An In-Vitro Study on the Use of an Arterial Bubble Trap Incorporated into the Venous Lines

by

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Abstract

Cardiopulmonary bypass has been linked to neurocognitive dysfunction in numerous studies. Air emboli are one of the main causes of neurocognitive dysfunction. The air emboli can come from the surgical field, the heart lung machine, or from anesthesia. This study investigated the amount of venous air returning to the heart lung machine from the surgical field. Venous air can come from a number of sources, including; loose purse strings, a trans-atrial approach to a mitral valve repair or replacement, or openings in the venous lines of the heart lung machine. Air may also come from an atrial septal defect, a patent foramen ovale, or if the left atrium or left ventricle are opened.

Since venous air comes from a variety of sources and is harmful to the patient, a device to remove this air could limit the amount of air emboli and improve patient outcomes. The purpose of this study was to look at the effects of placing an arterial bubble trap in the venous lines to reduce these air emboli. This was done by comparing the use of a bubble trap placed in the venous line to a circuit without a venous bubble trap under conditions of three air injection doses. Additionally the study tested the efficiency of the bubble trap in the venous lines (it is usually placed in the arterial line) by comparing air volume amounts before and after the bubble trap as measured by probes on each side of the trap. For each set of experimental conditions ten trials were performed and the data were assessed using Minitab (version 14).

An arterial bubble trap placed in the venous lines was found to be effective at air removal when the volume amounts before and after the bubble trap were analyzed. There was a statistically significant reduction in air volume amounts when analyzing the injection site volume and after the arterial line filter with a bubble trap in the circuit as well as without a bubble trap in the circuit. There was not a statistically significant difference in the air volume amount removed when comparing a circuit with a bubble trap to one without a bubble trap in the venous lines. One possibility for this is the efficiency of the circuit's components at air removal. Based on the results of this study, the arterial bubble trap does reduce the amount of air before it enters the cardiotomy and should be considered as another form of prevention for air emboli reaching the patient.

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Nomenclature

ALF = Arterial Line Filter
ANOVA = Analysis of Variance
Adj SS = Adjusted Sum of Squares
Adj MS = Adjusted Mean Square
BSA = Body Surface Area
BT = Bubble Trap
C5a = Complement Protein C5 activated
CABG = Coronary Artery Bypass Grafting
CPB = Cardiopulmonary Bypass
DBT = Dynamic Bubble Trap
DF = Degrees of Freedom
EKG = Electro Kardio Gram
ECC = Extracorporeal Circulation
IABP = Intra aortic balloon pump
IL-6 = Interleukin 6
IL-8 = Interleukin 8
LDH = Lactate Dehydrogenase
MHz = Mega Hertz
NNECDSG = Northern New England Cardiovascular Disease Study Group
PAP = Plasmin - antiplasmin
RF = Radio Frequency
Reg SS = Regression Sum of Squares
Seq SS = Sequential Sum of Squares
TAT = Thrombin-Antithrombin
TNF = Tissue Necrosis Factor
Total SS = Total Sum of Squares

1.0 Introduction

Neurological injury is associated with cardiopulmonary bypass (CPB) [1-4]. These injuries are frequently caused by emboli becoming lodged in the vasculature of end organs and thereby causing downstream ischemia. Emboli can refer to all solid particulates or air [3]. When emboli are caused by air, ischemia can occur. The blood air interface also has the effect of initiating the inflammatory response, reacting with proteins and platelets, and disrupting the endothelium. Other examples of cerebral emboli may include: atherosclerotic debris, fat, calcium, platelet thrombi, or particles from the CPB tubing [5]. The severity of the patient outcome is often dependant on the amount of emboli and the location of the emboli in circulation. Emboli are most frequently detected by ultrasound devices today, which include; transcranial, transesophageal, doppler flow devices, and echo machines, but can also be found through the use of perioperative monitoring, computed tomography, and brain magnetic resonance imaging [6, 7]. Air emboli are associated with the CPB surgery. These emboli can occur through the CPB circuit, by actions carried out by anesthesia, or through air from the surgical field that is termed “surgical air” [8]. One common source of CPB air is air returning through the venous lines.

The causes of venous line air vary, but if not removed from the circuit can reach the patient and cause damage. Some sources include: loose purse string sutures, a trans-atrial approach to a mitral valve repair or replacement, or openings in the venous system [1]. Air can also be seen in the venous lines if the patient has an atrial septal defect or a patent

foramen ovale and the left atrium or left ventricle is opened [1]. The source must be quickly identified to minimize harm to the patient.

Since prevention of these detrimental effects is optimal devices to eliminate venous air should be considered. In conventional circuits there is not a device in the venous lines to trap emboli. Therefore an arterial bubble trap shall be tested to look at its effectiveness in air removal.

2.0 Background

2.1 Criteria for the Detection of Stroke and Neuro-cognitive Dysfunction

Postoperative cognitive deficits have been documented after cardiopulmonary bypass. These deficits range from minor decreases in motor skills, subtle behavioral changes, and temporary memory loss, to effects as serious as severe strokes. The occurrence of a cognitive deficit is not well defined due to varying measures used to assess deficits.

The documented incidence of a stroke occurring after CPB varies slightly depending on the study and its criteria. For example, Taylor found that the incidence rate of a stroke is around 2 – 3%, Likosky *et al.* states that according to medical literature the incidence of stroke is between 1.3 – 4.3%, and Borger *et al.* found, in his literature review, that strokes occurred in 1.5 -3 % of patients [4, 9, 10]. These studies show the slight variation among literature in the incidence of stroke following CPB.

Although there is only a slight variation between studies on the incidence of strokes, there is significant variation in the documented occurrence of neurocognitive deficits. According to Borger and Feindel the "incidence of neuropsychological impairment is approximately 50-70% one week postoperatively and 30-40% three months postoperatively" [4]. Taylor found that the "incidence of cognitive defects is as high as 60% at 8 days postoperative with reduction to 25% to 30% incidence at 8 weeks and 12 months" [9]. Schonburg *et al.* found that "post operative psycho-neurological dysfunction occurs in 2-8% of all patients undergoing CPB" [3]. Three different studies show a broad range of neurocognitive deficits from as low as 2 to 8 % post CPB to up to

70 % incidence of neurocognitive deficit one week post operatively. The variability in these study results indicate the variability in assessment techniques that are currently used.

Since different study criteria and ways of assessing cognitive changes leads to varying results, a standard method must be developed to define cognitive impairment. Until a standard method is developed previous studies are difficult to compare. Some previously used methods of assessment include: the Johns Hopkins five high risk criteria, utilizing a comprehensive neurologic history and physical examination, and a preoperative stroke risk prediction model. These possibilities will be discussed further.

2.1.1 Preoperative Stroke Risk Prediction Model

The preoperative stroke risk prediction model was developed by Likosky *et al.* The initial model was developed by assessing 11,825 patients undergoing coronary artery bypass grafting (CABG) between the years of 1996 – 2001 [10]. All patients' data were accessed through the Northern New England Cardiovascular Disease Study Group (NNECDSG) which represents hospitals in Maine, New Hampshire, Vermont, and a medical center in Massachusetts [10]. The model was done to attempt to quantify the association between intraoperative and postoperative care and the chances of developing a stroke [10].

The study done by Likosky *et al.* had well defined criteria and validated their completed model with patient's data. Likosky *et al.* defined a stoke as "a new neurologic deficit

which appears and is still at least partially evident more than 24 hours after its onset, occurring during or following the coronary artery bypass grafting procedure and established before discharge" [10]. Likosky *et al.* revised their initial model to include estimated preoperative risk of a stroke as well as intraoperative and postoperative variables [10]. After developing the revised model they calculated the predicted risk of stroke for each patient and validated the model using a bootstrapping technique, which provided a virtually unbiased estimation or the predictive accuracy or their model [10].

The study found several preoperative, intraoperative, and postoperative variables were associated with an increased chance of stroke. The preoperative variables that were found to be significant included: age, diabetes, urgent or emergency surgery, renal failure or a creatinine of $\geq$ two mg/dl, ejection fraction $<$ 40%, and vascular disease [10]. The intraoperative and postoperative factors that were associated with an increase in stroke in the model were broad. Patients receiving cold cardioplegia, intra aortic balloon pump (IABP) insertion, patients in a high quartile for duration of bypass, patients going back on pump, prolonged inotropic support, low cardiac output syndrome, and atrial fibrillation had an increased chance of stroke [10]. These factors were considered as possible variables for the model.

After assessing the preoperative, intraoperative, and postoperative variables, four easily assessable variables were determined to be excellent indicators of stroke. The four that the model was based on consisted of: cardiopulmonary bypass duration, atrial fibrillation, prolonged inotrope use, and estimated preoperative risk [10]. This model is beneficial for

predicting a patient's risk of stroke and may be useful for determining a baseline to compare similar research in the future.

2.1.2 A Comprehensive Neurologic History and Physical Examination

Since frank ischemic stroke is a relatively uncommon occurrence and does not account for a variety of neurological deficits that could occur due to CPB, Hammon *et al.* developed an 11 part neurobehavioral battery to try to assess deficits following surgery. The neurobehavioral battery was conducted preoperatively, at five to seven days postoperatively, and at one month post surgery [11]. In addition to the neurobehavioral battery patients also were instrumented with a continuous wave carotid Doppler transducer (5-MHz) intraoperatively so an estimation of the amount of cerebral emboli could be determined [11]. The 11 part neurobehavioral examination was administered by a psychologist and included a battery of tests which were a sensitive measure of the patient's attention, concentration, memory, language, higher cortical function, and psychomotor skills [11]. The tests were structured to dismiss confounding variables such as IQ and secondary education [11]. This 11 part examination was used to create a baseline for all patients in the study.

A patient was classified as having a postoperative neurobehavioral deficit if either a new neurological deficit or a new neurophysical deficit was found. In the study done by Hammon *et al.* a new neurobehavioral deficit was defined as a "greater than or equal to 20% decline from preoperative performance on two or more neurobehavioral tests postoperatively" [11]. A new neurobehavioral deficit included a new postoperative

deficit found upon comprehensive examination at days five to seven or at one month, a worsening of a preoperative deficit found by comprehensive examination at days five to seven or at one month, or if death occurred before one month due to a neurological deficit [11]. Comprehensive neurologic history and physical examination is another way to classify neurological deficit following CPB that could be useful for future research.

2.1.3 Johns Hopkins Five High-Risk Criteria

Another method to determine the incidence of stroke following cardiac surgery includes the Johns Hopkins five high-risk criteria method. The five high-risk criteria include; age >70 years, hypertension, diabetes, previous cerebrovascular accident, and carotid bruit [9]. Documenting the preoperative risk factors prior to surgery may be useful for creating a baseline to correlate patients between studies.

Other factors, other than the high risk criteria, can also help predict post-surgical neurological dysfunction. Those mentioned in the study included: maintaining adequate cerebral blood flow, preventing micro and macro embolism, and limiting the systemic inflammatory response [9]. This and other studies also mention that the amount of manipulation of the aorta should also be limited, as it has been well documented to increase the chance of stroke [4, 9, 11]. Based on the various studies that have investigated post-surgical neurological dysfunction, it can be concluded that as the amount of predictive factors increase, the chance of neurocognitive deficit post surgery also increases. Therefore, the surgical team should minimize predictive factors when possible.

2.2 Main Sources of Air Emboli

There are three main sources of air that can reach the patient in CPB. These sources include: surgical air, anesthetic air, and cardiopulmonary bypass air. A timely identification of the source is essential to minimize damage to the patient; therefore, the possible causes for air at these sources will be discussed further.

2.2.1 Surgical Air

Air emboli occur most frequently when the chambers of the heart are open, such as in procedures including valvular repair, atrial septal defects, or ventricular septal repairs [7]. Surgical air has been a documented danger since 1914 when Carrel reported that opening of the pulmonary arteries, aorta, or the ventricles was followed by air entering the heart [8]. Pearson later defined the term "surgical air" as air "entering the arterial circulation from cannulation of the heart and aorta, after removal of the aortic clamp, air entrainment at the site of venous cannulation, after restoration of cardiac function, and during left atrial catherization" [7]. Surgical air has serious consequences to the patient, especially if not removed in a timely fashion.

2.2.2 Cardiopulmonary Bypass Air

Cardiopulmonary bypass air requires direct interventions of the perfusionist. Some of the causes of CPB air include; emptying of the CPB reservoir, rupture of the arterial roller pump head tubing, or pump creep which may also cause the reservoir to empty [8]. Punctures or openings in the lines, accidental disconnections, or open stopcocks may also pull air into circulation [8]. Air embolism due to cavitation has been documented due to

kinks or tubing clamps on the positive pressure arterial lines or due to high flows through a small cannula [8]. Other causes of CPB air may be due to an over pressurized circuit with an occluded vent or if the CPB reservoir is over pressurized and a vent valve is not in place [8]. There are numerous possibilities for CPB air that must be kept under the careful watch of the perfusionist to ensure the safety of the patient.

2.2.3 Anesthetic Air

Anesthetic air is most often given though intravenous or monitoring lines [8]. It is possible to cause air to enter the patient upon cannula insertion or when the left atrial monitoring line is inserted if there is inappropriate ventilation [8]. It is necessary for the anesthesiologist to fully expand the lungs to displace any pulmonary venous air while the surgeon is de-airing the heart [8]. Also, cell saver blood must not have a pressure bag on it or air could be forced into the patient when the bag is emptied [12]. There are a variety of ways for anesthetic air to be administered to the patient that are monitored to prevent their occurrence.

Whether the source is CPB, anesthetic, or surgical air, the effects vary depending if the air enters venous or arterial circulation. Arterial and venous air embolism occurs most frequently during hip replacements, cesarean section, craniotomy, and CPB [13]. The common link between these cases is an incised vascular bed that has a hydrostatic pressure gradient which favors the entry of gas into the vasculature [13]. Since air entry into the venous or arterial circulation has potentially different effects on the patient, they will be discussed further.

2.3 Venous Air Embolism

Venous air embolism may occur from a number of sources and cause a variety of problems. The most common cause is through the insertion or removal of the central venous catheter [13]. Another cause is through lung trauma that occurs from mechanical ventilation [13]. Venous air embolism leads to air bubble entrapment in the pulmonary capillary bed, which can cause; a decrease in the rate of gas exchange, an increased chance of cardiac arrhythmias, pulmonary hypertension, right ventricle strain, possible cardiac failure, or arterial gas embolism [6].

If the bubbles become trapped in the pulmonary microcirculation, a series of adverse events ensue. Pulmonary vascular obstruction initiates the release of vasoactive mediators which leads to cellular damage and lung edema [6]. These adverse effects are due to activated neutrophils that sequester themselves in the lung tissues [6]. The activated neutrophils release thromboxane and leukotrienes that lead to lung edema by increasing the alveolar capillary permeability [6]. When the capillaries become leaky, they cause a decrease in surfactant, which leads to alveolar collapse, atelectasis, and impaired gas exchange which may result in the need for mechanical ventilation [6]. Surfactant coats the alveoli and aids in gas exchange. Without surfactant the gas exchange ability of alveoli is greatly impaired. Venous gas embolism has a significant impact on the patient's outcome and can lead to long term damage or death.

2.4 Arterial Air Embolism

Arterial air embolism occurs through a variety of ways and can have more detrimental effects than venous air embolism. It can occur though manipulation of the aorta, cannula insertion, right to left shunts, such as a patent foramen ovale, and when the lung filter becomes overloaded, allowing for the bubbles to break through into the arterial circulation [6,13,14]. The lung filter is the pulmonary capillary bed that traps the air bubbles [6]. After the lung filter reaches a threshold and is overloaded the air bubbles spill over into the arterial circulation [6]. This occurrence is also termed a paradoxical embolism [6]. No matter what the source of the air is, the consequences can be severe.

