A Comparison of the Efficacy of 1:4 del Nido Cardioplegia and Micro del Nido Cardioplegia in Adult Single Valve Repair or Replacement Surgery: A Single-Center Retrospective Analysis

by

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Abstract

To adequately protect immature hearts during periods of ischemia necessitated by cardiac surgery, Pedro del Nido developed a novel cardioplegia formulation, called 1:4 del Nido cardioplegia, which arrested the heart to reduce myocardial energy demand and utilized supportive additives to help resume normal function upon reperfusion. Traditional 1:4 del Nido cardioplegia has been shown in numerous research studies to offer improved myocardial protection over many common cardioplegia formulations while possessing a favorable redosing strategy. Adoption of 1:4 del Nido cardioplegia has therefore become common in the United States in both pediatric and adult cardiac surgery centers, although many clinicians still hold reservations toward its associated hemodilution. In recent years, the desire to develop a cardioplegia formula that has the benefits of 1:4 del Nido cardioplegia without the perceived negative effects of hemodilution has led clinical sites to begin experimentation with a microplegia variant of del Nido, called Micro del Nido, that replaces the crystalloid component of 1:4 del Nido cardioplegia with whole blood from the cardiopulmonary bypass circuit.

Before Micro del Nido cardioplegia is adopted on a wider scale, it would be beneficial to have additional evidence supporting its safety and efficacy, specifically in relation to the more proven formulation of 1:4 del Nido cardioplegia. To support this goal, this project reviewed the historic origins of cardioplegia, the causes and severity of ischemic injury, the mechanisms of action of cardioplegia additives, and the perceived negative effects of hemodilution. A literature review was conducted that found limited studies about the safety and efficacy of Micro del Nido. In an attempt to provide additional evidence supporting its adoption, a retrospective study was initiated at Ascension Columbia St. Mary's Hospital in Milwaukee, which transitioned from 1:4 del Nido cardioplegia to Micro del Nido cardioplegia for adult single valve repair and replacement surgeries. Unfortunately, the study was unable to be completed as planned due to data accessibility and integrity concerns, leading to a final recommendation of further comparative studies, specifically prospective studies where confounding factors can be reduced.

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1.0 Introduction

Myocardial ischemia is characterized by a disruption of blood flow to the heart, interrupting the delivery of oxygen to cardiomyocytes and creating an imbalance between energy supply and demand. While the heart is resilient to brief periods of ischemia, extended periods are detrimental and have the potential to cause myocardial injury and death. During cardiac surgery, planned myocardial ischemia is often necessary to allow the surgeon to enter the heart, as well as to provide a quiet, bloodless field to work. While the heart is generally intolerant to the duration of ischemia required by cardiac surgery, the use of cardioplegia lowers myocardial energy demand and offers the necessary support for the heart to withstand these ischemic periods and resist injury during and after arrest.

Cardioplegia generally consists of three main components – hypothermia to reduce myocardial energy demand, an arrest agent to stop the heart from beating, and additives to support the arrest and help the heart resume normal function upon reperfusion. Since its inception, cardioplegia's formulation has been a hotly debated topic. As an example, cardiac arrest induced via hyperkalemic cardioplegia was discovered and utilized in humans as early as the 1950s and is still the most common arrest agent used today, yet surgeons imposed a 10-year moratorium on its use during the 1960s due to left ventricular dysfunction, ventricular fibrillation, and cell death [1, 2]. Further, despite hyperkalemic arrest's prominence within cardioplegia formulations, opponents continue to push for a safer arrest agent that caters to an aging patient demographic with sicker hearts [2].

