 306-310.

Edmunds LH Jr. 1995. Breaking the blood-biomaterial barrier. *ASAIO J*, 41(4), 824-830.

Edmunds LH Jr, Colman RM. 2006. Thrombin during cardiopulmonary bypass. *Ann Thorac Surg*, 82(6), 2315-22.

Edmunds LH Jr, Colman RM. 2008. Bleeding that won't stop. *Ann Thorac Surg,* 85, 1153-4.

Egeberg O. 1962. Blood coagulation and intravascular hemolysis. *Scand J Clin Lab Invest*, 14, 217-22.

Faurschou M, Borregaard N. 2003. Neutrophil granules and secretory vesicles in inflammation. *Microbes Infect,* 5(14), 1317-27.

Ferraris VA, Ferraris SP. 1996. Risk factors for postoperative morbidity. *J Thorac Cardiovasc Surg,* 111, 731-741.

Fontes Ml, Mathew JP, Rinder HM, Zelterman D, Smith BR, Rinder CS. 2005. Atrial fibrillation after cardiac surgery/cardiopulmonary bypass is associated with monocyte activation. *Anesth Analg*, 101(1), 17-23.

Franke A, Lante W, Fackeldey V *et al*. 2005. Pro-inflammatory cytokines after different kinds of cardio-thoracic surgical procedures: is what we see what we know? *Eur J Card Thorac Surg*, 28(4), 569-575.

Fraser IS, Porte RJ, Kouides PA, Lukes AS. 2008. A benefit-risk review of systemic haemostatic agents: part 1: in major surgery. *Drug Saf*, 31(3), 217-30.

Frechet JM. 1994. Functional polymers and dendrimers: reactivity, molecular architecture, and interfacial energy. S*cience*, 263(5154), 1710-5.

Frustaci A, Chimenti C, Bellocci F, Morgante E, Russo MA, Maseri A. 1997. Histological substrate of atrial biopsies in patients with lone atrial fibrillation. *Circulation,* 96(4), 1180-4.

Fujii Y, Fujii K, Nakano K, *et al*. 2003. Crosslinking of CD44 on human osteoblastic cells upregulates sICAM-1 and sVCAM-1. *FEBS Lett,* 539(1-3), 45-50.

Fukutomi M, Kobayashi S, Niwaya K, *et al.* 1996. Changes in Platelet, Granulocyte, and Complement Activation During Cardiopulmonary Bypass Using Heparin-coated Equipment. *Artif Organs*, 20(7),767-76.

Furie B, Furie BC. 2005. Thrombus formation in vivo. *J Clin Invest*, 115(12), 3355-62.

Furnary AP, Guangqlang G, Grunkemeier GL, *et al.* 2003. Continuous insulin infusion reduces mortality in patients with diabetes undergoing coronary bypass grafting. *J Thorac Cardiovasc Surg,* 125, 1007.

Gadaleta D, Fahey AL, Verma M, *et al.* 1994. Neutrophil leukotriene generation increases after cardiopulmonary bypass. *J Thorac Cardiovasc Surg*, 108(4), 642-647.

Galea J, Rebuck N, Finn A, Manché A, Moat N. 1998. Expression of soluble adhesion molecules in clinical cardiopulmonary bypass. *Perfusion,* 13(5), 314-321.

Gawaz M, Neumann FJ, Dickfeld T, *et al.* 1998. Activated platelets induce monocyte chemotactic protein-1 secretion and surface expression of intercellular adhesion molecules-1 on endothelial cells. *Circulation*, 98(12), 1164-1171.

Gearing AJ, Newman W. 1993. Circulating adhesion molecules in disease. *Immunol,* 14, 506-512.

Gibbon JH Jr. 1954. Application of a mechanical heart and lung apparatus to cardiac surgery. *Minn Med*, 37(3), 171-85.

Gibbon JH Jr, Miller BJ, Dobell AR, *et al*. 1954. The closure of interventricular septal defects in dogs during open cardiotomy with the maintenance of the cardiorespiratory functions by a pump oxygenator. *J Thorac Surg*, 28, 235-240.

Gibbon JH Jr. 1968. Development of the artificial heart and lung extracorporeal circuit. *JAMA,* 206, 1983-1986.

Glowinska B, Urban M, Peczynska J, Florys B. 2005. Soluble adhesion molecules (sICAM-1, sVCAM-1) and selectins (sE selectin, sP selectin, sL selectin) levels in children and adolescents with obesity, hypertension, and diabetes. *Metabolism,* 54(8), 1020-6.

Gorbet MB, Sefton MV. 2004. Biomaterial-associated thrombosis: role of coagulation factors, complement, platelets and leukocytes. *Biomaterials,* 25(26), 5681-5703.

Gorman RC, Ziats N, Rao AK, *et al.* 1996. Surface-bound heparin fails to reduce thrombin formation during clinical cardiopulmonary bypass. *J Thorac Cardiovasc Surg*, 111(1), 1-11.

Gravlee GP, Davis RF, Stammers AH, Ungerleider RM. 2008. *Cardiopulmonary bypass*. Philadelphia, PA: Lippincott Williams & Wilkins.