Unlike venous air embolism, where the air often lodges in the lungs, in arterial air embolism the air is more likely to end up in an organ or vessel. Where the air lodges has significant impact on the patient's outcome. Arterial air embolism can lodge in skeletal muscle, coronary circulation, cerebral circulation, or blood vessels in the body. When the air bubble lodges in the vessels located in the skeletal muscles or viscera the body can usually tolerate it, but when the bubbles become lodged in the coronary or cerebral circulation complications, even as severe as death, may occur [6].

When the air bubble becomes lodged in a vessel it interacts with the blood and the endothelium causing the inflammatory response [6]. This leads to hypoxia and ischemia downstream which causes neuronal cell death [6]. The blood brain barrier may also be affected by air embolism. When the blood brain barrier is affected it leads to the activation and adhesion of leukocytes, which in turn causes vessel obstruction [6]. These

interactions are examples of the deleterious effects of air bubbles located in the arterial circulation.

Some of the effects of arterial air embolism are easily identifiable while others are more difficult to pinpoint. The effects can be seen as: changes in the echocardiogram, defined areas of pallor on the tongue, marbling of the skin, air in retinal vessels, delayed recovery, or as various cognitive changes, such as headaches, disorientation, motor weakness, coma, or convulsions [6,13]. Other complications may include: hemianopia, asymmetry of the pupils of the eyes, cardiac arrhythmias, circulatory failure, and Cheyne-Stokes breathing [13]. The presence of anesthetic agents or central anticholinergic syndrome can act like mild cerebral arterial gas embolism and make diagnosing a neurological problem difficult [13]. The effects of arterial gas embolism can be dramatic and potentially difficult to identify.

Arterial and venous air embolisms are detrimental to the patient's health. The major cause of harm from arterial and venous embolism is due to the reactions that occur at the blood air interface. Therefore, a closer look at the blood air interface is necessary to understand the reason behind air embolism's detrimental effects.

2.5 Blood Air Interface

The blood air interface leads to many detrimental effects and can cause complications. The blood air interface leads to contact activation and the systemic inflammatory response [15]. This response is initiated when blood is exposed to a non endothelial surface, such as the extracorporeal circuit or air [15]. Some studies suggest that neurological injuries may be due to secondary thromboinflammatory responses which are activated by the blood air interface [16]. When the endothelium is damaged by air or when there is an interaction between a blood constituent, like platelets or proteins, and air these responses can occur [16]. When air becomes lodged in the vasculature ischemia can ensue downstream. The adhesion of the bubble in the vasculature depends on multiple factors including; bubble residence time, the perfusion solution, and the endothelium [17]. Other negative events include: adsorption of phospholipids and fibrinogen, red blood cell clumping, lipid peroxidation, micro thrombi production, and phospholipase activation [7].

2.5.1 Platelets

Platelets are affected by the blood air interface. Platelets have been observed adhering to air, which in turn leads to platelet activation [7, 18, 19]. A study done by Eckmann *et al.* looked at platelet-platelet and platelet-bubble binding in platelet rich plasma (PRP) with and without exposure to air bubbles [19]. Platelets have previously been shown to affect the adhesive strength between the endothelial surface and air bubbles by up to 61% [19, 20]. The study found that platelet-platelet and platelet-bubble binding were enhanced when the PRP was exposed to microbubbles [19]. They also found that the microbubbles

initiated platelet-platelet binding away from the microbubbles surface [19]. This may be due to biochemical signaling or indirectly through thrombin generation [19].

2.5.2 Proteins

Proteins interact with air bubbles at the gas-liquid interface in the vasculature. The gas-liquid adsorption of proteins can cause conformational changes in the protein, such as unfolding [21]. This can expose areas of the protein that can signal the immune system to respond [21]. Proteins can also trigger the activation of biochemical pathways that could affect vessel tone or initiate blood coagulation [21]. The coagulation process can lead to vessel occlusion and limit distal perfusion [21]. Protein layer adsorption to a bubble may also effect adhesion interactions between the surface of the bubble and the vasculature and may slow the process of gas reabsorption from the bubbles [21]. Albumin is an example of one such protein [17]. There are regions on albumin can bind to the gas-liquid interface due to hydrophobic interactions as a result of its tertiary structure [17]. This interaction is essentially an irreversible process and causes a protein layer to form around the air bubble which makes surface-surface adhesion to the vasculature easier and can slow gas efflux from the bubble [17].

2.5.3 Endothelial Membrane

The endothelial membrane is also affected by air in the vasculature. Bubbles in the vasculature can denude the endothelium and the bubbles that are adhering to the endothelial surface can completely occlude vascular blood flow [19]. These bubbles can strip the “endothelium from the basement membranes and disrupt the oligomellar luminal

surfactant lining that is considered to contribute to the integrity of the blood brain barrier" [18]. This damage to the endothelium may lead to harmful responses by leukocytes in the brain [18]. When the vasculature comes in contact with an air bubble it irritates the arterial endothelium and generates the foreign body response through cellular and humoral immune mechanisms [13]. The larger the surface area of the bubble can increase activation of the thromboinflammatory pathways [16]. This activation can lead to platelet aggregation and local neutrophils sequestering [16]. Due to air bubble obstruction, as shown in Figure 1, the neurons metabolic processes begin to fail [13]. This leads to cytotoxic edema and cellular injury from sodium and water entering the vessel [13]. Due to the edema there is further impairment of perfusion [13].

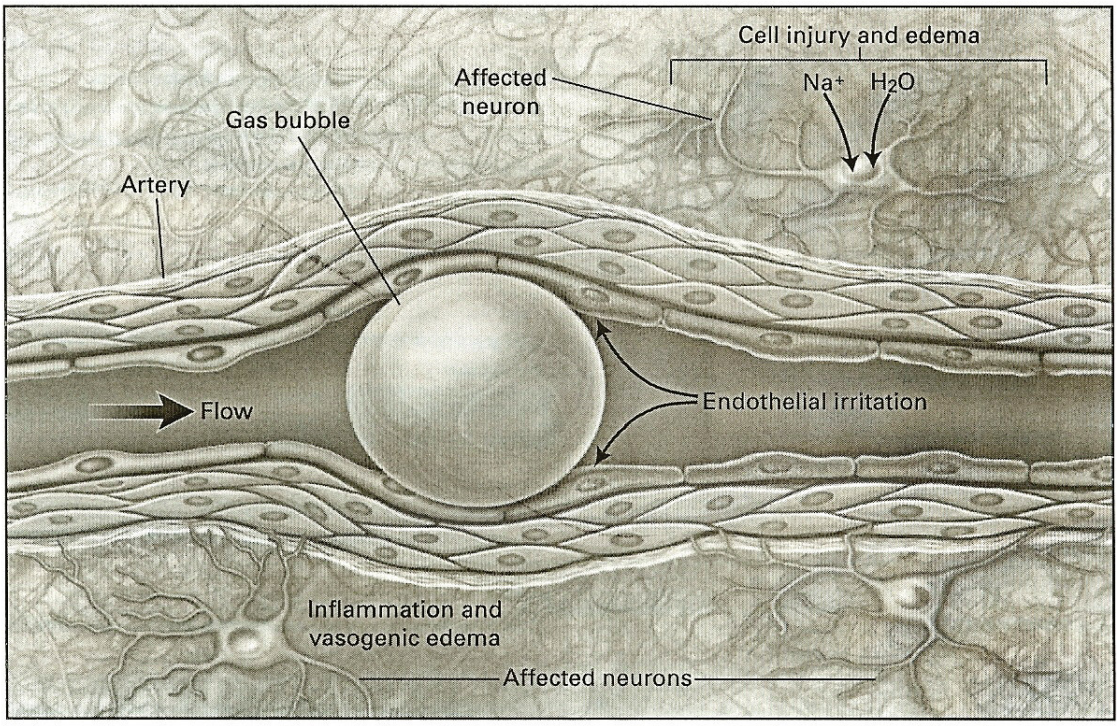

Figure 1. Bubble Obstructing Cerebral Blood Flow. The figure represents a bubble in the cerebral vasculature that is obstructing distal blood flow leading to ischemia [13].

2.5.4 Ischemia

Vessel obstruction from air embolism can lead to cerebral ischemia [16, 22]. Cerebral ischemia leads to neuronal cell death through energy failure or excitotoxicity [14]. The ischemic cell causes the release of excitatory amines that lead to an increase in intracellular calcium and sodium [14]. While this is occurring, glutamate stimulation of metabotrophic receptors will continue to increase the calcium levels and cause the cells to be more sensitive to excitatory amines [14]. These calcium activated enzymes cause the degradation of ribonucleic acids, proteins, and phospholipids, which in turn causes vasoconstriction, vessel occlusion, and the spreading of ischemia to surrounding cells [14].

The blood air interface leads to many potentially devastating effects for the patient. Proteins and platelets can bind to the air bubble increasing adhesion forces and allow the bubble to lodge in the vasculature. This can lead to ischemia, edema, and decreased perfusion downstream. The inflammatory response may also be activated. Due to these adverse effects, every precaution is taken to minimize exposure of blood to air and non-treated surfaces.

2.6 Air in the Venous Lines

Gaseous micro emboli in the venous lines can come from many sources. Some sources include: loose purse string sutures, a trans-atrial approach to a mitral valve repair or replacement, or openings in the venous system [1]. Air can also be seen in the venous lines if the patient has an atrial septal defect or a patent foramen ovale and the left atrium

or left ventricle is opened [1]. The source must be quickly identified to minimize harm to the patient.

Air can also be present through other mechanisms. Temperature gradients greater than 10-12 degrees Celsius, cavitation, vortexing of the blood, cardiotomy suction, and low reservoir volumes can lead to air formation. These sources are thought to produce gaseous micro emboli less than 45 microns in size [1].

The amount and size of air entering the patient that can lead to injury is uncertain. It is generally accepted that as the number of micro emboli created increases the neurophysical deficits increase [1, 5]. It has been reported that gaseous micro emboli with a diameter between 35-40 microns have been linked to morbidity during cardiopulmonary bypass [1]. A second source suggests that 10 – 40 microns is the "clinically interesting" range of bubbles [23]. Gaseous micro emboli greater than 100 microns should be considered macro emboli due to the high correlation with postoperative complications [1]. There is not a set range of bubbles that are known to cause significant neurophysical deficit, but general ranges have been established.

2.7 Bubble Flow Dynamics

The flow patterns of bubbles as they move through blood are dependant on many factors. The buoyancy, position of the patient, and the blood flow greatly affect how a bubble will act. In order to minimize the damage caused by bubbles, an understanding of their flow patterns is essential.

Buoyancy has a significant impact on the position of a bubble in the bloodstream. The buoyancy minimally affects bubbles if the patient is in a horizontal position or if the patient is in a head down position [18]. In these cases the bubbles would move to the higher point and are less likely to reach cerebral circulation where they would cause damage. Since very small bubbles will displace less fluid they are less buoyant and act more like particles allowing for their distribution to be determined mainly by blood flow [18]. In theory, in a typical gravity drained cardiopulmonary bypass circuit, the air remains whole and can be dissipated within the venous reservoir [1]. The reservoir is capable of handling whole air due to the buoyancy of the air and the screen filter the air passes through [1]. Buoyancy is an important factor in bubble distribution.

The fate of a bubble when it reaches a vessel is often dependant on the size of bubbles present. If a bubble is larger than the vessel it will form an elongated oblong shape as it moves through the vessel [16, 18]. If a bubble is small, for example <15 microns, it is likely to cause little or no interruption of flow as it passes through the microvasculature [18]. Bubbles greater than 15 microns, but not considered macro emboli may cause a slight interruption of flow in the capillaries, but are often cleared relatively quickly [18]. Bubbles that are greater than 200 microns may lodge in arterioles for varying lengths of time causing damage downstream from the blockage [18]. This lodging can be exacerbated by vasoconstriction that can occur due to the irritated endothelium and may persist for the duration of the resorption [16]. The increase in internal pressure causes the bubble to elongate further [16]. As the bubbles decrease in size the cylindrical portion of

the bubble disappears and the caps fuse to form a sphere [16]. It is not until this spherical shape is achieved that the internal pressure changes [16]. These larger bubbles may also break free and cause further damage by getting trapped in vessels down stream [18]. Bubbles may break free in cerebral vasculature due to a combination of bubbles shrinking, reflex cerebral vasodilation, and systemic hypertension which may be present [18]. The size of a bubble as it enters circulation is of utmost importance in the outcome.

The amount of bubbles present can also have an effect on the patient's outcome. If bubbles are in the presence of other bubbles they may coalesce. This may cause blockages in larger vessels downstream. If a bubble comes in contact with turbulent flow it may break up and form smaller bubbles [18]. The amount of bubbles present helps determine the severity of the patient's outcome.

Bubble redistribution can lead to devastating effects for the patient. A bubble that broke free will most likely take on the shape of the vessel it is in if it is large enough. This cylinder shaped bubble's redistribution is encouraged through surface tension forces at the leading end of the bubble [18]. These "surface tension forces are inversely proportional to the radius of the hemispherical ends of the bubble" [18]. This means that if the vessel and the bubble have the same radius then the surface tension forces will cancel each other out [18]. If the bubble is at a branching point and the leading part of the bubble is in a section of a smaller diameter than the trailing end the "surface tension forces opposing forward movement at the leading end may exceed both mean arterial blood pressure and surface tension forces at the trailing end, causing the bubble to trap"

[18]. This shows that the size of the bubble in cerebral circulation is a good indicator of the bubbles ability to be trapped and redistributed.

2.8 Treatments for Air Embolism

A variety of drug treatments have been found to help manage air embolism. Although there are is a wide array of treatments the strategy varies greatly between different centers and countries. Some common treatments used include; intravenous fluid administration, fluorocarbons, avoiding glucose administration, avoiding dextran, giving barbiturates, and administering Lidocaine [6, 13]. Steroids and aminophlline were given in the past, but are now contraindicated [6]. Other options may include a visit to the hyperbaric chamber and administering 100 percent oxygen [13].

2.8.1 Intravenous Fluid

Intravenous fluid administration may be beneficial since neurological damage can be minimized by diluting the hematocrit to roughly 30 percent [6, 13]. Since crystalloid solutions may lead to cerebral edema a colloid solution is preferred [6, 13]. When fluid administration to achieve normothermia is carried out it is necessary to maintain an approximate central venous pressure of 12 mmHg and a urine output of one to two ml per kilogram body weight per hour to assure adequate volume status [13]. Intravenous fluid administration is one of many ways to attempt to minimize air embolism damage.

2.8.2 Glucose

Glucose solutions should be avoided due to the potential to make neurological traumas worse [6]. This usually occurs due to an increase in lactate production which leads to intracellular acidosis [6]. It is recommended to avoid giving any glucose or lactate containing solutions during the acute phase of cerebrovascular air embolism [6].

2.8.3 Dextrans

Dextran solutions were used previously to improve microcirculation and reduce the chances of sludging [6]. However, these benefits were outweighed by the chance of causing acute volume overload, anaphylaxis, and the potential for lung congestion [6]. Due to these adverse effects dextran solutions are not often used.

2.8.4 Barbituates

Barbituates are used to treat seizures due to cerebral air embolism that are unresponsive to benzodiazepines [6]. Barbituates reduce cerebral oxygen consumption, lower intra cranial pressure, and inhibit the release of endogenous catecholamines which results in post ischemic cerebral protection [6, 14]. Barbituates are also thought to reduce the production of oxygen free radicals, further benefiting the patient [13]. Whenever barbiturates are used ventilatory support must be available due to the chance of high doses depressing respiration [13]. Barbituates are an alternate treatment to seizures due to air embolism if benzodiazepines are not having the desired effect.

2.8.5 Aminophlline

Aminophlline was initially used for its ability to relieve some symptoms of venous air embolism. It was beneficial for its capability to act as a pulmonary vasodilator which treats chest pain, tachypnea, and dyspnea which are seen in venous air emboli [6]. It is now contraindicated because pulmonary vasodilation can lead to an increased release of trapped bubbles into the systemic circulation [6].