One mainstay of the cardioplegia debate is whether crystalloid cardioplegia, blood cardioplegia (i.e., microplegia), or a combination of crystalloid and blood is superior. Crystalloid cardioplegia formulations add the arrest agent and additives to a crystalloid base to be delivered at hypothermic temperatures into the coronary circulation for cardiac arrest. Microplegia is also delivered at hypothermic temperatures and can contain arrest agents and additives similar to crystalloid cardioplegia but uses whole blood from the cardiopulmonary bypass circuit in place of the crystalloid base. Crystalloid-based cardioplegia formulations have been historically used since the development of cardioplegia, while microplegia is a more recent development. Proponents of both cardioplegia types offer arguments for their cardioplegia's superiority, but continued studies must be done to eliminate confounding factors and isolate the debate between microplegia and crystalloid-based cardioplegias from secondary debates about delivery methods, arrest agents, and additives.

One prominent example of a cardioplegia formulation that primarily possesses a crystalloid base is 1:4 del Nido cardioplegia, which is delivered at a ratio of one part blood to four parts crystalloid. The crystalloid, PlasmaLyte, is supplemented with potassium to provide cardiac arrest, as well as additional additives – lidocaine, mannitol, bicarbonate, and magnesium. In recent years, to counter the perceived negative effects of crystalloid-based cardioplegia formulations, Micro del Nido was developed. Micro del Nido possesses near identical drug concentrations as 1:4 del Nido but uses a specialized cardioplegia delivery system to inject the arrest agent and additives directly into whole blood from the cardiopulmonary bypass circuit for delivery to the coronary circulation.

Although both methods of cardioplegia have been used in the Milwaukee area and appear to be safe and effective, a limited number of studies exist about Micro del Nido cardioplegia's overall performance in comparison to 1:4 del Nido cardioplegia. Ascension Columbia St. Mary's Hospital began using 1:4 del Nido in 2015 for their adult single valve repair or replacement surgeries before transitioning to Micro del Nido for these same types of surgeries in 2019. A retrospective study at this facility therefore serves two purposes: (1) to assess the efficacy and safety of Micro del Nido in adults and (2) to compare the efficacy of a blood-based cardioplegia to a crystalloid-based cardioplegia without the confounding factor of varying additive formulations. Additionally, confounding factors like surgeon, facility, and procedure can be reduced or eliminated. This project therefore aims to discuss myocardial injury and protection strategies and compare the safety and efficacy of 1:4 del Nido cardioplegia and Micro del Nido cardioplegia in adult single valve repair or replacement surgeries.

2.0 Background

Before the use of hypothermia, the invention of the heart-lung machine, and the development of cardioplegia, cardiac surgery was thought to be an impossible endeavor. As advancements were made by doctors and researchers, a better understanding of the causes and severity of ischemic injury were developed. Additionally, insights have been gained on what can be done to reduce or prevent myocardial injury during periods of ischemia necessitated by intracardiac surgery. To fully understand the mechanisms of del Nido cardioplegia, it is helpful to first understand the historical development of core cardioplegia principles before diving into concepts of ischemic injury and its prevention.

2.1 Early Origins of Myocardial Protection

For centuries the heart was considered to be surgically untouchable due to its significance within the human body and relative intolerance to being handled [1]. Theodor Billroth, a German surgeon and a founding father of modern abdominal surgery, is even quoted as saying, “No surgeon who wished to preserve the respect of his colleagues would ever attempt to suture a wound of the heart” in 1881 [1]. While there is debate as to whether Billroth said the above quote, it is well-documented that surgeons of the late 19th century possessed sentiments that opposed the concept of cardiac surgery [1].

Just a decade later in the early 1890s, Henry Dalton in St. Louis and Daniel Hale Williams in Chicago separately rebuked Billroth’s claim by successfully repairing injuries to human hearts. In the more well-documented repair by Williams, an African-American man named James Cornish was stabbed during a bar fight after a heated game

of cards and was brought to the hospital with a faint heartbeat and symptoms of shock [3]. Williams proceeded to cut a small hole in Cornish's chest and suture a severed artery, as well as repair a short laceration in the pericardium, allowing Cornish to continue living for decades. With the work of Dalton and Williams, the levy had been broken and by 1906, Ludwig Rehn of Germany was able to compile a list of 124 cases of cardiac wound repairs in Europe, with an astonishing survival rate of 40% [4].