Gregoretti S. 1996. Suction-induced hemolysis at various vacuum pressures: implications for intraoperative blood salvage. *Transfusion*, 36(1):57-60.

Grelich PE, Brouse CF, Beckman J, Jessen ME, Martin EJ, Carr ME. 2002. Reductions in platelet contractile force correlate with duration of cardiopulmonary bypass and blood loss in patients undergoing cardiac surgery. *Thromb Res,* (6), 523-9.

Groom RC, Forest RJ, Cormack JE, Niimi KS, Morton J. 2002. Parallel replacement of the oxygenator that is not transferring oxygen: the PRONTO procedure. *Perfusion*, 17(6), 447-50.

Gu YJ, Boonstra PW. 2006. Selection of priming solutions for cardiopulmonary bypass in adults. *European Association for Cardiothoracic Surgery*. doi:10.1510/mmcts.2005.001198

Gunaydin S. 2004. Clinical significance of coated extracorporeal circuits: a review of novel technologies. *Perfusion*, 19, SupplS33-S41.

Gunaydin S. 2004. Emerging Technologies in Biocompatible Surface Modifying Additives: Quest for Physiologic Cardiopulmonary Bypass. *Curr Med Chem.* 2, 295-302.

Gunaydin S, Mccusker K, Vijay V. 2005. Clinical performance and biocompatibility of novel hyaluronan-based heparin-bonded extracorporeal circuits. *J Extra Corpor Technol*, 37(3), 290-5.

Hackman A, Abe Y, Insull W Jr, *et al.* 1996. Levels of soluble cell adhesion molecules in patients with dyslipidemia. *Circulation,* 93(7), 1334-8.

Hafezi-Moghadam A, Thomas KL, Prorock AJ, Huo Y, Ley K. 2001. sL-selectin shedding regulates leukocyte recruitment. *J Exp Med*, 193, 863-72.

Haga Y, Hatori N, Yoshizu H, Okuda E, Uriuda Y, Tanaka S. 1993. Granulocyte superoxide anion and elastase release during cardiopulmonary bypass. *Artif Organs*, 17(10), 837-842.

Hajjar LA, Vincent JL, Galas RE, *et al*. 2010. Transfusion Requirements After Cardiac Surgery: The TRACS Randomized Controlled Trial. *JAMA,* 304(14): 1559.

Hambsch J, Osmancik P, Bosci J, *et al.* 2002. Neutrophil adhesion molecule expression and serum concentration of soluble adhesion molecules during and after pediatric cardiovascular surgery with or without cardiopulmonary bypass. *Anesthesiology,* 96(5), 1078-1085.

Harig F, Feyrer R, Mahmoud FO, Blum U, von der Emde J. 1999. Reducing the post-pump syndrome by using heparin-coated circuits, steroids, or aprotinin. *Thorac Cardiovasc Surg,* 47(2), 111-8.

Harpaz Y, Chothia C. 1994. Many of the immunoglobulin superfamily domains in cell adhesion molecules and surface receptors belong to a new structural set which is close to that containing variable domains. *J Mol Biol,* 238(4), 528–39.

Hasleton PS. 1972. The internal surface area of the adult lung. *J Anat,* 112(Pt 3), 391-400.

Haworth WS. 2003. The development of the modern oxygenator. *Ann Thorac Surg*, 76:S2216-S2219.

Helmsworth JA, Clark LC, Jr, Kaplan S *et al*. 1953. An oxygenator pump for use in total bypass of heart and lungs. *J Thorac Surg*, 26, 617-631.

Hemmingway A. 1913. Some observation of the perfusion of the isolated kidney by a pump. *J Physiol*, 71, 201-213.

Hessel EA II, Edmunds LH Jr. 2003. Extracorporeal perfusion systems. *Cardiac surgery in the adult.* New York, NY: McGraw-Hill.

Hill-Eubanks DC, Parker CG, Lollar P. 1989. Differential proteolytic activation of factor VIII-von Willebrand factor complex by thrombin. *Proc Natl Sci U S A,* 86(17), 6508-12.

Hirayama T, Yamaguchi H, Allers M, *et al*. 1985. Evaluation of red cell damage during cardiopulmonary bypass. *Scand J Cardiovasc Surg*, 19, 263-265.

Hirose T, Burman SD, O'Connor RA. 1964. Reduction of perfusion hemolysis by use of atraumatic low pressure suction. *J Thorac Cardiovasc Surg*, 47, 242-247.

Hodges PC, Seleers RD, Story JL. 1958. The effects of total cardiopulmonary bypass procedures upon cerebral function evaluated by electroencephalogram and a blood brain barrier test. Allen JG, ed. *Extracorporeal circulation.* Springfield, Il: Charles C Thomas Publishers.

Horbett TA. 1993. Principles underlying the role of adsorbed plasma proteins in blood interactions with foreign materials. *Cardiovasc pathol*, 2(3), 137S-148S.

Hwang SJ, Ballantyne CM, Sharrett AR*, et al.* 1997. Circulating adhesion molecules sVCAM-1, sICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk in Communities (ARIC) study. *Circulation,* 96(12), 4219-25.

Ikuta T, Fujii H, Shibata T, *et al.* 2004. A new poly-2-methoxyethylacrylate-coated cardiopulmonary bypass circuit possesses superior platelet preservation and inflammatory suppression efficacy. *Ann Thorac Surg*, 77(5), 1678-83.