2.8.6 Steroids

Steroids were used in the past to treat cell swelling. Cerebral air embolism damages the blood brain barrier and allows fluid to cross [6]. These cells swell due limited energy to maintain osmotic integrity [6]. It was thought that steroids would reduce the cell swelling, improving cerebral air embolism [6]. It is now known that corticosteroids cause more harm than good due to its vessel occluding effects [6]. Due to these negative effects it is no longer recommended.

2.8.7 Lidocaine

Lidocaine is thought to improve cerebral function. It has been shown to reduce the rise in blood pressure and intracranial pressure, reduce infarct size, preserves cerebral blood flow, reduces cerebral edema, and preserves neuro-electrical function [6]. It is recommended to administer a bolus dose of 1.5 mg per kilogram and maintaining a therapeutic concentration through IV administration when severe arterial gas embolism has occurred [13]. The levels of lidocaine should be monitored to be kept within therapeutic levels, as a lidocaine overdose may lead to central nervous system depression,

cerebral convulsions, and bradyarrhythmias [13]. Lidocaine should be considered following air embolism.

2.8.8 Fluorocarbons

Fluorocarbons are recommended to treat cerebral air embolism. They have a high gas dissolving capability, low viscosity, and chemical and biological inertness which make them highly valuable as a treatment [6]. Their administration increases tissue oxygen delivery and shrink the existing gas bubbles due to a higher diffusion gradient [6]. It is thought that fluorocarbons reduce brain infarct size and improve cardiovascular function post air embolism [6]. Fluorocarbons are a valuable tool in the treatment of cerebral air embolism.

2.8.9 Hyperbaric Chamber

Using the hyperbaric chamber or utilizing 100 percent oxygen benefits patients post gas embolism. Currently the hyperbaric chamber is the standard treatment for gas embolism [17, 21]. When 100 percent oxygen is administered at pressures above atmospheric levels the ambient pressure rises and systemic hyperoxia occurs causing a decrease in the size of the gas bubble [13]. Hyperoxia causes a large diffusion gradient, in the case of nitrogen bubbles, and pulls nitrogen out of the bubble and oxygen into the bubble and leads to an increase in oxygen delivery to the tissues [13]. This increase of oxygen in the plasma and increase in oxygen delivery to the tissues through the large diffusion gradient counteract the gas emboli's insult to the microvasculature [13]. The hyperbaric chamber

has other proposed benefits, such as, preventing cerebral edema by maintaining the integrity of the blood brain barrier and decreasing the permeability of blood vessels [13].

Treatments vary from hospital to hospital. An optimal treatment for air or gas embolism is yet to be determined. Some beneficial treatments include the use of lidocaine in therapeutic levels, using barbiturates, a visit to the hyperbaric chamber or administering 100 percent oxygen, using fluorocarbons, and maintaining normovolemia through administration of a colloid solution. Glucose or lactate containing solutions, Aminophlline, dextrans, and steroids should be avoided due to there adverse traits. Although these treatments may minimize the damage to the patient preventing air embolism altogether is ideal.

2.9 Prevention

Many safety devices have also been employed in the attempt to prevent air embolism in cardiac patient. In CPB circuits an arterial line filter, bubble detectors, low level alarms, bubble traps, and one way valves have been incorporated to reduce the incidence of air embolism [7].

2.9.1 Techniques to De-air the Heart

Many techniques have been developed in attempt to de-air the heart and circulation. Some techniques include inserting a needle into the ventricle, using a vent to de-air the ventricle, finishing to sew the left atrium while blood is coming out of it, expanding the lungs to clear the pulmonary venous blood, placing the patient in the Trendelenburg

position, or by letting the right lung collapse to stop air from entering the pulmonary veins located on the right side [7]. If there is air in the coronaries leading to myocardial dysfunction the patient will need to be placed back on CPB to meet oxygen requirements [8]. If the air embolism occurs during the CPB surgery there are a variety of options such as: hypothermia, venting, retrograde coronary sinus perfusion, retrograde cerebral perfusion, or cardiac massage [8]. These techniques can effectively de-air the heart.

2.9.2 Arterial Line Filters

Arterial line filters are often seen as the last line of defense in the CPB circuit. Blood enters the arterial filter at the side and exits via the tubing on the bottom. This encourages air to be removed through the purge line on top of the arterial filter. The arterial line filter is an effective way to remove air from the CPB circuit.

2.9.3 Bubble Detectors

Bubble detectors are placed on the arterial line and are programmed to shut off the pump if air is detected to reduce the chances of air reaching the patient. Before bubble detectors were used there was little chance of reacting fast enough, as the perfusionist would have to see the bubble, shut off the pump, and clamp the arterial line before the bubble flowing an average of five liters a minute reached the patient. Bubble detectors greatly decrease the chances of air reaching the patient.

2.9.4 Low Level Alarms

Low level alarms have been incorporated into CPB circuits to reduce the chance of reservoir emptying, leading to air embolism. When the blood level reaches a designated point an alarm will sound alerting the perfusionist. Since incorporating level alarms in CPB circuits the incidence of air embolism due to perfusionist neglect has greatly decreased.

2.9.5 One Way Valves

One way valves are incorporated into sucker and vent lines during CPB cases. They prevent air from being pumped into circulation if the lines were reversed. They are also used for additional vent lines during valve cases. One way valves are a useful tool to prevent air embolism.

2.9.6 Bubble Traps

Bubble traps are another form of prevention for air embolism. Like an arterial line filter, they are used as a last line of defense to remove air before it would enter the patient. Studies have been done on different manufacturers bubble traps in the arterial line to test their efficiency and biocompatibility. Three studies will be discussed further, two on efficiency, one on biocompatibility.

2.10 Studies on Bubble Traps

2.10.1 Biocompatibility of a Dynamic Bubble Trap

The biocompatibility of a dynamic bubble trap (DBT) was tested in an in-vitro study. An illustration of a dynamic bubble trap is shown in Figure 2. The bubble trap works by centrifugal forces and consists of a 3/8 inlet, a tube, a 3/8 outlet, a site for collecting the micro bubbles at the top of the device connected to a recirculation line, and a diffuser chamber [2]. The efficiency of the bubble trap is dependant on blood viscosity, temperature, the micro bubble size, the micro bubbles position in the bloodstream, and the velocity [2]. The model made for this study simulated normal physiological conditions that would occur during bypass, including normal pressures, flows, and recirculation time [2]. The study used healthy donor blood with a period between the blood collection and the start of recirculation not exceeding 20 minutes [2]. The study looked at blood samples taken at baseline, 1, 60, 120, and 180 minutes after starting to recirculate [2]. They took 10 samples with the bubble trap and 10 samples without the bubble trap [2]. The parameters looked at consisted of: hemoglobin, hematocrit, erythrocytes, leukocytes, thrombocytes, lactate dehydrogenase (LDH), potassium, free hemoglobin, thrombin-antithrombin (TAT), Plasmin antiplasmin (PAP), D-dimer, C5a, Interleukin 6 (IL-6), Interleukin 8 (IL-8), and tissue necrosis factor (TNF) [2].

The results of the study were informational. It was found that hemoglobin, erythrocyte, and hematocrit had no significant difference over the 180 minute time period [2]. The number of leukocytes decreased in both groups over time with no significant difference [2]. The thrombocyte, potassium, LDH, free hemoglobin levels, C5a, TAT, PAP, D-

dimer, IL 6, IL8, and TNF levels all showed no significant difference between the bubble trap group and the control group [2]. The study concluded that the in vitro model of the dynamic bubble trap has no adverse effect on hemocompatibility [2].

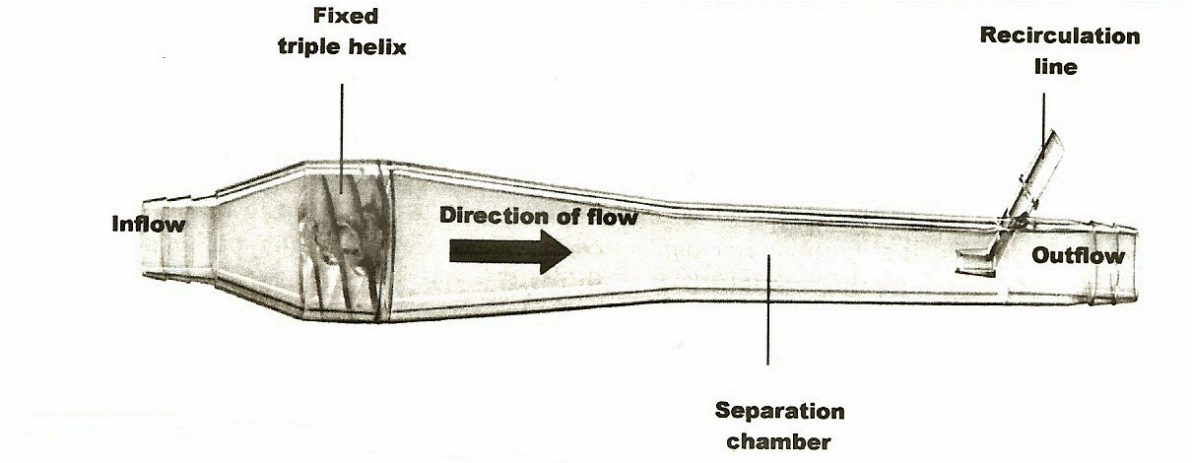

Figure 2. Dynamic Bubble Trap. Illustration of the dynamic bubble trap used in the experiment [24].

2.10.2 Efficiency of a Dynamic Bubble Trap

A study by Schonburg *et al.* was done to evaluate the efficiency of a micro bubble removal device during CPB. The study looked at 31 patients (DBT=17, placebo = 14) undergoing coronary artery bypass grafting by one surgeon [3]. The patients were randomly assigned using a double blind protocol to the dynamic bubble trap group or the placebo group [3]. The study looked at the amount of bubbles > five microns found before and after the bubble trap as well as the amount of HITS in the left and right cerebral arteries [3]. There was no significant difference between the two groups based on the enrollment variables, which consisted of: gender, age, body surface area (BSA), weight, extracorporeal circulation (ECC) time, number of bypasses, cross clamp time, haemoglobin before ECC and during, minimum venous temperature, and mean flow [3].

The results showed that the reduction in bubble counts on the outflow side of the bubble trap was significantly reduced ($p<0.001$) when compared to no bubble trap in the circuit [3]. In the study the placebo bubble trap was five times worse at removing bubbles than the dynamic bubble trap [3]. These results were also seen in the amount of HITS detected in the patient's left and right cerebral arteries. The mean value of HITS detected in the placebo group was 77, while the HITS in the DBT group was found to be 51 ($p = 0.04$) [3]. The results showed that the DBT reduced the number of micro bubbles that were detected in the arterial line as well as the micro bubbles reaching the patient.

2.10.3 Effectiveness of a Dynamic Bubble Trap

In a study done by Perthel *et al.* 12 patients undergoing two and three vessel coronary artery bypass grafting were studied with a DBT placed after the arterial filter in the arterial line [24]. The purge line for the DBT in this study was opened only when air bubbles were detected in the Quart air collecting chamber [24]. The middle cerebral artery was monitored for HITS throughout the case by Doppler sonography technology (Pioneer TC 4040 Medilab, Wurzburg, Germany) [24]. Air bubbles ranging in size from 10 – 120 micrometers were monitored [24]. The HP medica bubble counter probes were placed on the arterial line before and after the DBT or fixed in two places on the arterial line when the DBT was not used in the circuit [24]. There was no significant difference between patients when their characteristics, including gender, age, bypass time, cross clamp time, and number of grafts was compared [24]. The study found that with the use of a DBT in circulation there was a reduction in micro bubbles of 65.7% in the circuit

which corresponds with a reduction in the amount of micro embolic signals by a dramatic 86.2% [24].

These studies have shown that the use of a dynamic bubble trap is beneficial and safe to use in a CPB circuit. A significant reduction in the amount of bubbles seen reaching the patient was noted in both studies. The use of DBT's should be considered as a method to reduce the amount of air bubbles bypassing the arterial filter and ultimately reaching the patient.

2.11 Hypothesis

Placing a bubble trap in the arterial line has been proven to reduce micro air [3, 24]. Since the arterial bubble trap reduces micro air in the arterial lines it could potentially reduce air when placed in the venous lines. With growing concern about how much air actually reaches the patient and the potentially devastating effects on the patient every precaution should be taken. This study looks at the use of an arterial bubble trap in the venous lines and the potential reduction in air reaching the patient with its use. The three hypotheses are listed below.

2.11.1 Hypothesis #1

It is hypothesized that there will be a significant reduction in air volume amounts when comparing before and after the BT with a arterial BT placed in the venous lines.

2.11.2 Hypothesis #2

It is hypothesized that there will be a significant reduction in the volume amount of air measured after the ALF when a BT is used in the circuit than when the BT is not incorporated.

2.11.3 Hypothesis #3

It is hypothesized that low reservoir volumes will increase the amount of microbubbles found after the ALF significantly.

3. Materials and Methods

3.1 Test Circuit

A variety of components were used to construct the circuit for this in vitro experiment. The pieces of the circuit consisted of a Pemco pump, an integrated Terumo SX-25 oxygenator and hard shell reservoir, a 40 micron arterial filter, and a 5/8 inch silastic arterial boot that were all connected using Terumo X coated tubing. The arterial filter was fitted with a purge line that returned to the front of the Terumo hard shell reservoir. The arterial line (3/8 in x 3/32 in x 74 in) from the cardiotomy lead to a 4.5 L Terumo hard shell reservoir that served as the "patient" in the study. The "patient" reservoir was attached to an IV pole coming off the pump. The height of this reservoir and the cardiotomy reservoir were at a height difference of 30 inches to mimic the height of a patient in CPB surgery. The venous line exited the "patient" reservoir and a 3/8 3/8 luer lock was placed three inches below the start of the venous lines. This luer lock, with an attached injection port, was the site of air injection for the experiment. A Capiox bubble trap, intended for use in the arterial line, was placed in the venous line. It was located 15 inches prior to the entrance of the CPB reservoir and a pre-constructed bypass line was placed around the bubble trap. A stop cock attached to ¼ inch tubing was placed on top of the bubble trap, fitted through a roller head pump on the Pemco system, and returned to the cardiotomy reservoir to purge air from the bubble trap. The venous line (3/8 in x 3/2 x 72 inches) became ½ in tubing three inches prior to entry into the cardiotomy reservoir. All components were chosen to closely mimic the conditions during cardiac surgery.

Several monitoring devices were incorporated into the circuit. To monitor the hematocrit, hemoglobin, and venous saturations a CDI 500 was utilized. The sensor was placed five inches prior to the venous lines entry into the cardiotomy. Pressure was monitored off of the stop cock at the site of the arterial filter purge using a dlp pressure display 60000. The flow rate was measured with a flow probe off an A-Med (model # MCC01). A flow probe was placed on the arterial line 11 inches below the "patient" reservoir. Temperature was measured at the outlet of the oxygenator through a temperature probe attached to an Electromedic, Inc. Dual Display thermometer (Model # TM-147T). The temperature was maintained during the experiment through the use of a Sarns 3M heater/cooler. These monitoring devices were put in place to try to maintain conditions throughout the experiment.

The injection site for venous air was through a 3/8 3/8 luer lock connector with an injection port attached at the luer lock. A 25 gauge needle with a stop cock was attached to the injection port. The "slow" and "medium" doses were administered through the use of a Baxter syringe pump and the "bolus" dose was delivered by injecting the four cc bolus from a five cc syringe every 10 seconds. The same person did all the bolus injections to limit human error. The interval between the injections was controlled through the use of a stopwatch to minimize error.

3.2 BC 100 Bubble Counter

Bubble counts and sizes were measured with a BC 100 bubble counter made by GAMPT ltd. The bubble counter consists of a personal computer to display the analysis, two probes that fit over 3/8 inch tubing, and a console. A picture is shown in Figure 3.