In the early 20th century, advances in the field of cardiac surgery were limited as continued progress required a safe method for opening the heart. The first major revolution in the development of intracardiac surgery was Wilfred Bigelow's observation during the 1940s that natural hibernators, like bears, had reduced energy demands during the cold winter months. In a paper presented in 1950, Bigelow demonstrated that a period of ten minutes of circulatory arrest was tolerated by dogs at 20°C, which allowed for simple intracardiac repairs that could be completed within that timeframe [5]. Thus, systemic hypothermia was introduced to cardiac surgery to reduce the heart's energy demand, allowing brief periods of myocardial ischemia for the surgeon to enter the heart and perform necessary repairs. Amongst the first successful and documented cases of hypothermia being used for intracardiac surgery was John Lewis's successful repair of an atrial septal defect in 1952 [5].

In the following year, the next major revolution in cardiac surgery would arrive – the invention and successful use of the heart-lung machine by John Gibbon. Gibbon's design was capable of circulating and oxygenating the patient's blood in an extracorporeal

circuit, allowing the work of the heart to be offloaded and the heart to be emptied during cardiac repairs [6]. Reducing the workload of the heart further reduced myocardial energy demand when compared with hypothermia alone. John Gibbon and his heart-lung machine can be seen in Figure 1.

Figure 1. Dr. John Gibbon and his Heart-Lung Machine [7]. On May 6, 1953, Gibbon successfully closed a large secundum atrial septal defect by using cardiopulmonary bypass for 26 minutes [8].

Despite the advancements of both hypothermia and the heart-lung machine, the demands of cardiac surgery left much to be desired. While the advances made the heart more tolerant to periods of ischemia, the heart was generally still beating or fibrillating during the intracardiac repair, leaving the myocardium inadequately protected. The next major advancement required the heart to be stopped or “arrested” to further reduce myocardial energy demand and extend tolerated ischemic periods.

British physiologist, Sidney Ringer (for whom Lactated Ringer's solution is named), detailed the use of hyperkalemia to induce "diastolic arrest" in frog hearts as early as 1883, but its use in humans was slow to be adopted [9]. With a need to further reduce the energy demands of the heart, Denis Melrose borrowed Ringer's concept in 1955 and proposed that hyperkalemia could be used during cardiac surgery to induce "elective reversible cardiac arrest" and provide the surgeon with the quiet, bloodless field that was desired [10, 11]. Figure 2 illustrates how increased potassium levels induce electrocardiogram (ECG) changes and cause arrest. The "Melrose Technique" would take hold within the industry and provide the earliest utilized example of what is now known as "cardioplegia" – a term that would be introduced by Conrad Lam in 1957 [7].