Imhof BA, Dunon D. 1995. Leukocyte migration and adhesion. *Adv Immunol*, 58:345-416.

Ivetic A, Florey O, Deka J, Haskard DO, Ager A, Ridley AJ. 2004. Mutagenesis of the ezrin-radixin-moesin binding domain of L-selectin tail affects shedding, microvillar positioning, and leukocyte tethering. *J Biol Chem,* 279(32), 33263-72.

Jaffe EA. 1994. Biochemistry, immunology, and cell biology endothelium. *Hemostasis and thrombosis*. Philadelphia, Pa: J.B. Lippincott.

Jones RH, Hanna EL, Hammermeister KE, *et al*. 1996. Identification of preoperative variables needed for risk adjustment of short-term mortality after coronary artery bypass graft surgery. The working group panel of the cooperative CABG database project. *Am Coll Cardiol,* 28, 1478-87.

Jordan SW, Chaikof EL. 2007. Novel thromboresistant materials. *J Vasc Surg,* 45, 104A-115A.

Jung TM, Dailey MO. 1990. Rapid modulation of homing receptors (gp90MEL-14) induced by activators of protein kinase C. Receptor shedding due to accelerated proteolytic cleavage at the cell surface. *J Immunol*, 144, 3130-6.

Jutila MA, Rott L, Berg EL, *et al*. 1989. Function and regulation of the neutrophil MEL-14 antigen in vivo: comparison with LFA-1 and MAC-1. *J Immunol,* 143,3318-24.

Kadoi Y, Saito S, Fuijta N, *et al*. 2005. Risk factors for cognitive dysfunction after coronary artery bypass graft surgery in patients with type 2 diabetes. *J Thorac Cardiovasc Surg,* 129, 576-83.

Kalawaski R, Majewski M, Kaszkowiak E, Wysocki H, Siminiak T. 2003. Transcardiac release of soluble adhesion molecules during coronary artery bypass grafting: effects of crystalloid and blood cardioplegia. *Chest,* 123(5), 1355-60.

Kannel WB, McGee DL. 1979. Diabetes and cardiovascular risk factors: the Framingham study. *Circulation,* 59(1), 8-13.

Kansy A, Tobota Z, Maruszewski P, Maruszewski B. 2010. Analysis of 14,843 neonatal congenital heart surgical procedures in the European Association of Cardiothoracic Surgery Congenital Database. *Ann Thorac Surg*, 89(4), 1255-9.

Kawahito S, Maeda T, Yoshikawa M, *et al*. 2001. Blood trauma induced by clinically accepted oxygenators. *ASAIO J,* 47(5), 492-5.

Kim SW. 1996. Nonthrombogenic treatment and strategies. *Biomaterial*s s*cience. An introduction to materials in medicine.* San Diego: Academic Press.

Kincaid EH, Jones TJ, Stump DA, *et al*. 2000. Processing scavenged blood with a cell saver reduces lipid microembolization. *Ann Thorac Surg,* 70(4), 1296-300.

Kirkland JW, Barrat-Boyes BG, eds. 1993. Hypothermia, circulatory arrest, and cardiopulmonary bypass. *Cardiac Surgery*, 2nd ed. New York: Churchhill Livingstone.

Kishimoto TK, Jutila MA, Berg EL, *et al.* 1989. Neutrophil MAC-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors. *Science,* 245, 1238-41.

Klings ES, Anton BD, Rosenman D, *et al.* 2008. Pulmonary arterial hypertension and left-sided heart disease in sickle cell disease: clinical characteristics and association with soluble adhesion molecule expression. *Am J Hematol,* 83(7), 547-53.

Koizumi A, Hashimoto S, Kobayashi T, Imai K, Yachi A, Horie T. 1995. Elevation of serum soluble vascular cell adhesion molecule-1 (sVCAM-1) levels in bronchial asthma. *Clin Exp Immunol*, 101,468-73.

Kolev K, Machovich R. 2003. Molecular and cellular modulation of fibrinolysis. *Thromb Haemost*, 89, 610-621.

Kraus J, Oschmann P, Leis S, Neundörfer B, Heckmann JG. 2002. High concentrations of sVCAM-1 and sICAM-1 in the cerebrospinal fluid of patients with intracerebral haemorrhage are associated with poor outcome. *J Neurol Neurosurg psychiatry*, 73(3), 346-347.

Kurusz M, Wheeldon DR. 1990. Risk containment during cardiopulmonary bypass. *Semin Thorac Cardiovasc Surg*, 2(4), 400-409.

Laffey JG, Boylan JF, Cheng DC. 2002. The systemic inflammatory response to cardiac surgery: implications for the anesthesiologist. *Anesthesiology*, 97(1), 215-52.

Lappegård KT, Riesenfeld J, Brekke OL, *et al.* 2005. Different effect of heparin coating and complement inhibition on artificial surface-induced eicosanoid production. *Ann Thorac Surg,* 79(3), 917-23.

Levy JH, Tanaka KA. 2003. Inflammatory response to cardiopulmonary bypass. *Ann Thorac Surg*, 75(2), S715-20.