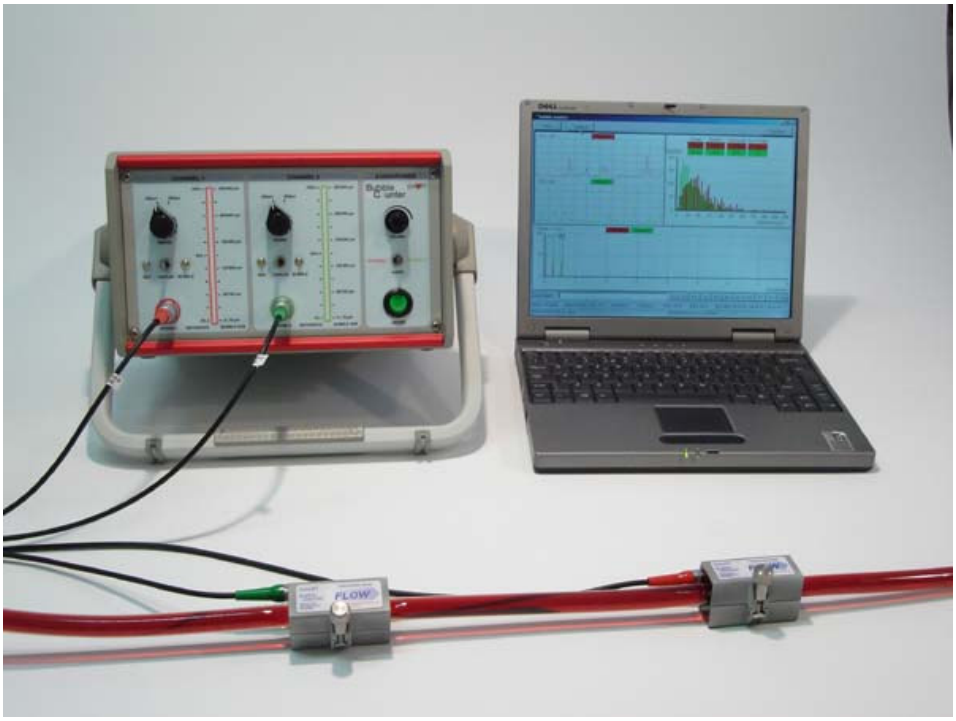

Figure 3. BC 100 Bubble Counter. Picture of the BC 100 Bubble Counter flow probes, personal computer, and console [25].

In the first part of the experiment which addresses hypothesis #1 the efficiency of air emboli removal by the arterial bubble trap in the venous lines is tested. The probes were placed four inches before the inlet of the bubble trap and four inches after the exit from the bubble trap. The second part of the experiment which addresses hypothesis #2 investigated the efficiency of a venous bubble trap placed in the CPB circuit. The probes were placed seven inches before the inlet of the cardiotomy reservoir and seven inches

after the arterial filter. A diagram of the circuit set up and the locations of the flow probes are shown in Figure 4.

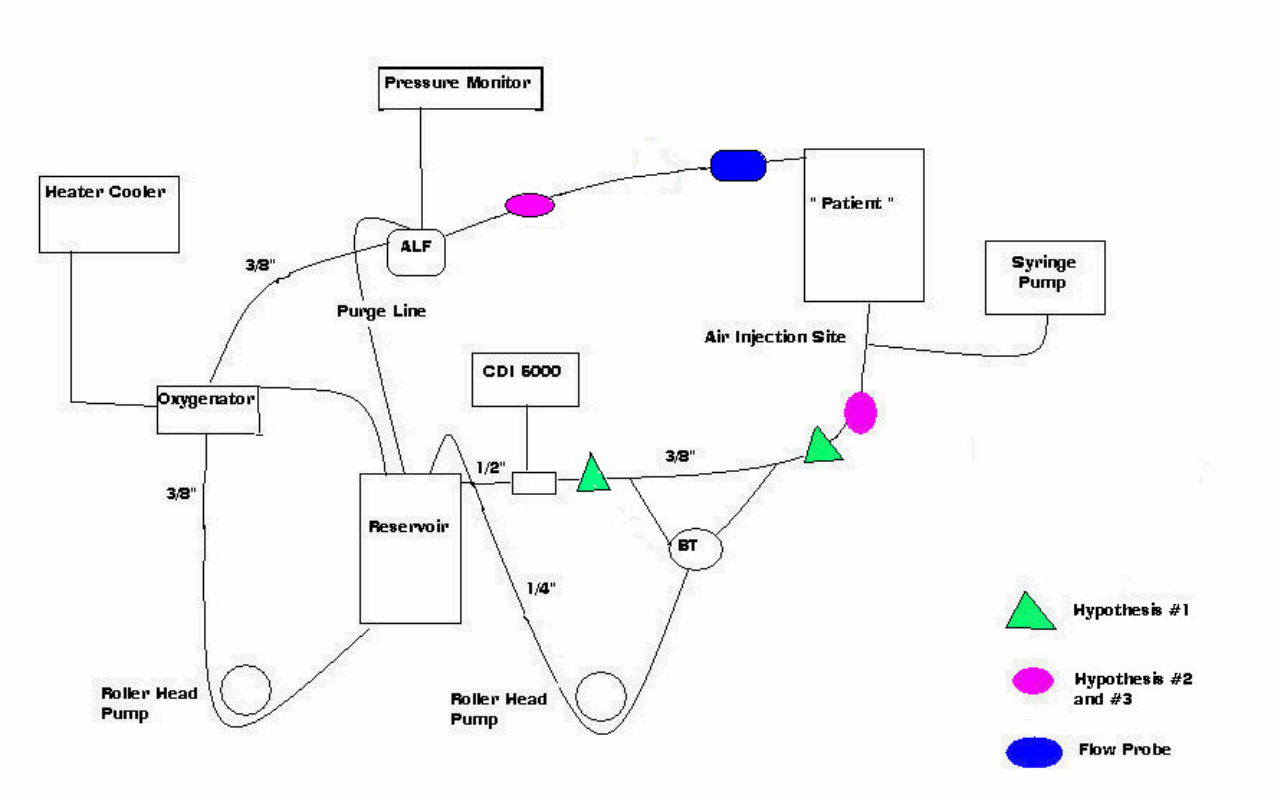

Figure 4. Circuit Diagram. Visual of the circuit set up to show flow probe, and the locations of the probes for the BC 100 Bubble Counter for both parts of the experiment.

The BC 100 bubble counter is a self calibrating system that can be used to detect and classify micro bubbles down to 5 µm in size [26]. It has two settings and is capable of switching between micro bubble measuring ranges of either 5-250 µm or 10-500µm [25]. This technology is possible through the use of ultrasound Doppler technology which uses a measurement method that scatters the ultrasound waves onto the bubbles to detect their presence [26]. The Doppler signal is audible and gives an estimation of the amount of bubbles present and their size [26]. The BC 100 has full sensitivity in flows between 1 – 8 Liters / minute [26]. The BC 100's traits make it exceptional for this experiment.

3.2.1 Self Calibrating System

The bubble counters self calibrating system uses a specially developed generator system that is capable of producing known sizes for distribution [26]. These bubbles of known size are injected into a tube system where they are sized using a microscopic camera. The sizes that the camera sees are compared to the sizes the probe sees and adjusted so the probe is accurate [26]. This allows for the calibration of a whole measurement range by simply altering the mean size of the generated bubble [26].

3.2.2 Detection of Micro Bubbles

The sleeve that houses the sensor is pressed around the 3/8 inch tubing. The two MHz transducer emits an ultrasound beam that is transmitted at a defined angle through a polystyrene window and then through the 3/8 inch tubing [27]. If the ultrasound beam does not come in contact with a bubble it will reach the other side of the tubing where a reference reflector is placed. The sensor also receives these reflected pulses, which contain information about the acoustical properties of the tubing itself [26]. Through the information received by the sensor the BC 100 bubble counter is able to perform measurements that are independent of the tubing material. If a bubble is present a small amount of the incident energy will be scattered by the bubble and sent back to the sensor [26]. The sensor will then take the back scattered ultrasound pulse and transform the signal into electrical radio frequency (RF) pulses [26]. The cross sectional area of the bubbles correlates with the amplitude of the pulses [26]. The RF signal is then processed by the devices internal micro processor to yield a histogram; this process is shown in Figure 5 [26].

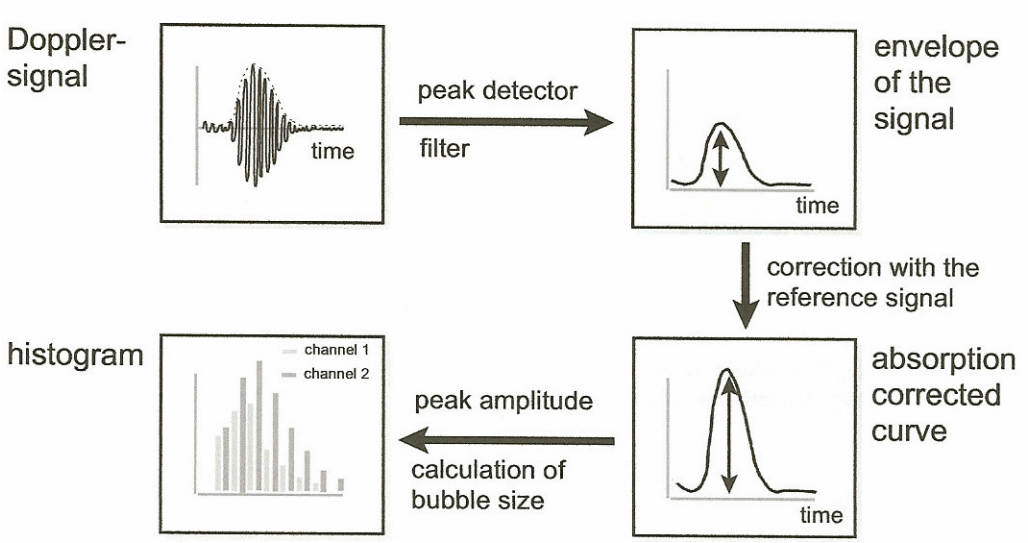

Figure 5. Schematic of the BC 100 Bubble Counter Sizing Process. A block schematic of the bubble sizing process used by the BC 100 bubble counter showing how the Doppler signal is converted to a histogram that is displayed on a personal computer [23].

3.2.3 Conversion to Bubble Volume for Data Analysis from the Histogram

The BC 100 Bubble Counter recorded the bubbles counted at two locations and classified them by bubble size. The bubble range counted for this experiment was between 5 and 500 microns, as shown by the bottom numbers on Figure 6. The bubbles above 500 microns were counted, but specific sizes could not be quantified due to limitations of the equipment. The red bars in Figure 6 represent the bubbles located after the bubble trap. The green bars on the histogram refer to the bubbles detected before the bubble trap.

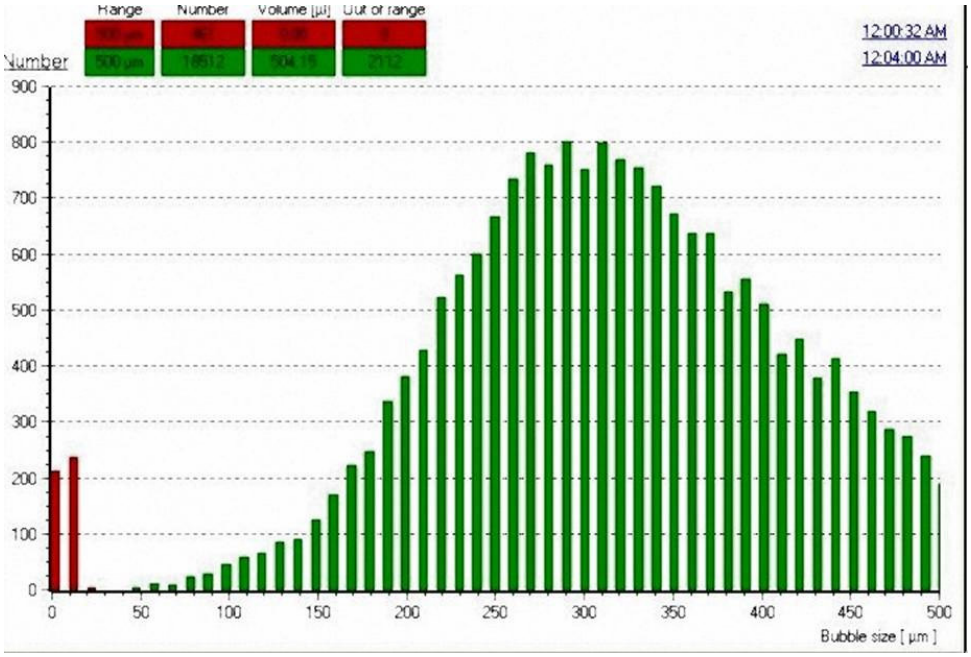

Figure 6. Histogram of BC 100 Bubble Counter Data. Bubble count showing the output from the two probe locations using the BC 100 bubble counters data analysis system. The red bars represent the bubbles located after the bubble trap. The green bars on the histogram refer to the bubbles detected before the bubble trap.

On the top of Figure 6 the categories range, number, volume, and out of range are written. The range is 5-500 microns and is detected by the bubble counter, the number is the overall count of bubbles, and the volume is the total volume in microliters for that trial. The out of range category is the total count for over 500 micron bubbles detected. This count is not included in the total volume. The data were then converted to a form that could be more easily analyzed.

The experiment data were transferred to Microsoft Office Excel (2003) from the BC 100 bubble counters program. All microbubbles between 5 and 500 microns were

individually converted to volume amounts. Equation (1) was used to convert the bubble counts to volume amounts.

$$= 1/6 * PI() * \text{Bubble Size} * \text{Bubble Size} * \text{Bubble Size}, \quad (1)$$

where:

PI() = Pi = 3.14,
Bubble Size is in microns.

These volume amounts were then summed and the total volume amounts for each trial were analyzed. The experimental data were analyzed using MiniTab (version 14), a statistical analysis program.

3.3 Circuit Preparation and Set Up

Once the circuit was assembled it was flushed with carbon dioxide. It was then primed with Plasmalyte A and allowed to circulate for a minimum of three hours. The occlusion of the roller head was verified with the crystalloid prime dropping at one cm per minute. The circuit was then chased with bovine blood to allow for minimal dilution. The bovine blood was purchased through lampire.com and was anticoagulated with Citrate Phosphate Dextrose (CPD) at the time it was drawn. All six liters purchased were from the same cow and two were initially used to prime the circuit. The hematocrit, determined through the use of a CDI 5000, was 24. The heater cooler was set at 38 degrees Celsius which resulted in a perfusate temperature of 37 degrees Celsius. The pressure monitoring site off the arterial filter was zeroed. Ultrasonic gel was when placing the probes onto the 3/8 inch tubing. The BC 100 bubble counter is a self calibrating system with two settings (5 – 250µm and 10 – 500µm). For this experiment the range of 10 – 500µm was chosen.

The BC 100 Bubble counter utilizes ultrasound Doppler technology and is able to simultaneously measure bubbles at both probe location.

3.4 Study Protocol

This study looked at the effects of having a bubble trap in the venous lines. It compared how effective an arterial bubble trap was at removing air in the venous lines (Hypothesis #1) and also how the bubble trap affected the circuit as a whole (Hypothesis #2). The study compared three air injection rates. The "low dose" used the syringe pump to administer air at a rate of three cc / min for four minutes. The "medium dose" also utilized the syringe pump to administer air at a rate of six cc / min for two minutes. The first two trials used the syringe pump to minimize human error. The bubble trap started counting bubbles once air was detected in the circuit and counted for the necessary amount of time. The "bolus dose" was administered using a five cc syringe and the 10 second intervals were measured through the use of a stop watch. A four cc bolus was delivered every 10 seconds, with the first dose starting the timer for the 10 seconds. The bubble counter measured the bubble count for 25 seconds. One person injected the bubbles to minimize error for the bolus experiment. Each circuit was made to be used for two days of testing due to limitations of the lab.