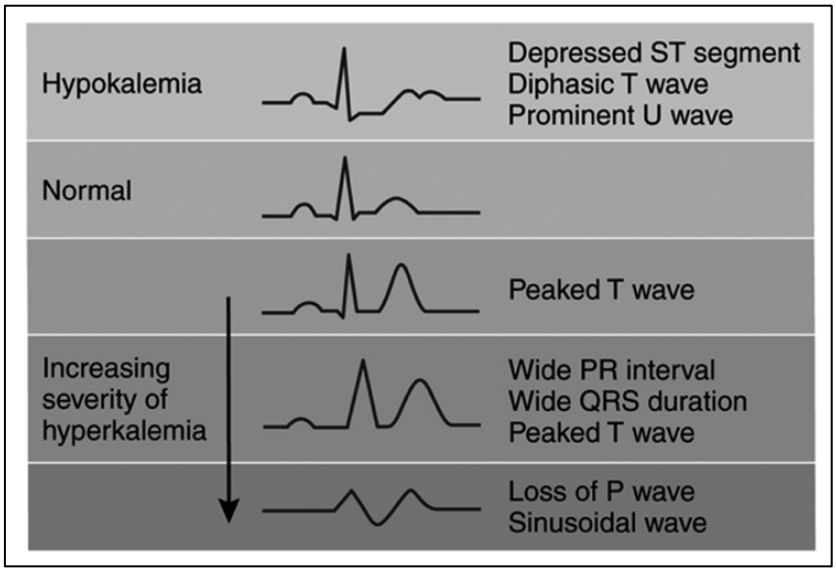

Figure 2. ECG Changes Associated with Hyperkalemia [12]. Increasing serum potassium results in flattened P waves, ST depression, and prolonged QRS duration. Eventually, a diastolic arrest is induced, which can be useful during cardiac surgery.

Despite community enthusiasm for the Melrose Technique, studies during the 1960s demonstrated that cardioplegia solutions high in potassium could lead to permanent myocardial injury (i.e., “stone heart” syndrome) and cell death, leading to a 10-year moratorium on the use of hyperkalemic cardioplegia solutions in humans [1, 2, 13]. Hyperkalemic cardioplegia solutions would eventually be reintroduced during the 1970s with more moderate potassium concentrations that did not induce the occurrence of stone heart syndrome seen in the decades prior [13].

Since the reintroduction of hyperkalemic arrest, formulations for cardioplegia have been continually debated and revised to reduce ischemic injury and meet the needs of changing patient demographics. One of the most notable formulations to arise was the development of 1:4 del Nido cardioplegia by Pedro del Nido in the 1990s, which still utilizes the historical developments of hypothermia, decompression utilizing the heart-lung machine, and a hyperkalemic arrest. To understand what distinguishes del Nido cardioplegia from other common cardioplegias, it is first necessary to discuss the causes and levels of severity of myocardial injury, means of injury prevention, and types of arrest.

2.2 Myocardial Injury

Under normal physiological conditions, the heart requires a constant supply of oxygenated blood to meet the energy demands of cardiomyocytes. Assuming a sufficient oxygen supply, cardiomyocytes will produce ATP through aerobic respiration, which includes the Krebs cycle and oxidative phosphorylation [14]. For every mole of glucose consumed during aerobic respiration, 36 moles of adenosine triphosphate (ATP) are

generated, as seen in Figure 3. Overall oxygen consumption for normal ventricular myocardium is approximately 8 to 13 mL of O_2 per 100g of myocardium per minute.

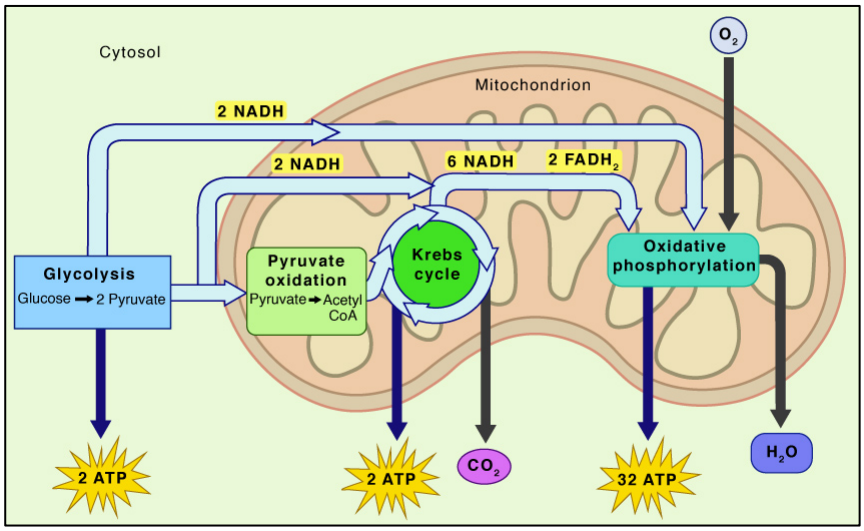

Figure 3. Aerobic Respiration [15]. Aerobic respiration is a biological process in which glucose is broken down into ATP, carbon dioxide, and water in the presence of oxygen. Aerobic respiration is preferable to anaerobic respiration due to the high ratio of ATP produced to glucose consumed.