Lewis FJ, Tauffic M. 1953. Closure of atrial septal defects with the aid of hypothermia; experimental accomplishments and the report of one successful case. *Surgery*, 33, 52-59.

Liles WC, Van Voorhis WC. 1995. Review: nomenclature and biologic significance of cytokines involved in inflammation and the host immune response. *J Infect Dis*, 172(6), 1573-80.

Lillehei CW, Cohen M, Warden HE, *et al.* 1955. Direct vision intracardiac surgery: by means of controlled cross circulation or continuous arterial reservoir perfusion for correction of ventricular septal defects, atrio-ventricularis communis, isolated infundibular pulmonic stenosis, and tetrology of Fallot. *Proceedings of Henry Ford Hospital symposium.* Philadelphia: WB Saunders.

Lin CY, Yang TL, Hong GJ, *et al*. 2010. Enhanced intracellular heat shock protein 70 expression of leukocytes and serum interleukins release: comparison of on-pump and off-pump coronary artery surgery. *World J Surg*, 34(4), 675-81.

Liu JY, Birkmeyer NJ, Sanders JH, *et al*. 2000. Risks of morbidity and mortality in dialysis patients undergoing coronary artery bypass surgery. *Circulation,* 102(24), 2973-2977.

Lytle BW, Loop FD, Cosgrove DM, *et al*. 1985. Long-term (5 to 12 years) serial studies of internal mammary artery and saphenous vein coronary bypass grafts*. J Thorac Cardiovasc Surg,* 89, 248–58.

Mahoney CB. 1998. Heparin-bonded circuits: clinical outcomes and cost. *Perfusion*, 3, 192-204.

Mangano CM, Diamondstone LS, Ransey JG, *et al.* 1998. Renal dysfunction after myocardial revascularization: risk factors, adverse outcomes, and hospital resource utilization. The Multicenter Study of Perioperative Ischemia Research Group. *Ann Intern Med,* 28(3), 194-203.

Marcoux J, Sohn N, Meng Q, *et al*. 2009. Outcomes comparison of 5 coated cardiopulmonary bypass circuits versus an uncoated control group of patients undergoing cardiac surgery. *Perfusion,* 24(5), 307-15.

Marlin SD, Springer TA. 1987. Purified intercellular adhesion molecule (sICAM-1) is a ligant for lymphocyte function-associated antigen- (LFA-1). *Cell*, 51, 813-819.

Matata BM, Sosnowski AW, Galinanes M. 2000. Off-pump bypass graft operation significantly reduces oxidative stress and inflammation. *Ann Thorac Surg*, 69, 785–91.

Mayer G. 2007. Immunology: Innate (non specific) Immunity. *Microbiology and Immunology* [e-book]. Los Angeles, CA:USC School of Medicine.

McKeating EG, Andrews PJ. 1998. Cytokines and adhesion molecules in acute brain injury. *Br J Anaesth*, 80, 77-84.

Menasche P, Edmunds LH, Jr. Inflammatory response. 2003. *Cardiac surgery in the adult*. 2nd ed. New York, NY: McGraw-Hill.

Mihm MJ, Yu F, Carnes CA, *et al.* 2001. Impaired myofibrillar energetics and oxidative injury during human atrial fibrillation. *Circulation*, 104(2), 174-80.

Miller BE, Levy JH. 1997. The inflammatory response to cardiopulmonary bypass. *J Cardiothorac Vasc Anesth,* 11(3), 355-366.

Moller JT, Cluitmans P, Rasmussen LS, *et al*. 1998. Long-term post-operative cognitive dysfunction in the elderly ISPOCD1 study. ISPOCD Investigators. International Study of Post-Operative Cognitive Dysfunction. *Lancet,* 21;351(9106), 857-861.

Morisaki, N, Kanzaki T, Tamura K, *et al.* 1995. Specific inhibition of vascular cell adhesion molecule-1 expression by type IV collagen in endothelial cells. *Biochem Biophys Res Commun*, 214, 1163-7.

Morisaki N, Saito I, Tamura K, *et al.* 1997. New indices heart disease and aging: studies on the serum levels of soluble adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) in patients with hypercholesterolemia and ischemic heart disease. *Atherosclerosis*, 131(1), 43-48.

Myers GJ, Weighell PR, McCloskey BJ, *et al.* 2003. A multicenter investigation into the occurrence of high-pressure excursions. *J Extra Corpor Technnol,* 35(2), 127-32.

Naito M. 1993. Macrophage heterogeneity in development and differentiation. *Arch Histol Cytol*, 56(4), 331-51.

Nalae H, Endo S, Inada K, Takakuwa T, Kasai T. 1996. Changes in adhesion molecule levels in sepsis. *Res Commun Mol Pathol Pharmacol*, 91(3), 329-38.

Nelson DL, Cox MM. 2008. *Lehninger Principles of Biochemistry*, Fifth Edition. New York: W.H. Freeman and Co.

Nelson SM, Sattar N, Freeman DJ, *et al.* 2007. Inflammation and endothelial activation is evident at birth in offspring of mothers with type I diabetes. *Diabetes,* 56(11), 2697-704.