The variables for the study were decided after careful review of literature. The dependent variable was the amount of bubbles detected by the BC 100 Bubble Counter. Hypothesis #1 looked at how efficient the arterial bubble trap was in the venous lines. For this part, the independent variables were: 1) the rate of air injection and 2) the before the bubble

trap and after the bubble trap air detection sites. The independent variables for Hypothesis #2 of the experiment, where the overall effect of the bubble trap in the circuit was investigated, included: 1) if the bubble trap was included in the circuit or if it was bypassed, 2) the reservoir volume, either being between 200-300 or between 900-1100 ml, 3) the rate of air injection, and 4) the air detection sites (at the injection point and after ALF). These reservoir volumes were chosen due to a study done by Mitchell, Willcox, and Gorman that suggests that at a level of 1000 ml there is no bubble formation expected from the reservoir and that a reservoir volume of 400 ml or less bubble formation would be expected [28]. Another study done by Martens *et al.* reinforced the importance of this variable as they noted that lower blood levels in the reservoir were related to an increase in bubbles found in the arterial line [27]. The controlled variables for the experiments include: a flow rate of four L/min, hematocrit maintained at 24, temperature of the blood at 37 degrees Celsius, and a line pressure maintained between 100 – 250 mmHg (through the use of C clamps prior to and after the "patient" reservoir). For the circuit to be considered free of air and acceptable to perform another trial a baseline of <50 bubbles/minute was confirmed with the BC100 bubble counter.

Trials were randomized within hypothesis #1 and hypothesis #2. Hypothesis #3 was randomized because of its inherent relationship with hypothesis #2. Hypothesis #2 of the experiment was completed first and hypothesis #1 was completed second to minimize possible error due to moving the probes around multiple times unnecessarily. When the bubble trap was in use the flow rate of the roller pump head that acted as a purge for the

bubble trap was maintained at 30 rpm for all trials**.** The 30 rpm rate was calculated to be 17.5 cc/min.

3.5 Statistical Analyses

Ten trials were completed for all sets of data; however, since the BC 100 bubble counter did not record some trials completely, those sections have less than 10 trials analyzed. For all statistical tests a p-value of less than 0.05 was considered significant.

3.5.1 Hypothesis #1

Hypothesis #1 compared the volume amounts before and after the bubble trap at three different air injection rates. This was done to determine if the arterial bubble trap could significantly reduce the amount of air when placed in the venous lines. The independent variables included the air injection rates and the detection sites (before the bubble trap and after the bubble trap). The dependent variable was the bubble counts, which were then converted to air volume amounts. To test hypothesis #1 a 3 x 2 way repeated measures ANOVA was performed. For the 3 x 2 way ANOVA there were three levels of air injection rates and two levels for the probe locations (being before the bubble trap and after the bubble trap).

3.5.2 Hypothesis #2

This part of the experiment compared the results from having a bubble trap in the circuit to not having a bubble trap in the circuit. It looked at the bubble counts at two locations, at the air injection site and after the ALF, with and without a bubble trap in the venous

lines. The independent variables for this set of data included the reservoir volume, air dose, and the two probe locations at the air injection site and after the ALF. The dependant variable was the bubble counts which were later converted to total volume amount to be analyzed. To test hypothesis #2 a 3 x 2 x 2 x 2 way repeated measures ANOVA was performed. For the 3 x 2 x 2 x 2 way ANOVA there were three levels of air injections rates, two reservoir levels, two levels due to a bubble trap being in the circuit and one not in the circuit, and two levels for the location of the probes.

3.5.3 Hypothesis #3

This part of the experiment compared air bubble formation at "high" reservoir volumes to "low" reservoir volumes to see if there was a significant increase in microbubbles at low reservoir volumes. The independent variables for this set of data included the reservoir volume, air dose, and the two probe locations at the air injection site and after the ALF. The dependant variable was the bubble counts which were later converted to total volume amount to be analyzed. To test hypothesis #2 a 3 x 2 x 2 x 2 way repeated measures ANOVA was performed. The levels were the same for hypothesis #1 and hypothesis #2.

3.5.4 Other Tests Performed

A Tukey test was performed for all three hypotheses due to the three levels of air injection and a p-value of less than 0.05 for the air injection rates. The Tukey test compares the interactions between the slow and medium dose, the slow and bolus dose, and the medium and bolus dose. For each ANOVA an r-squared was calculated by

taking the regression sum of squares (Reg SS) divided by the total sum of squares (Total SS) [29]. The R-squared was calculated in order to determine the goodness of fit [29].

4. Results

4.1 Hypothesis #1: Comparing Air Volume Amounts before and After a BT Placed in the Venous Lines

Hypothesis #1 predicted that there would be significant reduction in air volume amounts when comparing before and after the BT with an arterial BT placed in the venous lines. The data for hypothesis #1 are shown in the interaction plot found in Figure 7. The solid line represents the air volume amounts before the bubble trap at the three air injection rates. Number one is the slow injection rate, two is the medium, and three is the bolus dose. The dotted line represents the air volume amounts after the bubble trap. There was significantly more air removed from the slow dose injection rates than from the bolus dose. This was because the BC 100 bubble counter could not accurately count bubble above 500 microns. Also, there is a significant difference between the location prior to the BT and after the BT, indicating that the BT was removing air when placed in the venous lines.

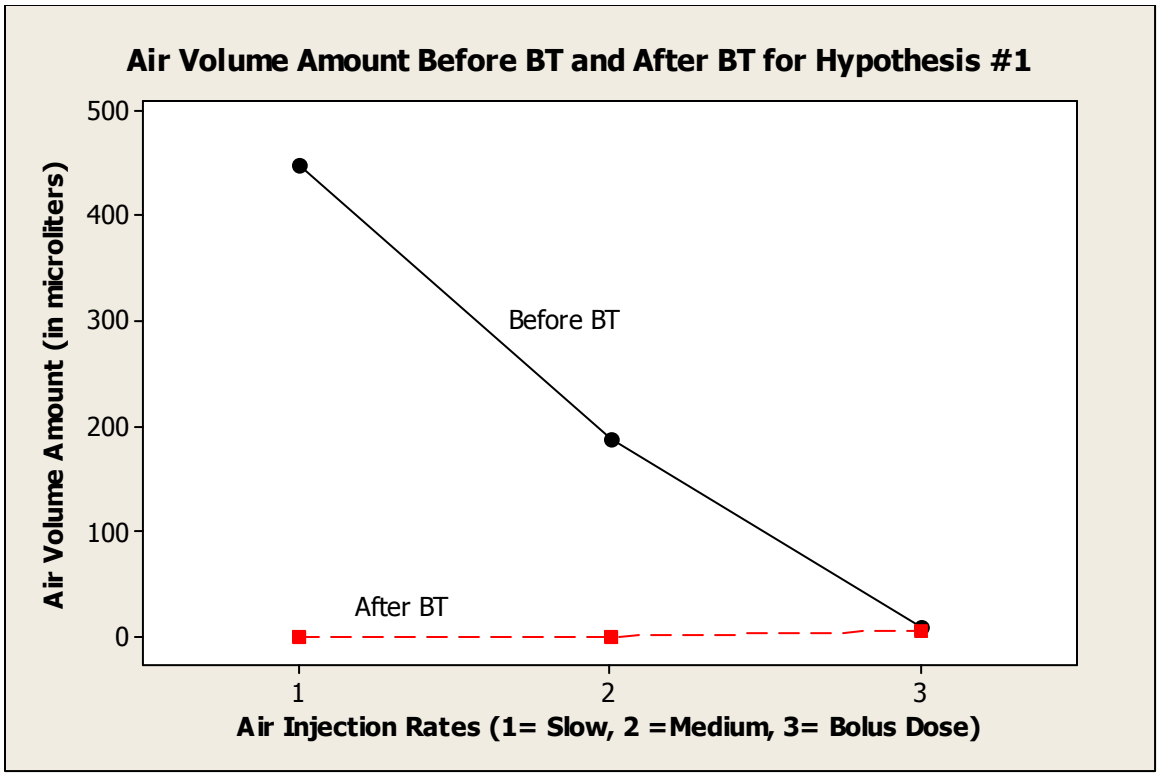

Figure 7. Air Volume Amount before the BT and After the BT for Hypothesis #1. The Figure shows the air removal at three air injection rates for hypothesis #1.

The summarized results of the 3 x 2 way repeated measures ANOVA are shown in Table 1. The full ANOVA source table is found in Appendix D. The individual trials used to test hypothesis #1 did not statistically vary (p-value of 0.407), indicating consistency among experimental trials. The BT, air injection dose, and the interaction between the air and the bubble trap were found to be statistically significant (p-value <0.001), indicating that the bubble trap was able to remove air when placed in the circuit. The r-squared value was 99.39%, meaning that 99.39% of the proportion of variability was explained. A Tukey test was performed on the data for hypothesis #1 due to the three levels of air injection rate and a significant p-value noted from the ANOVA. It revealed significance between all three levels of air injection doses (p-value < 0.05).

Table 1: Results from a 3 x 2 Way Repeated Measures ANOVA for Hypothesis #1.

Source	P-value
Trials	0.407
BT	<0.001
Air	<0.001
BT * Air	<0.001
R-Squared 99.39%	

4.2 Hypothesis #2: Comparison of a Bubble Trap and Without a Bubble Trap in the Venous Lines

Figure 8 shows the probe before the BT, indicated by the solid line, and the probe after the ALF, indicated by the dotted line. The numbers 1-3 shows the air dose. One is the slow injection, two is the medium injection, and three is the bolus dose. The data at the injection point and after the ALF combines both the BT data and the WOBT data. This graph shows the significant difference in air volume amounts at the injection point compared to the air volume amounts after the ALF for the three doses of air injection. The bolus dose has the smallest difference because the over 500 micron bubbles could not be quantified by sizes accurately.

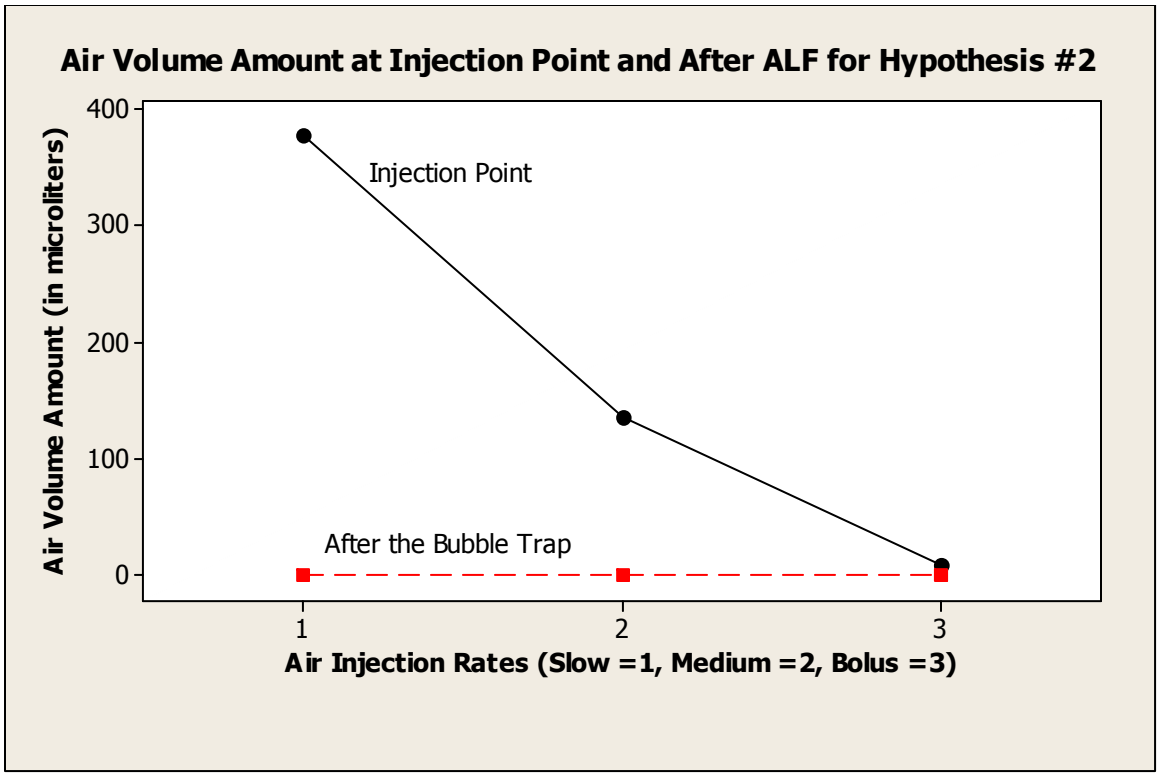

Figure 8. Air Volume Amount at the Injection Point and After the ALF for Hypothesis #2. The Figure shows the air removal volumes at three air injection rates.

Figure 9 compares the air volume amounts at the injection point, indicated by the number one, to the point after the ALF, indicated by the number 2 with a bubble trap in the circuit and without a bubble trap in the circuit. In Figure 9 the solid line indicates the circuit with the bubble trap bypassed and the dotted line indicates the circuit with a bubble trap included in the circuit. It shows the slight variation in the injection point and after the ALF when a bubble trap is used.

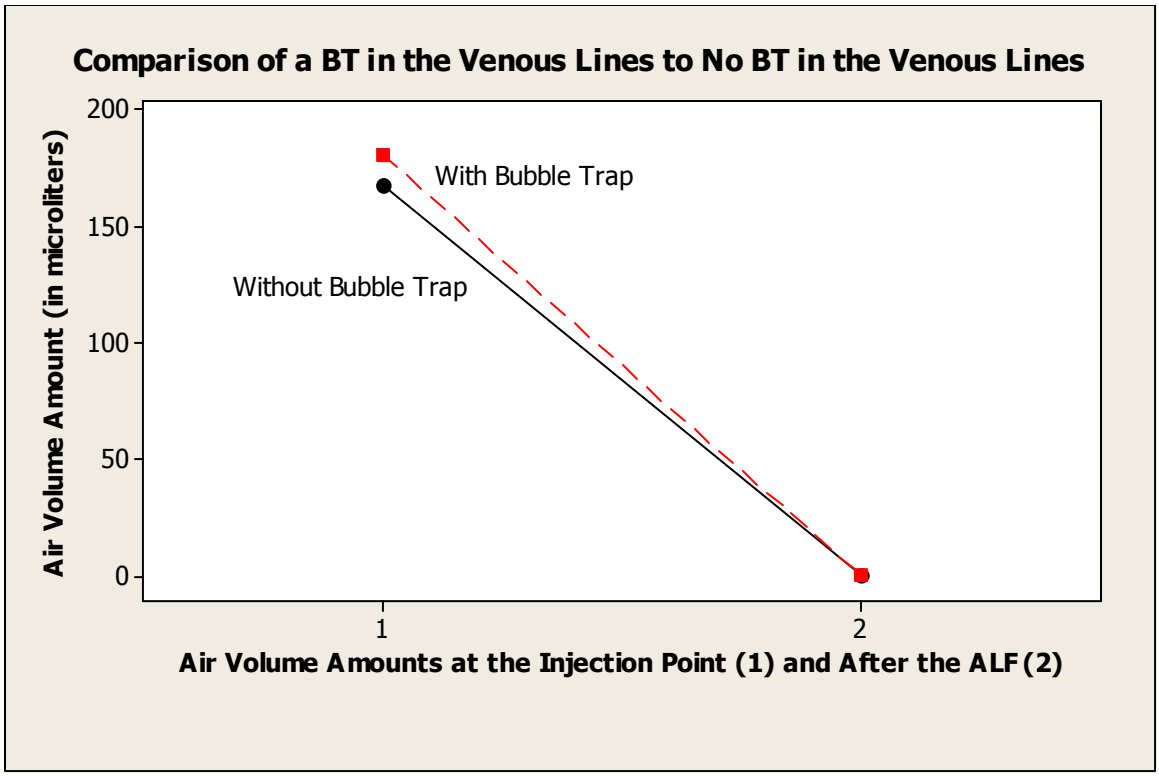

Figure 9. Comparison of a BT in the Venous Lines to No BT in the Venous Lines. Comparison of the air volume amounts at the injection point and after the ALF with a bubble trap in the venous lines compared to not having a bubble trap in the venous lines.