Given normal oxygen extraction by the heart is 75% and the physiological limit of oxygen extraction is approximately 95%, limited additional amounts of energy can be gained through increased oxygen extraction. In other words, energy supply has a limited surplus relative to energy demand under normal physiological conditions [14]. During times of increased energy demand by cardiomyocytes, it therefore becomes necessary to increase coronary blood supply to provide more oxygen for the production of additional ATP. While coronary blood supply can normally increase 4- to 5-fold to meet the energy demands of cardiomyocytes, natural (e.g., coronary artery disease or myocardial infarction) and artificial (e.g., aortic cross-clamping) obstruction are possible and result in

insufficient blood flow to meet the demands of the heart, resulting in a hypoxic condition in the myocardial tissue. If oxygenated blood flow is insufficient and an imbalance between energy supply and demand of cardiac tissue is present, it is termed “ischemia”.

During periods of ischemia, the cardiomyocytes must resort to anaerobic respiration for the production of ATP. For every mole of glucose consumed during anaerobic respiration, only 2 moles of ATP are generated – an insufficient source of ATP for the normal function of cardiomyocytes (Figure 4). In addition, byproducts of anaerobic respiration, lactate and hydrogen, accumulate in the tissue, further limiting glycolysis and reducing cell function [16]. Extended reliance on anaerobic respiration for ATP production results in “ischemic injury” (i.e., cell damage or death caused by ischemia) as detailed in the next paragraph.

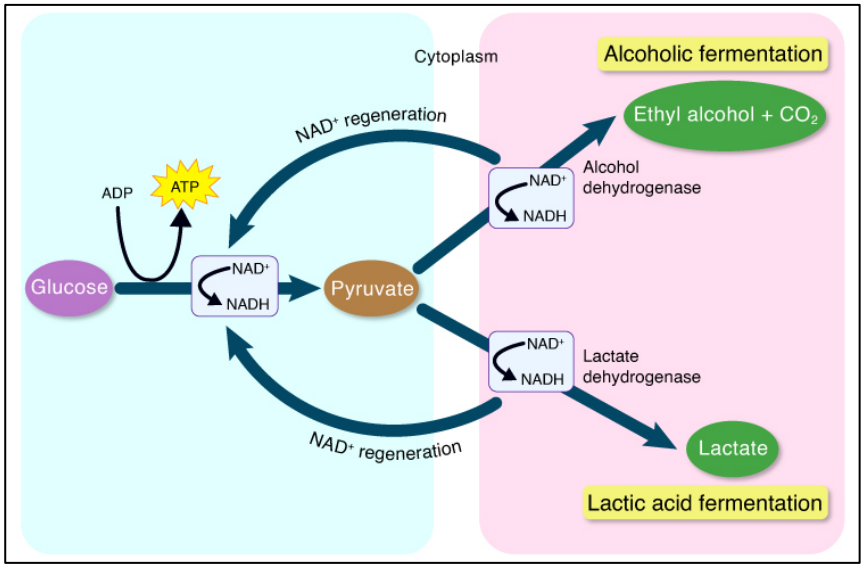

Figure 4. Anaerobic Respiration [16]. Anaerobic respiration is a biological process in which glucose is broken down into ATP, carbon dioxide, alcohol, and lactate in the absence of oxygen. Anaerobic respiration’s energy production is limited in comparison to aerobic respiration.

As lactate and hydrogen accumulate in cardiomyocytes during ischemia, the pH of the cell decreases, and the cell becomes acidic. To compensate for the acidity and maintain normal cell pH, the sodium-hydrogen exchanger excretes accumulated hydrogen ions, leading to an influx of sodium ions [17]. The insufficient ATP supply available during times of ischemia also inactivates ATPases, leads to an intracellular accumulation of calcium due to increased sodium-calcium exchanger activity, and limits calcium reuptake by the endoplasmic reticulum – the cell eventually becomes overloaded with calcium. Next, the mitochondrial permeability transition (MPT) pore is opened and dissipates the mitochondrial membrane potential, further decreasing ATP production. Intracellular proteases that damage cellular contractile proteins are then activated and create contractile band necrosis, resulting in cell dysfunction or death.