Newman MF, Croughwell ND, Blumenthal JA, *et al.* 1995. Predictors of cognitive decline after cardiac operation. *Ann Thorac Surg,* 59(5), 1326-1330.

Newman MF, Kirchner JL, Phillips-Bute B*, et al.* 2001. Longitudinal assessment of neurocognitive function after coronary artery bypass grafting. *N Engl J Med,* 344(6), 395-402.

Nieuwland R, Berckmans RJ, Rotteveel-Eijkman RC, *et al.* 1997. Cell-derived microparticles generated in patients during cardiopulmonary bypass are highly procoagulant. *Circ,* 96(10), 3534-3541.

Novo E, Parola M. 2008. Redox mechanisms in hepatic chronic wound healing and fibrogenesis. *Fibrogenesis Tissue Repair*, 1(1), 5.

Ohga E, Nagase T, Tomita T, *et al.* 1999. Increased levels of circulating ICAM-1, sVCAM-1, and sL-selectin in obstructive sleep apnea syndrome. *J Appl physiol,* 87(1), 10-14.

Oliveira DC, Ferro CR, Oliveira JB, *et al*. 2008. Risk factors from stroke after coronary artery bypass grafting. *Arq Bras Cardiol,* 91(4), 213-216.

Ott I. 2005. Soluble tissue factor emerges from inflammation. *Circ Res*, 12, 1217-8.

Pantoni L, Sarti C, Inzitari D. 1998. Cytokines and cell adhesion molecules in cerebral ischemia: experimental bases and therapeutic perspectives. *Arterioscler Thromb Vasc Biol,* 18, 503-513.

Papayianni A, Alexopoulos E, Giamalis *P*, *et al.* 2002. Circulating levels of sICAM-1, sVCAM-1, and MCP-1 are increased in haemodialysis patients: association with inflammation, dyslipidaemia, and vascular events. *Nephrol Dial Transplant*, 17, 435–441.

Parham P. 2005.*The immune* s*ystem.* 2nd ed. New York: Garland Science.

Parolari A, Pesce LL, Trezzi M, *et al*. 2009. Performance of EuroSCORE in CABG and off-pump coronary artery bypass grafting: single institution experience and meta-analysis. *Eur Heart J*, 30(3), 297-304.

Pasnik J, Baj Z, Moll JA, *et al.* 2004. Effects of cardiopulmonary bypass on the expression of adhesive molecules in children undergoing cardiac surgery due to congenital heart disorders. *Kard pol,* 60(2), 123-31.

Patterson SW, Starling EH. 1914. On the mechanical features which determine the output of the ventricles. *J Physiol*, 48, 357-379.

Paul WE. 1999. *Fundamental Immunology*, 4th ed. Philadelphia: Lippincott-Raven.

Pierangeli A, Masieri V, Bruzzi F, *et al*. 2001. Haemolysis during cardiopulmonary bypass: how to reduce the free haemoglobin by managing the suctioned blood separately. *Perfusion,* 16(6), 519-24.

Pigott, R, Dillon LP, Hemingway H, *et al*. 1992. Soluble forms of E-selectin, sICAM-1 and sVCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. *Biochem Biophys Res Commun,* 187, 584-9.

Pixley RA, Schapira M, Colman RW. 1985. Effect of heparin on the inactivation rate of human activated factor XII by antithrombin III. *Blood,* 66(1), 198-203.

Poston RN, Haskard DO, Coucher JR, *et al.* 1992. Expression of the adhesion molecule sICAM-1 in atherosclerotic plaques: implications for positive-feedback monocyte recruitment in the pathogenesis of the plaque. *Am J pathol*, 140(3), 665-73.

Pugsley W, Klinger L, Paschalis C, *et al.* 1994. The impact of microemboli during cardiopulmonary bypass on neuropsychological functioning. *Stroke,* 25(7), 1393-1399.

Puskas JD, Kilgo PD, Lattouf OM, *et al.* 2008. Off-pump coronary bypass provides reduced mortality and morbidity and equivalent of 10-year survival. *Ann Thorac Surg*, 86,1139-46.

Raja SG, Dreyfus GD. 2006. Impact of off-pump coronary artery bypass surgery on postoperative bleeding: current best available evidence. *J Card Surg*, 21, 35–41.

Ranucci M, Pazzaglia A, Bianchini C, Bozzetti G, Isgrò G. 2008. Body Size, Gender, and Transfusions as Determinants of Outcome after Coronary Operations. *Ann Thorac Surg*, 85, 481-486.

Ranucci M, Balduini A, Dita A, *et al*. 2009. A Systematic Review of Biocompatible Cardiopulmonary Bypass Circuits and Clinical Outcome. *Ann Thorac Surg,* 87, 1311-9.

Reston JT, Tregear SJ, Turkelson CM. 2003. Meta-analysis of short-term and mid-term outcomes following off-pump coronary artery bypass grafting. *Ann Thorac Surg*, 76, 1510-5.

Remadi JP, Rakitiarivello Z, Marticho *P*, *et al.* 2004. Aortic valve replacement with the minimal extracorporeal circulation (Jostra MECC System) versus standard: A randomized prospective. *J Thorac Cardiovasc Surg*, 128(3), 436-441.