A 3 x 2 x 2 x 2 way repeated measures ANOVA was performed on the data comparing having a BT in the venous lines to a circuit without a BT in the venous lines. The results discussed are summarized in Table 2**.** The full ANOVA source table is found in Appendix E. The injection point air volume amount compared to the air volume after the ALF, the air injection dose, and the interaction between the air and the before bubble trap and after ALF volume amounts were found to be statistically significant (p-value <0.05). This indicates that there was significant air removal with a BT in the venous lines and without a BT in the venous lines and that the air doses were significant. The comparison between the circuit with a bubble trap and without a bubble trap in the venous lines was found to not be statistically significant at a p-value of 0.332. This indicates that the BT

did not have a statistically significant effect on the circuit when it was placed in the venous lines. The trials for hypothesis #2 were not found to be statistically significant with a p-value of 0.359, indicating consistency among experimental trials. The R-Squared value was 92.92%. This means that 92.92% of the proportion of variability was explained.

Table 2: Results of a 3 x 2 x 2 x 2 Way Repeated Measures ANOVA Performed for Hypothesis #2 and Hypothesis #3.

Source	P-value
BT/WOBT	0.332
Trials	0.359
Inject Point/ALF	< 0.001
Air	< 0.001
Reservoir	0.697
Inject Point/ALF *Air	< 0.001
R-Squared	92.92%

A Tukey Test was performed for hypothesis #2. It revealed significance between all three levels of air injection doses. This means that the three air doses were all significantly different from each other with a p-value of < 0.001 for all three comparisons.

4.3 Hypothesis #3: Comparing Air Volume Amounts at High and Low Reservoir Volumes

Figure 10 shows the air volume amounts at the injection point and after ALF when comparing the "low" and "high" reservoir volumes. For this figure the data for BT and without BT were combined. The reservoir volumes are not significantly different from each other, which indicated that the reservoir volumes had little impact on the amount of

bubbles formed. This is shown by the lines being almost level indicating little difference. The volume before the BT and after the ALF was significantly different, which is shown by the large distance between the parallel lines in Figure 10.

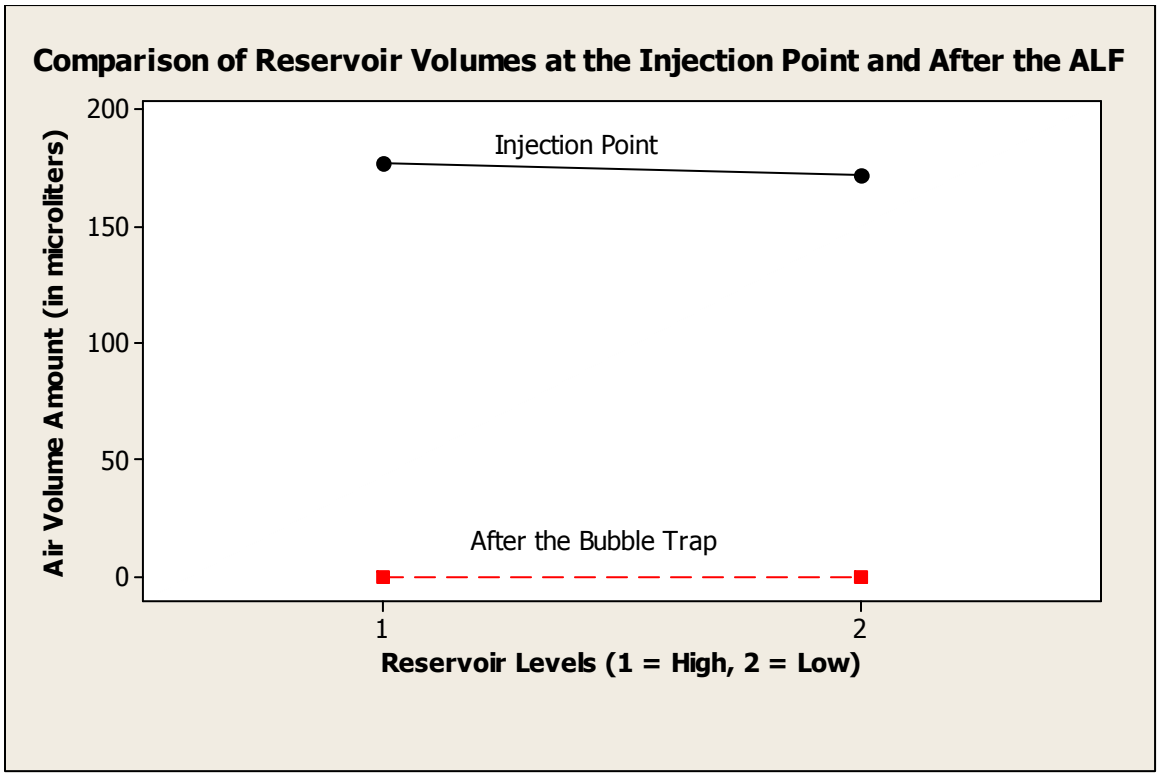

Figure 10. A Comparison of High and Low Reservoir Volumes at the Injection Point and After the ALF. This Figure shows the difference in air volume amounts at the injection point and after the ALF when comparing the high and low reservoir volumes.

Figure 11 compares the three air doses at the two reservoir volumes. The line with the circle end points is the slow dose, the line with the square end points is the medium dose, and the line with the triangle end points is the bolus dose. Figure 11 shows that the three air doses are statistically significant from each other by the large distance between lines. It also shows that at all three air doses the reservoir levels did not significantly impact the amount of air detected. For this Figure the BT and without BT data were combined.

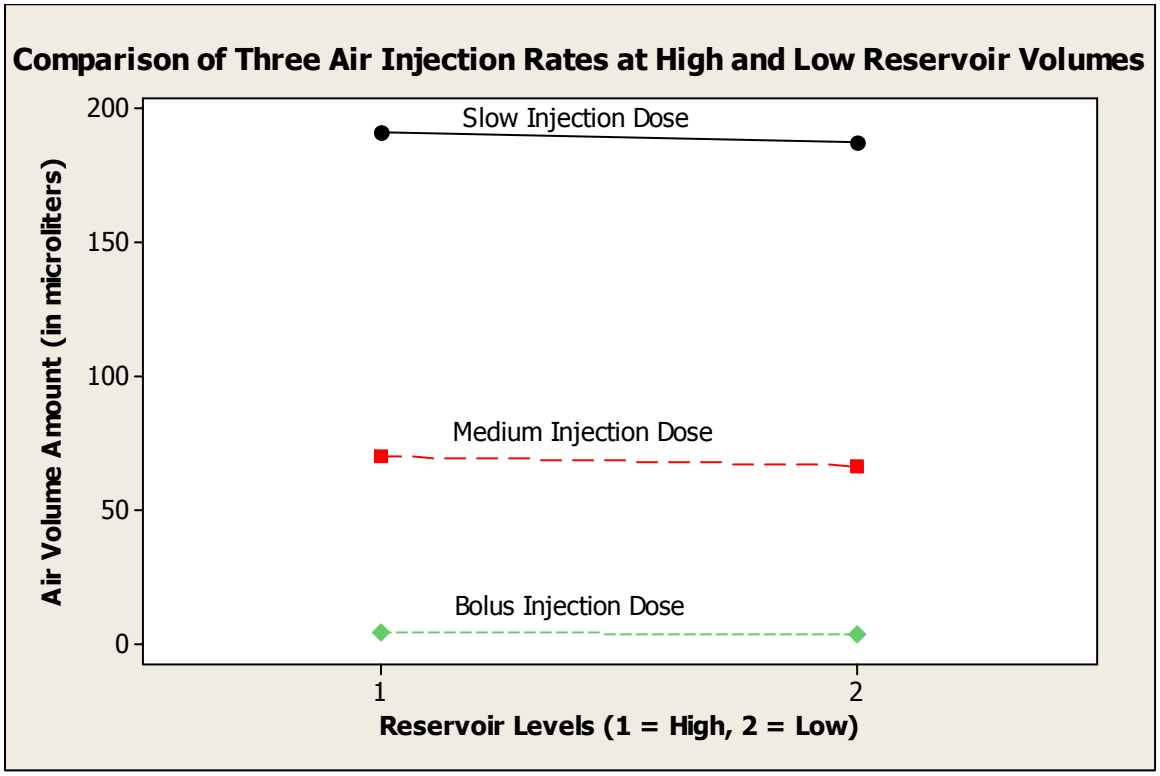

Figure 11. Comparison of Three Air Injection Rates at High and Low Reservoir Volumes. The Figure shows the three air injection rates at the high and low reservoir levels.

Hypothesis #3 also utilized the same 3 x 2 x 2 x 2 way repeated measures ANOVA performed on the data for hypothesis #2. Hypothesis #3 stated that at low reservoir volumes there would be a significant increase in the amount of microbubbles found after the ALF. The summarized results are shown in Table 2, with the full results found in Appendix E. The reservoir volumes were found to not be statistically significant with a p-value of 0.697. This indicates that the reservoir volumes did not affect the air volume amounts detected with this circuit.

5. Discussion

5.1 Controlled Variables

Certain variables were controlled during the experiment to minimize variations in trials. The controlled variables for the experiments include: a flow rate of four L/min, hematocrit maintained at 24, temperature of the blood at 37 degrees Celsius, and a line pressure maintained between 100 – 250 mmHg (through the use of C clamps prior to and after the "patient" reservoir). For the circuit to be considered free of air and acceptable to perform another trial a baseline of <50 bubbles/minute was confirmed with the BC100 bubble counter.

5.2 Hypothesis #1

The results from hypothesis #1 showed that the arterial bubble trap could efficiently remove air when placed in the venous lines. This was shown by the significant p-value (<0.05) when comparing the air volume amounts at the injection point and after the ALF. The initial air volume amount for the bolus dose was reduced due to the limitations of the bubble counter. The sizes above 500 microns could not be accurately sized and were not included in the total volume amounts. The bolus dose had a larger number of over 500 micron bubbles, so the initial dose may appear smaller than it was. The trials for the hypothesis #1 were not statistically significant (p-value of 0.407). This shows that the trials were consistent and did not vary significantly from each other. A tukey multiple comparison test was performed for hypothesis #1. It revealed that the three air doses were all significantly different from each other with a p-value of < 0.001 for all three comparisons.

5.3 Hypothesis #2

A comparison was done evaluating the effectiveness of incorporating a bubble trap in the venous lines to a traditional circuit without a bubble trap in the venous lines. The study found that there was not a significant difference (p-value > 0.05) between the circuit with the bubble trap to the circuit without. There was, however, a significant reduction in the air volume amounts for both when comparing the injection point to the point after the ALF. This may be due to the efficiency of the Terumo circuits at air removal. Another possibility is the amount of circuit components, such as the reservoir, oxygenator, and ALF that the air went through before the second probe detected the bubble count. Two probes were able to be placed on the circuit due to limitations in the lab. If probes were placed in multiple points in the circuit the major source of air reduction would be more easily identifiable. Another possibility for these results could be because the bubble trap was designed for use in the positive pressure arterial line. Even though the bubble trap was placed under positive pressure through the use of a roller head it may have different effects then the same device placed in the arterial line. If a device, such as a bubble trap, were designed specifically for use in the venous lines it may cause a significant reduction in air emboli.

5.4 Hypothesis #3

The reservoir level was found to not have a statistically significant effect (p-value >0.05) on the air volume amounts. This may be due to the reservoir used, as Terumo reservoirs have been noted to have negligible bubble formation, even at low reservoir levels [28]. The levels for the experiment were determined by a study done by Mitchell, Willcox, and

Gorman who suggested that at a level of 1000 ml there is no bubble formation and at a level of 400 cc or less bubble formation would be expected [28]. Another study by Martens *et al.* reinforced the importance of this variable as they noted that lower blood levels in the reservoir were related to an increase in bubbles found in the arterial line [27].

6. Conclusion

Microbubbles that are introduced into the CPB circuit can have potentially damaging effects on the patient. Air coming back in the venous lines is one possible source. If the air is not removed by the circuit it could reach the patient. This study looked at the effects of placing an arterial bubble trap in the venous lines.

Hypothesis #1, looking at the bubble trap's air removal capabilities in the venous lines, found a statistically significant reduction in air volume amounts when comparing the locations before and after the bubble trap. This showed that when the arterial bubble trap was incorporated into the venous lines it did remove a statistically significant volume amount of air. In hypothesis #2 a 3 x 2 x 2 x 2 way repeated measures ANOVA was performed on the data comparing a circuit with a bubble trap in the venous lines to a circuit without one. The results showed that there was not a statistically significant reduction in air volume amounts (range 10 – 500 microns) with a bubble trap incorporated into the circuit. Therefore, the circuit used was effective at removing the air, so the bubble trap, although able to remove a significant air volume amount, was not statistically significant when placed in the circuit.

7. Future Research Considerations

This study was done to serve as a template for further studies on the use of a device, such as a bubble trap, in the venous lines. Future studies may wish to look at a bubble trap's effectiveness at varying base flows, temperatures, or hematocrits. The temperature and the hematocrit greatly affect bubbles and altering these components may affect the results substantially. Varying the base flows could change the flow pattern of the bubbles thereby effecting how efficiently the bubble trap removes air. These alterations may greatly affect the bubble trap's capabilities and would be interesting to study.

Another possibility would be to look at certain ranges of bubbles. Some ranges to consider include: 1) fewer than 40 microns, since the average ALF is only able to remove air bubbles above 40 microns, or 2) bubbles above 100 microns, that have been linked to postoperative complications. Incorporating a bubble trap may reduce certain ranges of bubbles significantly when compared to a circuit without a bubble trap.

It would also be interesting to look at different injection rates, as the actual rate of air seen in surgery is not yet quantifiable. Since the amount of air seen in an average case is not known, different ranges should be looked at to test the effectiveness of the bubble trap at varying doses of air.

Another interesting thing to consider would be to compare different reservoirs with a bubble trap in the venous lines. The reservoirs may affect the efficiency of the bubble trap.

Air bubbles can originate from a number of sources during CPB. One common source is through air in the venous lines. Future research may lead to a bubble removal device in the venous lines that effectively removes air. This could dramatically reduce the amount of micro air in the circuit and thereby decrease potential harm to the patient.

8. References

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9. Appendix

Appendix A: Capiox Arterial Bubble Trap Information.

The bubble trap being studied is a Terumo Capiox bubble trap. The device is rated for up to six hours, per manufacturer, and is intended for use in the arterial line with a vent line purging to the cardiotomy reservoir.[1] The bubble trap is made of polycarbonate housing and a polyester filter inside.[1] The pore size is 170 micrometers, with a prime volume of 150 ml.[1] The maximum flow rate through the bubble trap is 6.5 liters per minute.[1] Figure A-1 shows a diagram of the device.

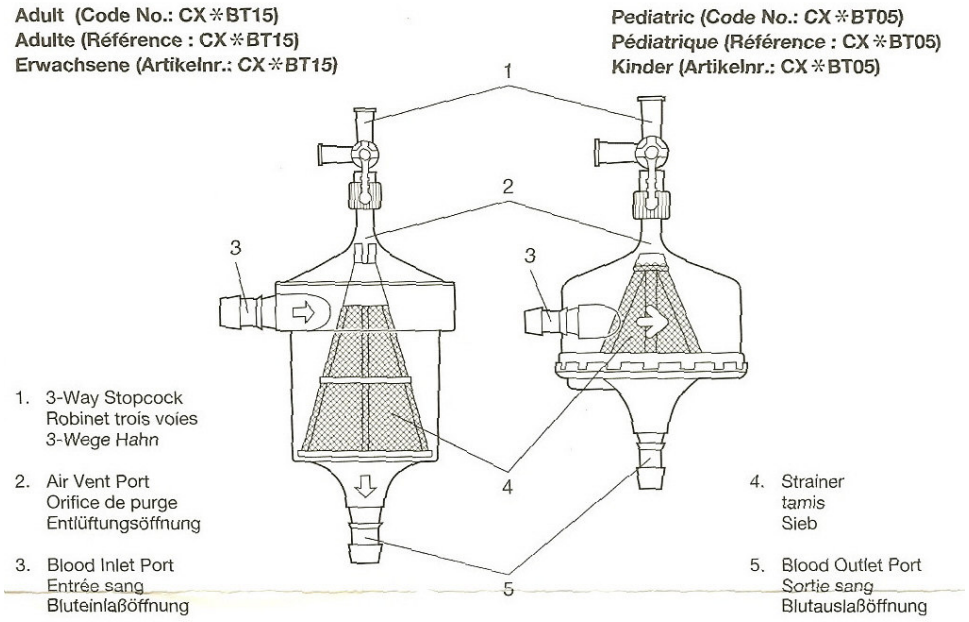

Figure A-1. Capiox Bubble Trap.[1]

[1] Terumo. February 1998. "Capiox Bubble Trap." Published brochure. Available from the author.

Appendix B: Appendix for the Conditions during the Experiment.

Parameters with Bubble Trap in Circuit for Low Level in Reservoir

	Low Dose	Medium Dose	Bolus Dose
Trial 1	HCT: 24 Temp: 34.5 ° C Line: 334 mmHg Flow: 3.92 L/min Reservoir: 280 cc	HCT: 24 Temp: 37.1 ° C Line: 360 mmHg Flow: 3.96 L/min Reservoir: 320 cc	HCT: 24 Temp: 37.2 ° C Line: 360 mmHg Flow: 3.96 Reservoir: 320 cc
Trial 2	HCT: 24 Temp: 37.2 ° C Line: 355 mmHg Flow: 3.94 L/min Reservoir: 250 cc	HCT: 24 Temp: 37.1 ° C Line: 350 mmHg Flow: 3.91 Reservoir: 300 cc	HCT: 24 Temp: 37.2 ° C Line: 356 mmHg Flow: 3.94 L/min Reservoir: 250 cc
Trial 3	HCT: 24 Temp: 37.2° C Line: 360 mmHg Flow: 3.96 L/min Reservoir: 250 cc	Data Did Not Save For This Trial	HCT: 24 Temp: 37.2 ° C Line: 362 mmHg Flow: 3.96 L/min Reservoir: 250 cc
Trial 4	HCT: 23 Temp: 37.1 ° C Line: 218 mmHg Flow: 3.98 L/min Reservoir: 350 cc	HCT: 23 Temp: 37.2 ° C Line: 219 mmHg Flow: 3.93 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.3° C Line: 342 mmHg Flow: 3.92 L/min Reservoir: 300 cc
Trial 5	HCT: 23 Temp: 37.3 ° C Line: 200 mmHg Flow: 3.98 L/min Reservoir: 300 cc	Data Did Not Save For This Trial	HCT: 23 Temp: 37.2 ° C Line: 180 mmHg Flow: 3.98 L/min Reservoir: 250 cc
Trial 6	HCT: 23 Temp: 37.3 ° C Line:148 mmHg Flow: 3.99 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.3 ° C Line: 140 mmHg Flow: 3.98 L/min Reservoir: 250 cc	HCT: 23 Temp: 37.2 ° C Line: 142 mmHg Flow: 3.92 L/min Reservoir: 300 cc
Trial 7	HCT: 23 Temp: 37.2 ° C Line: 142 mmHg Flow: 3.91 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.2 ° C Line: 144 mmHg Flow: 3.92 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.2 ° C Line:137 mmHg Flow: 3.91 Reservoir: 300 cc
Trial 8	HCT: 23 Temp: 37.2 ° C Line: 160 mmHg Flow: 3.95 L/min Reservoir: 300 cc	HCT: 23 Temp:37.1 ° C Line: 140 mmHg Flow: 3.95 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.3 ° C Line: 164 mmHg Flow: 3.98 L/min Reservoir: 300 cc
Trial 9	HCT: 23 Temp:37.4 ° C Line: 162 mmHg Flow: 3.95 L/min Reservoir: 280 cc	HCT: 23 Temp: 37.3 ° C Line: 199 mmHg Flow: 3.96 Reservoir: 300 cc	HCT: 23 Temp: 37.3 ° C Line: 198 mmHg Flow: 3.96 L/min Reservoir: 300 cc
Trial 10	HCT: 23 Temp: 37.4 ° C Line: 202 mmHg Flow: 3.97 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.2 ° C Line: 216 mmHg Flow: 3.99 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.2 ° C Line: 216 mmHg Flow: 3.99 L/min Reservoir: 300 cc

Parameters without Bubble Trap in Circuit for Low Level in Reservoir

	Low Dose	Medium Dose	Bolus Dose
Trial 1	HCT: 24 Temp: 37.3 ° C Line: 369 mmHg Flow: 4.0 L/min Reservoir: 300 cc	Data Did Not Save For This Trial	HCT: 24 Temp: 37.2 ° C Line: 365 mmHg Flow: 4.0 L/min Reservoir: 280 cc
Trial 2	HCT: 24 Temp: 37.2 ° C Line: 355 mmHg Flow: 3.95 L/min Reservoir: 300 cc	HCT: 24 Temp: 37.2 ° C Line: 354 mmHg Flow: 3.95 L/min Reservoir: 300 cc	HCT: 24 Temp: 37.2 ° C Line: 358 mmHg Flow: 3.90 L/min Reservoir: 320 cc
Trial 3	HCT: 24 Temp: 37.2 ° C Line: 353 mmHg Flow: 3.95 L/min Reservoir: 250 cc	HCT: 24 Temp: 37.3 ° C Line: 362 mmHg Flow: 3.97 L/min Reservoir: 300 cc	HCT: 24 Temp: 37.3 ° C Line: 360 mmHg Flow: 3.95 L/min Reservoir: 300 cc
Trial 4	HCT: 23 Temp: 37.3 ° C Line: 222 mmHg Flow: 3.97 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.2 ° C Line: 222 mmHg Flow: 3.98 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.3 ° C Line: 220 mmHg Flow: 3.98 L/min Reservoir: 300 cc
Trial 5	HCT: 23 Temp: 37.2 ° C Line: 214 mmHg Flow: 4.0 L/min Reservoir: 400 cc	HCT: 23 Temp: 37.2 ° C Line: 180 mmHg Flow: 3.99 L/min Reservoir: 250 cc	HCT: 23 Temp: 37.2 ° C Line: 200 mmHg Flow: 3.99 L/min Reservoir: 300 cc
Trial 6	HCT: 23 Temp: 37.3 ° C Line: 156 mmHg Flow: 3.99 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.1 ° C Line: 146 mmHg Flow: 3.92 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.3 ° C Line: 140 mmHg Flow: 3.98 L/min Reservoir: 300 cc
Trial 7	HCT: 23 Temp: 37.3 ° C Line: 144 mmHg Flow: 3.94 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.3 ° C Line: 140 mmHg Flow: 3.91 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.2 ° C Line: 132 mmHg Flow: 3.96 L/min Reservoir: 300 cc
Trial 8	HCT: 23 Temp: 37.3 ° C Line: 165 mmHg Flow: 3.96 L/min Reservoir: 280 cc	HCT: 23 Temp: 37.2 ° C Line: 166 mmHg Flow: 3.97 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.3 ° C Line: 136 mmHg Flow: 3.97 L/min Reservoir: 300 cc
Trial 9	HCT: 23 Temp: 37.5 ° C Line: 198 mmHg Flow: 3.95 L/min Reservoir: 250 cc	HCT: 23 Temp: 37.5 ° C Line: 168 mmHg Flow: 3.96 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.3 ° C Line: 196 mmHg Flow: 3.88 L/min Reservoir: 300 cc
Trial 10	HCT: 23 Temp: 37.5 ° C Line: 199 mmHg Flow: 3.94 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.4 ° C Line: 215 mmHg Flow: 4.0 L/min Reservoir: 300 cc	HCT: 23 Temp: 37.3 ° C Line: 202 mmHg Flow: 3.96 L/min Reservoir: 250 cc

Parameters with Bubble Trap in Circuit for High Level in Reservoir

	Low Dose	Medium Dose	Bolus Dose
Trial 1	HCT: 24 Temp: 37.2 ° C Line: 365 mmHg Flow: 3.86 L/min Reservoir: 750 cc	HCT: 24 Temp: 37.2 ° C Line: 363 mmHg Flow: 3.95 L/min Reservoir: 850 cc	HCT: 24 Temp: 37.2 ° C Line: 360 mmHg Flow: 3.84 L/min Reservoir: 800 cc
Trial 2	HCT: 24 Temp: 37.2 ° C Line: 354 mmHg Flow: 3.97 L/min Reservoir: 800 cc	HCT: 24 Temp: 37.1 ° C Line: 350 mmHg Flow: 3.97 L/min Reservoir: 750 cc	HCT: 24 Temp: 37.3 ° C Line: 369 mmHg Flow: 3.98 L/min Reservoir: 900 cc
Trial 3	HCT: 24 Temp: 37.2 ° C Line: 360 mmHg Flow: 3.96 L/min Reservoir: 780 cc	HCT: 24 Temp: 37.1 ° C Line: 359 mmHg Flow: 3.98 L/min Reservoir: 800 cc	HCT: 23 Temp: 37.2 ° C Line: 357 mmHg Flow: 3.96 L/min Reservoir: 800 cc
Trial 4	HCT: 23 Temp: 37.2 ° C Line: 250 mmHg Flow: 3.92 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.3 ° C Line: 220 mmHg Flow: 4.02 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.2 ° C Line: 220 mmHg Flow: 3.99 L/min Reservoir: 1000 cc
Trial 5	HCT: 23 Temp: 37.2 ° C Line: 207 mmHg Flow: 4.0 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.3 ° C Line: 184 mmHg Flow: 4.01 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.2 ° C Line: 202 mmHg Flow: 4.0 L/min Reservoir: 1000 cc
Trial 6	Data Did Not Save For This Trial	HCT: 23 Temp: 37.3 ° C Line: 151 mmHg Flow: 4.02 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.3 ° C Line: 145 mmHg Flow: 3.96 L/min Reservoir: 1000 cc
Trial 7	HCT: 23 Temp: 37.1 ° C Line: 140 mmHg Flow: 3.95 L/min Reservoir: 900 cc	HCT: 23 Temp: 37.3 ° C Line: 141 mmHg Flow: 3.96 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.3 ° C Line: 137 mmHg Flow: 3.96 L/min Reservoir: 1000 cc
Trial 8	HCT: 23 Temp: 37.2 ° C Line: 136 mmHg Flow: 4.03 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.2 ° C Line: 162 mmHg Flow: 3.99 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.2 ° C Line: 160 mmHg Flow: 3.96 L/min Reservoir: 1000 cc
Trial 9	HCT: 23 Temp: 37.2 ° C Line: 163 mmHg Flow: 4.02 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.3 ° C Line: 196 mmHg Flow: 4.0 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.4 ° C Line: 197 mmHg Flow: 4.01 L/min Reservoir: 1000 cc
Trial 10	HCT: 23 Temp: 37.2 ° C Line: 197 mmHg Flow: 3.96 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.3 ° C Line: 199 mmHg Flow: 4.01 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.5 ° C Line: 198 mmHg Flow: 4.0 L/min Reservoir: 1000 cc

Parameters without Bubble Trap in Circuit for High Level in Reservoir

	Low Dose	Medium Dose	Bolus Dose
Trial 1	Data Did Not Save For This Trial	HCT: 24 Temp: 37.1 ° C Line: 386 mmHg Flow: 3.84 L/min Reservoir: 900 cc	HCT: 24 Temp: 37.3 ° C Line: 371 mmHg Flow: 4.0 L/min Reservoir: 800 cc
Trial 2	HCT: 24 Temp: 37.4 ° C Line: 355 mmHg Flow: 3.98 L/min Reservoir: 800 cc	HCT: 24 Temp: 37.3 ° C Line: 355 mmHg Flow: 3.97 L/min Reservoir: 900 cc	HCT: 24 Temp: 37.3 ° C Line: 356 mmHg Flow: 3.97 L/min Reservoir: 900 cc
Trial 3	HCT: 24 Temp: 37.2 ° C Line: 362 mmHg Flow: 4.0 L/min Reservoir: 800 cc	HCT: 24 Temp: 37.4 ° C Line: 357 mmHg Flow: 3.97 L/min Reservoir: 800 cc	HCT: 24 Temp: 37.1 ° C Line: 361 mmHg Flow: 4.0 L/min Reservoir: 900 cc
Trial 4	HCT: 23 Temp: 37.2 ° C Line: 220 mmHg Flow: 4.0 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.2 ° C Line: 217 mmHg Flow: 4.04 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.3 ° C Line: 251 mmHg Flow: 3.93 L/min Reservoir: 1000 cc
Trial 5	HCT: 23 Temp: 37.2 ° C Line: 207 mmHg Flow: 4.0 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.3 ° C Line: 215 mmHg Flow: 4.01 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.4 ° C Line: 185 mmHg Flow: 4.03 L/min Reservoir: 900 cc
Trial 6	HCT: 23 Temp: 37.3 ° C Line: 151 mmHg Flow: 3.93 L/min Reservoir: 1100 cc	HCT: 23 Temp: 37.2 ° C Line: 150 mmHg Flow: 4.03 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.3 ° C Line: 149 mmHg Flow: 3.94 L/min Reservoir: 1000 cc
Trial 7	HCT: 23 Temp: 37.2 ° C Line: 138 mmHg Flow: 3.92 L/min Reservoir: 1100 cc	HCT: 23 Temp: 37.2 ° C Line: 130 mmHg Flow: 4.03 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.3 ° C Line: 134 mmHg Flow: 3.98 L/min Reservoir: 1000 cc
Trial 8	HCT: 23 Temp: 37.2 ° C Line: 170 mmHg Flow: 3.97 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.4 ° C Line: 157 mmHg Flow: 3.99 L/min Reservoir: 1100 cc	HCT: 23 Temp: 37.2 ° C Line: 156 mmHg Flow: 3.96 L/min Reservoir: 1000 cc
Trial 9	HCT: 23 Temp: 37.3 ° C Line: 188 mmHg Flow: 4.0 L/min Reservoir: 1100 cc	HCT: 23 Temp: 37.2 ° C Line: 196 mmHg Flow: 4.02 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.5 ° C Line: 196 mmHg Flow: 4.0 L/min Reservoir: 1100 cc
Trial 10	HCT: 23 Temp: 37.3 ° C Line: 198 mmHg Flow: 3.99 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.3 ° C Line: 202 mmHg Flow: 3.94 L/min Reservoir: 1000 cc	HCT: 23 Temp: 37.2 ° C Line: 217 mmHg Flow: 4.0 L/min Reservoir: 1000 cc