Ridker PM, Hennekens CH, Roitman-Johnson B, *et al.* 1998. Plasma concentration of soluble intercellular adhesion molecule-1 and risks of future myocardial infarction in apparently healthy men. *Lancet,* 351(9096), 88-92.

Riet V, Van Camp B. 1993. The involvement of adhesion molecules in the biology of multiple myeloma. *Leuk Lymph*, 9, 441-452.

Rinder CS, Fontes M, Mathew JP, *et al*. 2003. Neutrophil CD11b upregulation during cardiopulmonary bypass is associated with postoperative renal injury. *Ann Thorac Surg.* 75(3), 899-905.

Rodriguez HG, Vanderwielen AJ. 1979. Molecular weight determination of commercial heparin sodium USP and its sterile solutions. *J Pharm Sci,* 68(5), 588-591.

Roldán V, Marín F, Blann AD, *et al.* 2003. Interleukin-6, endothelial activation and thrombogenesis in chronic atrial fibrillation. *Eur Heart J,* 24(14), 1373-1380.

Romagnani S. 2002 Cytokines and chemoattractants in allergic inflammation. *Mol Immuno*, 38(12-13), 881-5.

Rosenberg RD. 1989. Biochemistry of heparin antithrombin interactions, and the physiologic role of this natural anticoagulant mechanism. *Am J Med,* 87(Suppl 3B), 2S-9S.

Ross R.1999. Atherosclerosis is an inflammatory disease. *Am Heart J,* 138(5 Pt 2), S419-20.

Rubens FD. 2002. Cardiopulmonary bypass technology transfer: musing of a cardiac surgeon. *J Biomater Sci,* 13, 485-499.

Rubens FD, Mesana T. 2004. The inflammatory response to cardiopulmonary bypass: a therapeutic overview. *Perfusion,* 19, S5-S12.

Sadler JE. 1998. Biochemistry and genetics of von Willebrand factor. *Annu rev Biochem*, 67, 396-424.

Sallusto F, Mackay CR. 2004. Chemoattractants and their receptors in homeostasis and inflammation. *Curr Opin Immunol,* 16(6), 724-31.

Sano Y, Hirai S, Katayama M, *et al.* 1994. *Mol Cell Biochem*, 139(2), 123-130.

Schleiffenbaum B, Spertini O, Tedder TE. 1992. Soluble sL-selectin Is Present in Human Plasma at High Levels and Retains Functional Activity. *J Cell Biol,* 119(1), 229-38.

Schmid-Schönbein H, Born G, Richardson P, *et al*. 1979. ADP release from red cells subjected to high shear stress. *Basic aspects of blood trauma.* The Hague, The Netherlands: Nijhoff.

Schreurs HH, Wijers MJ, Gy YJ, *et al*. 1998. Heparin-coated bypass circuits: effects on inflammatory response in pediatric cardiac operations. *Ann Thorac Surg,* 66, 166-171.

Schulze CJ, Han L, Ghorpade N, *et al*. 2009. Phosphorylcholine-coated circuits improve preservation of platelet count and reduce expression of proinflammatory cytokines in CABG: a prospective randomized trial. *J Card Surg,* 24(4), 363-8.

Seabra VF, Alobaidi S, Balk EM, *et al*. 2010. Off-Pump Coronary Artery Bypass Surgery and Acute Kidney Injury: A Meta-Analysis of Randomized Controlled Trials. *Clin J Am Soc Nephrol*, [Epub ahead of print].

Seeburger J, Hoffmann J, Wendel HP, *et al*. 2005. Gene expression changes in leukocytes during cardiopulmonary bypass are dependent on circuit coating. *Circulation,* 112(9 Suppl), 1224-8.

Serrano CV, Souza JA, Lopes NH, *et al*. 2010. Reduced expression of systemic proinflammatory and myocardial biomarkers after off-pump versus on-pump coronary artery bypass surgery: A prospective randomized study. *Critical care*, 25(1), 305-312.

Sezer O, Niemoller J, Eucker J, *et al*. Bone marrow microvessel density is a prognostic factor for survival in patients with multiple myeloma. *Ann Hematol*, 79, 574-577.

Singh RJ, Mason JC, Lidington EA, *et al.* 2005. Cytokine stimulated vascular cell adhesion molecule-1 (VCAM-1) ectodomain release is regulated by TIMP-s. *Cardiovasc Res,* 67(1), 39-49.

Skrabal CA, Khosravi A, Choi YH, *et al.* 2006. Pericardial suction blood separation attenuates inflammatory response and hemolysis after cardiopulmonary bypass. *Scand Cardiovasc J*, 40(4), 219-223.

Skrabal CA, Khosravi A, Westphal B, *et al.* 2006. Effects of poly-2-methoxyethylacrylate (PMEA)-coating on CPB circuits. *Scand Cardiovasc J,* 40(4), 224-9.

Smalley DM, Ley K. 2005 sL-selectin: mechanisms and physiological significance of ectodomain cleavage. *J Cell Mol Med*, 9(2), 255-66.

Soper DS. 2010. The free statistics calculators website online software. http://wwww.danielsoper.com/statcalc/

Sowers JR, Epstein M. 1995. Diabetes mellitus and associated hypertension, vascular disease and nephropathy. *Hypertension,* 26(6 Pt 1), 869-879.

Spijker HT, Graaf R, Boonstra PW, *et al.* 2003. On the influence of flow conditions and wettability on blood material interactions. *Biomaterials,* 24(26), 4717-4727.

Stables MJ, Gilroy DW. 2010. Old and new generation lipid mediators in acute inflammation and resolution. *Prog Lipid Res*, [Epub ahead of print].

Stadelmann WK, Digenis AG, Tobin GR. 1998. Physiology and healing dynamics of chronic cutaneous wounds. *Am J Surg*, 176(21 Suppl), 26s-38s.

Stallwood MI, Grayson AD, Mills K, *et al.* 2004. Acute renal failure in coronary bypass surgery: independent effect of cardiopulmonary bypass. *Ann Thorac Surg*. 77, 968-972.

Stefano GB, Bilfinger TV. 1993. Human neutrophil and macrophage chemokinesis induced by cardiopulmonary bypass: loss of DAME and IL-1 chemotaxis. J Neuroimmunol, 47(2), 189-97.

Stevens LA, Coresh J, Greene T, *et al*. 2006. Assessing kidney function--measured and estimated glomerular filtration. *N Engl J Med*, 354(23), 2473-83.

Stump DA, Rogers AT, Hammon JW, *et al*. 1996. Cerebral emboli and cognitive outcome after cardiac surgery. *J Cardiothorac Vasc Anesth,* 10(1), 113-118.

Sudhoff T, Wehmeier A, Kliche KO, *et al.* 1996. Levels of circulating endothelial adhesion molecules (sE-selectin and sVCAM-1-1) in adult patients with acute leukemia. *Leukemia*, 10, 682.

Suleiman MS, Zacharowski K, Angelini GD. 2008. Inflammatory response and cardioprotection during open-heart surgery: the importance of anaesthetics. *Br J pharmacol, 153(1),* 21-23.

Suzuki Y, Daitoku K, Minakawa M, *et al.* 2008. Poly-2-methoxyethylacrylate-coated bypass circuits reduce activation of coagulation system and inflammatory response in congenital cardiac surgery. *J Artif Organs,* 11(3), 111-6.

Svensson M, Pfeifer J, Stockinger B, Wick MJ. 1997. Bacterial antigen delivery systems: phagocytic processing of bacterial antigens for MHC-I and MHC-II presentation to T cells. *Behring Inst Mitt,* 98, 197-211.

Swan H, Zeavin I, Blount SG Jr, *et al*. 1953. Surgery by direct vision in the open heart during hypothermia. *JAMA*, 153, 1081-1085.

Takagi H, Matsui M, Umemoto T. 2010. Off-pump coronary artery bypass may increase late mortality: a meta-analysis of randomized trials. *Ann Thorac Surg*, 89(6), 1881-8.

Te Velthuis H, Baufreton C, Jansen PG, *et al.* 1997. Heparin coating of extracorporeal circuits inhibits contact activation during cardiac operations. *J Thorac Cardiovasc Surg,* 114(1), 117-22.

Tevaearai HT, Mueller XM, Seigneul I, *et al.* 1999. Trillium™ coating of cardiopulmonary bypass circuits improves biocompatibility. *Int J Artif Organs,* 22(9), 629-34.

Thomas ED, Blume KG. 1993. Thomas's hematopoietic cell transplantation. *Fundamental immunology*. New York, NY: Raven Press.

Tu L, Poe JC, Kadono T, Venturi GM *et al.* 2002. A functional role for circulating mouse sL-selectin in regulating leukocyte/endothelial cell interactions in vivo. *J Immunol,* 169: 2034-43.

Ueyama k, Nishimura K, Nishina T, *et al*. 2004. PMEA coating of pump circuit and oxygenator may attenuate the early systemic inflammatory response in cardiopulmonary bypass surgery. *ASAIO J,* 50(4), 369-72.

Van Beers EJ, Schaap MC, Berckmans RJ, *et al.* 2009. Circulating erythrocyte-derived microparticles are associated with coagulation activation in sickle cell disease. *Haematologica*, 94(11), 1513-9.

van den Goor J, Nieuwland R, van den Brink A, *et al*. 2004. Reduced complement activation during cardiopulmonary bypass does not affect the postoperative acute phase response. *Eur J Cardiothorac Surg*, 26(5), 926-931.

Van Uden P, Kenneth NS, Rocha S. 2008. Regulation of hypoxia-inducible factor-1 alpha by NF-kappa*ß*. *Biochem J*, 412(3), 477-84.

Vercaemst L. 2008. Hemolysis in cardiac surgery patients undergoing cardiopulmonary bypass: a review in search of a treatment algorithm. *J Extra Corpor Technol,* 40(4), 257-67.

Videm V, Mollnes TE, Bergh K, *et al*. 1999. Heparin-coated cardiopulmonary bypass equipment. II. Mechanisms for reduced complement activation in vivo. *J Thorac Cardiovasc Surg*, 117(4), 803-9.

Visser LH. 2006. Critical illness polyneuropathy and myopathy: clinical features, risk factors and prognosis. *Eur J Neurol*, 13(11), 1203-12.