Test Parameters for Bubble Trap Only part of study

	Slow	Medium	Bolus
Trial 1	HCT: 23 Temp:37.5 ° C Line:120 mmHg Flow: 3.97 L/min Reservoir: 750cc	HCT:23 Temp:37.5 ° C Line: 147 mmHg Flow: 3.90 L/min Reservoir: 700cc	Data Did Not Save For This Trial
Trial 2	HCT: 23 Temp: 37.5 ° C Line: 152 mmHg Flow: 3.97 L/min Reservoir: 750cc	HCT: 23 Temp: 37.3 ° C Line: 148 mmHg Flow: 3.88 L/min Reservoir: 750cc	HCT: 23 Temp: 37.4 ° C Line: 151 mmHg Flow: 3.95 L/min Reservoir: 750cc
Trial 3	HCT:23 Temp:37.5 ° C Line: 151 mmHg Flow: 3.95 L/min Reservoir: 750cc	HCT: 23 Temp: 37.4 ° C Line: 151 mmHg Flow: 3.96 L/min Reservoir: 750cc	HCT: 23 Temp:37.3 ° C Line: 153 mmHg Flow: 3.97 L/min Reservoir: 750cc
Trial 4	HCT:23 Temp: 37.3 ° C Line: 151 mmHg Flow: 3.96 L/min Reservoir: 750cc	HCT: 23 Temp: 37.4 ° C Line: 153 mmHg Flow: 3.97 L/min Reservoir: 800cc	HCT: 23 Temp: 37.5 ° C Line: 151 mmHg Flow: 3.96 L/min Reservoir: 800cc
Trial 5	HCT:23 Temp: 37.3 ° C Line: 149 mmHg Flow: 3.96 L/min Reservoir: 750cc	HCT: 23 Temp: 37.4 ° C Line: 153 mmHg Flow: 3.97 L/min Reservoir: 750cc	HCT: 23 Temp: 37.3 ° C Line: 153 mmHg Flow: 3.97 L/min Reservoir: 750cc
Trial 6	HCT:23 Temp: 37.3 ° C Line: 154 mmHg Flow: 3.96 L/min Reservoir: 750cc	HCT: 23 Temp: 37.3 ° C Line: 153 mmHg Flow: 3.96 L/min Reservoir: 750cc	HCT: 23 Temp: 37.4 ° C Line: 153 mmHg Flow: 3.96 L/min Reservoir: 750cc
Trial 7	HCT: 23 Temp: 37.3 ° C Line: 154 mmHg Flow: 3.95 L/min Reservoir: 750cc	HCT: 23 Temp: 37.3 ° C Line: 153 mmHg Flow: 3.93 L/min Reservoir: 750cc	HCT: 23 Temp: 37.3 ° C Line: 154 mmHg Flow: 3.96 L/min Reservoir: 750cc
Trial 8	HCT: 23 Temp: 37.5 ° C Line: 155 mmHg Flow: 3.97 L/min Reservoir: 750cc	HCT: 23 Temp: 37.5 ° C Line: 156 mmHg Flow: 3.98 L/min Reservoir: 750cc	HCT:23 Temp: 37.5° C Line: 155 mmHg Flow: 3.97 L/min Reservoir: 750cc
Trial 9	HCT: 23 Temp: 37.5 ° C Line: 155 mmHg Flow: 3.96 L/min Reservoir: 750cc	HCT: 23 Temp: 37.5 ° C Line: 154 mmHg Flow: 3.96 L/min Reservoir: 750cc	HCT: 23 Temp: 37.5 ° C Line: 153 mmHg Flow: 3.91 L/min Reservoir: 750cc
Trial 10	HCT: 23 Temp: 37.5 ° C Line: 155 mmHg Flow: 3.95 L/min Reservoir: 750cc	HCT: 23 Temp: 37.3° C Line: 153 mmHg Flow: 3.93 L/min Reservoir: 750cc	HCT: 23 Temp: 37.3 ° C Line: 155 mmHg Flow: 3.95 L/min Reservoir: 750cc

Appendix C: Raw Data.

Hypothesis #1 Data

Slow Dose		Medium Dose			Bolus Dose		
Total Bubble Volume		Total Bubble Volume			Total Bubble Volume		
5.68E+09	2.56E+11	2.49E+09	1.6E+11		Trial 1 did not save		
31214978	4.55E+11	1.46E+09	1.86E+11		6.62E+09	7.58E+09	
4.11E+08	4.71E+11	30564254	1.96E+11		5.5E+09	1.19E+10	
3.14E+08	4.55E+11	1.14E+09	2E+11		5.33E+09	1.28E+10	
28689303	4.14E+11	81919806	2.1E+11		4.38E+09	1.09E+10	
36440625	4.6E+11	2.21E+09	1.7E+11		5.52E+09	9.77E+09	All in [µm3]
23794460	4.8E+11	4.64E+08	1.84E+11		5.62E+09	2.52E+09	
19793627	4.77E+11	1.47E+08	1.81E+11		6.08E+09	8.85E+09	
1.88E+08	3.74E+11	2.98E+08	1.7E+11		7.2E+09	5.77E+09	
1.92E+08	4.39E+11	21558538	1.98E+11		5.67E+09	8E+09	
5.680349	256.3729	2.492568	160.1965	Trial 1 did not save			
0.031215	455.1286	1.459411	185.8639		6.619641	7.582412	
0.410598	471.0089	0.030564	196.2074		5.501142	11.86666	
0.313797	455.274	1.142722	200.027		5.326724	12.79676	
0.028689	414.0945	0.08192	209.9551		4.38304	10.92214	All in [µl]
0.036441	460.3391	2.211604	169.7336		5.517897	9.768451	
0.023794	480.1359	0.464308	183.6262		5.623714	2.518969	
0.019794	477.1805	0.147266	181.1739		6.080132	8.84952	
0.18755	373.8408	0.297715	170.3382		7.197498	5.771444	
0.192395	438.958	0.021559	198.2781		5.67144	7.99817	
After	Before	After	Before		After	Before	

Hypothesis #2 and #3
Data with No Bubble Trap Included
in the Circuit

Slow High Level Total Bubble Volume		Slow Low Level Total Bubble Volume		Medium High Level Total Bubble Volume	
Trial 1 did not save		913245.3	3.36E+11	354112.5	1.95E+11
624963.3	3.68E+11	462170.2	4.24E+11	423430.7	2.18E+11
430253.7	4.03E+11	482890.5	3.67E+11	455303.2	2.19E+11
2505282	4.49E+11	761900.6	4.19E+11	7386153	8.32E+10
5166481	4.03E+11	4136065	3.5E+11	4143661	1.4E+11
9901623	4.08E+11	3677965	4.64E+11	4511218	1.06E+11
10651375	4.34E+11	9529112	4.01E+11	6145185	1.04E+11
4124433	1.5E+11	3720656	1.36E+11	9662146	9.32E+10
17918032	4.67E+11	22076954	4.87E+11	23772251	1.63E+11
21553264	4.96E+11	21676160	4.78E+11	18991346	1.76E+11

Top in [µm3]

Trial 1 did not save		0.000913	335.7021	0.000354	194.6118
0.000625	367.7799	0.000462	423.6134	0.000423	218.4773
0.00043	402.924	0.000483	367.4512	0.000455	219.4917
0.002505	449.4263	0.000762	418.8198	0.007386	83.20864
0.005166	402.9511	0.004136	349.6682	0.004144	140.1138
0.009902	408.2666	0.003678	463.7009	0.004511	106.2083
0.010651	433.6159	0.009529	400.9716	0.006145	104.267
0.004124	149.8005	0.003721	135.8765	0.009662	93.16832
0.017918	466.6712	0.022077	487.4636	0.023772	162.5201
0.021553	496.0737	0.021676	477.5269	0.018991	175.7991
After	Before	After	Before	After	Before

Bottom in [µl]

Hypothesis #2 and #3
Data with No Bubble Trap Included in the Circuit

Medium Low Level Total Bubble Volume		Bolus High Level Total Bubble Volume		Bolus Low Level Total Bubble Volume	
Trial 1 did not save		203680.9709	2.55E+10	158713.3	8.17E+09
540680.1	2.21E+11	64280.65088	1.03E+10	147758	1.08E+10
559371	2.06E+11	138817.031	1.74E+10	106418.8	8.58E+09
9472790	7.43E+10	79657.69973	1.5E+10	169117.7	4.24E+09
8951949	7.37E+10	1249066.347	3.93E+09	1475869	4.31E+09
11209274	6.83E+10	1030102.575	6.35E+09	749473.5	8.35E+09
10193358	6.7E+10	479310.6968	6.23E+09	620435.8	6.47E+09
4774864	8.49E+10	859948.6807	7.69E+09	999357.9	5.17E+09
17973830	2.07E+11	1916313.923	6.48E+09	1554184	8.13E+09
20607203	1.7E+11	1537124.737	7.59E+09	1124594	7.47E+09

Top in [µm3]

Trial 1 did not save		0.000203681	25.48229	0.000159	8.170019
0.000541	221.2175	6.42807E-05	10.25633	0.000148	10.81236
0.000559	206.0843	0.000138817	17.42924	0.000106	8.579781
0.009473	74.34471	7.96577E-05	15.01583	0.000169	4.236401
0.008952	73.73864	0.001249066	3.933445	0.001476	4.313193
0.011209	68.26239	0.001030103	6.347926	0.000749	8.350046
0.010193	67.02284	0.000479311	6.234331	0.00062	6.471404
0.004775	84.88096	0.000859949	7.686001	0.000999	5.165533
0.017974	206.5392	0.001916314	6.481689	0.001554	8.132945
0.020607	169.7159	0.001537125	7.589084	0.001125	7.467211
After	Before	After	Before	After	Before

Bottom in [µl]

Data for Hypothesis #2 and #3
Bubble Trap Included in the Circuit

Slow High Level		Slow Low Level		Medium High Level	
Total Bubble Volume		Total Bubble Volume		Total Bubble Volume	
411576.9	3.21E+11	677952	2.99E+11	941519.1	1.61E+11
492086.5	3.67E+11	361692.1	3.82E+11	719320.5	2.09E+11
314290.2	3.53E+11	479853.7	3.85E+11	496719.3	2.03E+11
66424584	4.09E+11	4393123	4.01E+11	12644301	6.46E+10
5023134	4.21E+11	6897851	3.5E+11	8050258	8.38E+10
Trial did not save		8346195	4.24E+11	10034411	9E+10
4381367	3.44E+11	7378809	4.31E+11	5089234	1.4E+11
8932715	3.8E+11	8636195	2.78E+11	1971233	8.97E+10
18512363	4.37E+11	19824665	4.3E+11	23031179	1.61E+11
22881356	4.78E+11	21587249	4.69E+11	21100935	1.69E+11
		Top in [µm3]			
0.000412	321.0714	0.000678	299.0845	0.000942	161.2968
0.000492	366.9432	0.000362	381.7516	0.000719	209.1028
0.000314	353.0773	0.00048	385.0617	0.000497	203.4044
0.066425	408.5907	0.004393	401.4387	0.012644	64.6222
0.005023	420.8542	0.006898	350.3672	0.00805	83.79559
Did not save		0.008346	424.2768	0.010034	89.97581
0.004381	344.007	0.007379	430.977	0.005089	139.9899
0.008933	379.8904	0.008636	278.3331	0.001971	89.70666
0.018512	436.7478	0.019825	430.0313	0.023031	161.305
0.022881	477.5013	0.021587	468.854	0.021101	168.9593
After	Before	After	Before	After	Before
0.014153	389.8537	0.007858	385.0176	0.008408	137.2158
		Bottom in [µl]			

Date for Hypothesis #2 and #3
Bubble Trap Included in the Circuit

Medium Low Level		Bolus High Level		Bolus Low Level	
Total Bubble Volume		Total Bubble Volume		Total Bubble Volume	
1223055	2.12E+11	28708.92087	8.48E+09	136239.8778	9.43E+09
674798.9	2.07E+11	63542.90021	9.58E+09	107481.2151	7.37E+09
Trial did not save		78114.13054	9E+09	88336.34943	1.14E+10
750222.8	8.25E+10	2713975.817	5.44E+09	114257.6304	6.67E+09
Trial did not save		759941.8381	6.81E+09	846301.6022	6.91E+09
8657205	9.42E+10	659785.7699	9.45E+09	970969.947	9.6E+09
5589569	1.37E+11	1349649.148	4.67E+09	548526.2661	3.38E+09
3836551	7.77E+10	624200.9499	6.48E+09	647910.0261	4.85E+09
21803667	1.72E+11	1287146.638	7.7E+09	1367788.18	1.3E+10
18452460	1.77E+11	1423744.658	7.75E+09	988219.9087	7.44E+09
			Top in [µm3]		
0.001223	211.7444	2.87089E-05	8.483023	0.00013624	9.427484
0.000675	207.2824	6.35429E-05	9.580264	0.000107481	7.371861
Did not save		7.81141E-05	9.00321	8.83363E-05	11.35458
0.00075	82.4624	0.002713976	5.438321	0.000114258	6.674186
Did not save		0.000759942	6.80777	0.000846302	6.912901
0.008657	94.16509	0.000659786	9.449583	0.00097097	9.595566
0.00559	136.5552	0.001349649	4.666136	0.000548526	3.38195
0.003837	77.67203	0.000624201	6.479318	0.00064791	4.852445
0.021804	172.2565	0.001287147	7.701419	0.001367788	12.97266
0.018452	177.0372	0.001423745	7.745369	0.00098822	7.441697
After	Before	After	Before	After	Before
0.007623	144.8969	0.000898881	7.535441	0.000581603	7.998532
			Bottom in [µl]		

Appendix D: ANOVA Source Table for Hypothesis #1.

Table A-1: ANOVA source table showing the results of a 3 x 2 way repeated measures ANOVA performed on the data from hypothesis #1.

ANOVA Source Table for Hypothesis #1

Source	DF	Seq SS	Adj SS	Adj MS	F	P-value
Trials for BT only Study	8	1931	1931	241	1.06	0.407
BT	1	610187	610187	610187	2690.46	< 0.001
Air	2	427175	427175	213587	941.76	< 0.001
BT * Air	2	448309	448309	224154	988.35	< 0.001
Error	40	9072	9072	227		
Total	53	1496672				

R-Sq = 99.39%

Appendix E: ANOVA Source Table for Hypothesis #2 and #3.

Table 4: ANOVA source table showing the results of a 3 x 2 x 2 x 2 way repeated measures ANOVA run on the data for hypothesis #2 and hypothesis #3.

ANOVA Source Table Comparing Data for Bubble Trap and Without Bubble Trap

Source	DF	Seq SS	Adj SS	Adj MS	F	P-Value
BT/WOBT	1	1719	1719	1719	0.95	0.332
Trials	6	12090	12090	2015	1.11	0.359
Inject Point/ALF	1	1275276	1275276	1275276	703.60	<0.001
Air	2	992630	992630	496315	273.83	<0.001
Reservoir	1	276	276	276	0.15	0.697
BT/WOBT * Inject Point/ALF	1	1717	1717	1717	0.95	0.332
BT/WOBT * Air	2	1006	1006	503	0.28	0.758
BT/WOBT* Reservoir	1	436	436	436	0.24	0.624
Inject Point/ALF *Air	2	992553	992553	496276	273.81	<0.001
Inject Point/ALF *Reservoir	1	276	276	276	0.15	0.697
Air * Reservoir	2	81	81	40	0.02	0.978
BT/WOBT* Inject Point/ALF *Air	2	1005	1005	503	0.28	0.758
BT/WOBT * Inject Point/ALF *Reservoir	1	437	437	437	0.24	0.624
BT/WOBT * Air * Reservoir	2	597	597	298	0.16	0.848
Inject Point/ALF *Air * Reservoir	2	80	80	40	0.02	0.978
BT/WOBT * Inject Point/ALF* Air	2	597	597	298	0.16	0.848
Error	138	250124	250124	1812		
Total	167	3530899				

R-Sq 92.92 %

Perfusion Thesis Approval Form

MASTER OF SCIENCE – PERFUSION

Milwaukee School of Engineering

This thesis, entitled "An In-Vitro Study on the Use of an Arterial Bubble Trap Incorporated into the Venous Lines," submitted by the student, Julie Knechtsberger, has been approved by the following committee:

Faculty Advisor: _____ Date: _____

Faculty Member: _____ Date: _____

Faculty Member: _____ Date: _____