Wahba A, Black G, Koksch M, *et al.* 1996. Cardiopulmonary bypass leads to a preferential loss of activated platelets. A flow cytometric assay of platelet surface antigens. *Eur J Cardio Throrac Surg,* 10(9), 768-773.

Wagner DD, Burger PC. 2003. Platelets in inflammation and thrombosis. *Arterioscler Thromb Vasc Biol,* 23(12), 2131-7.

Wan IY, Arifi AA, Wan S, *et al*. 2004. Beating heart revascularization with or without cardiopulmonary bypass: evaluation of inflammatory response in a prospective randomized study. *J Thorac Cardiovasc Surg*, 127,1624–31.

Wan S, LeClerc JL, Vincent JL. 1997. Inflammatory response to cardiopulmonary bypass: mechanisms involved and possible therapeutic strategies. *Haemost Thromb*, (5-6), 263-268.

Warren O, Alexiou C, Massey R*, et al.* 2007. The effects of various leukocyte filtration strategies in cardiac surgery. *Eur J Cardiothorac Surg*, 31(4), 665-676.

Watts DD, Trask A, Soeken K, *et al*. 1998. Hypothermic coagulopathy in trauma: effect of varying levels of hypothermia on enzyme speed, platelet function, and fibrinolytic activity. *J Trauma,* 44(5), 864-854.

Wei M, Kuukasjärvi *P*, Laurikka J, *et al.* 2003. Soluble adhesion molecules and myocardial injury during coronary artery bypass grafting. *World J Surg,* 27(2), 140-4.

Wendel HP, Eckstein FS, Fink E, *et al.* 1999. Increased coagulation and bradykinin-release in shed pleural blood during cardiac surgery. *Immunopharmacology*, 44(1-2), 119-22.

Wendel HP, Ziemer G. 1999. Coating-techniques to improve the hemocompatibility of artificial devices used for extracorporeal circulation. *Eur J Cardiothorac Surg*, 16(3), 342-50.

Westaby S. 1983. Complement and the damaging effects of cardiopulmonary bypass. *Thorax,* 38(5), 321-325.

Westaby S. 1987. Organ dysfunction after cardiopulmonary bypass. A systemic inflammatory reaction. *Intensive Care Med*, 13(2), 89-95.

Whelan DM, van der Giessen WJ, Krabbendam SC, *et al*. 2000. Biocompatibility of phosphorylcholine coated stents in normal porcine coronary arteries. *Heart,* 83, 338-345.

Whitaker DC, Stygall JA, Newman SP*, et al*. 2001. The use of leukocyte depleting and conventional arterial line filters in cardiac surgery: a systematic review of clinical studies. *Perfusion,* 16, 433-446.

Wielogorske JW, Cross DE, Nwadlike EV. 1975. The effects of subatmospheric pressure on the hemolysis of blood. *J Biomechan*, 8, 321-325.

Wildhirt SM, Schulze C, Schulz C, *et al*. 2001. Reduction of systemic and cardiac adhesion molecule expression after off-pump versus conventional coronary artery bypass grafting. *Shock*, 16 Suppl, 1, 55-9.

Wolberg AS, Meng ZH, Monroe DM, *et al.* 2004. A systematic evaluation of the effect of temperature on coagulation enzyme activity and platelet function. *J Trauma,* 56(6), 1221-1228.

Wong W, Goehring AS, Kapiloff MS, *et al*. 2008. mAKAP compartmentalizes oxygen-dependent control of HIF-1alpha. Sci Signal, 1(51), ra18.

Wright G, Sanderson JM. 1979. Cellular aggregation and trauma in cardiotomy suction systems. *Thorax*, 34, 621-628.

Wu KK, Aleksic N, Ballantyne CM *et al.* 2003. Interaction Between Soluble Thrombomodulin and Intercellular Adhesion Molecule-1 in Predicting Risk of Coronary Heart Disease [Clinical Investigation and Reports]. *Circ,* 107, r66-r69.

Yang L, Froio RM, Sciuto TE, *et al*. 2005. sICAM-1 regulates neutrophil adhesion and transcellular migration of TNF-alpha-activated vascular endothelium under flow. *Blood*, 106(2), 584-92.

Yedgar S, Koshkaryev A, Barshtein G. 2002. The red blood cell in vascular occlusion. *Pathophysiol Haemost Thromb,* 32(5-6), 263-8.

Zerr KJ, Furnary AP, Grunkemeier GL, *et al*. 1997. Glucose control lowers the risk of wound infection in diabetics after open heart operations. *Ann Thorac Surg*, 63, 356-61.

Zimmermann AK, Aebert H, Reiz A, *et al*. 2004. Hemocompatibility of PMEA coated oxygenators used for extracorporeal circulation procedures. *ASAIO J,* 50(3), 193-9.

Zimmermann AK, Weber N, Aebert H, *et al*. 2007. Effect of biopassive and bioactive surface-coatings on hemocompatibility of membrane oxygenators. *J Biomed Mater Re*s *B Appl Biomater,* 80(2), 433-9.

Zuhdi N, McCollough B, Carey J, *et al*. 1961. Hypothermic perfusion for open heart surgical procedures-report of the use of a heart-lung machine primed with a five percent dextrose in water inducing hemodilution. *J Int Coll Surg*, 35, 319-326.