Once sufficient blood flow for aerobic respiration is restored to the cardiomyocytes, pH is rapidly normalized by the washing out of accumulated hydrogen ions. Restoration of blood flow and oxygen supply, known as reperfusion, is also detrimental to the myocytes. A study published in 1960 found that reperfusion accelerated necrosis in dog hearts subjected to occlusion and reperfusion of the coronary arteries [18]. While the mechanisms of reperfusion injury are complex and multifactorial, commonly documented causes of reperfusion injury include the generation of oxygen free radicals, calcium overload, dysfunction of endothelial cells, and the inflammatory response that accompanies ischemic injury [19]. The sum of cellular injury caused by interrupted blood flow is therefore a combination of ischemia and reperfusion, with causes summarized in Figure 5.

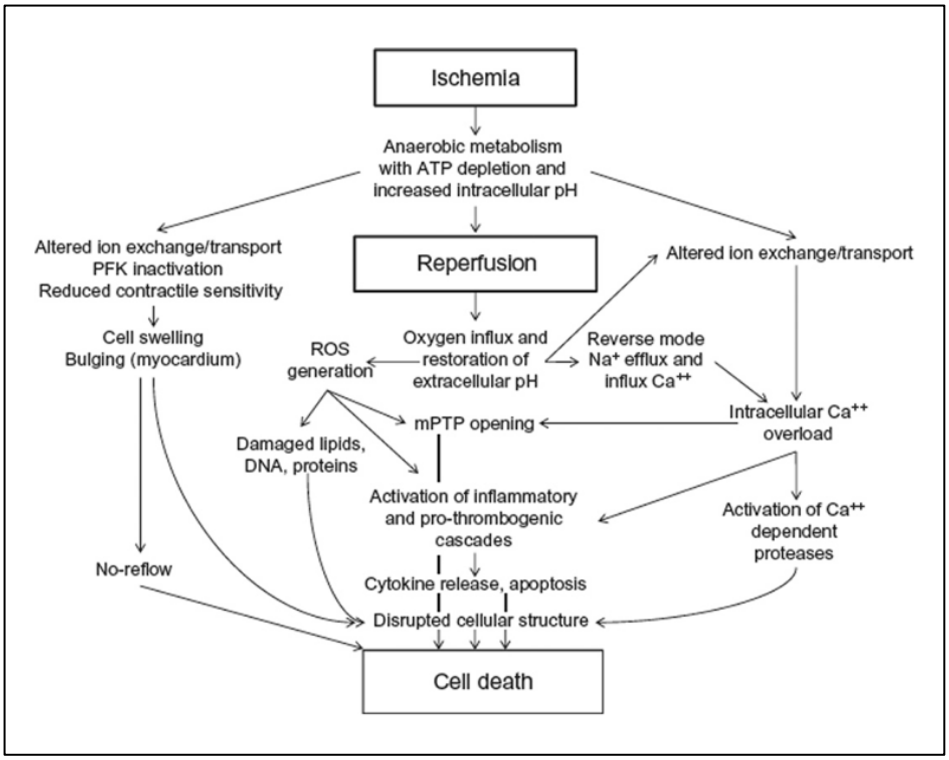

Figure 5. Mechanisms of Ischemia and Reperfusion Injury [17]. During ischemia, hydrogen and lactate accumulate within cardiomyocytes. To maintain a balanced pH, hydrogen ions are excreted for sodium ions, which are excreted for calcium ions and cause a calcium overload. Cellular damage occurs through the activation of inflammatory cascades and proteases. During reperfusion, the excess hydrogen is washed out, but further damage occurs due to the influx of oxygen free radicals and other biological processes.

Ischemia and reperfusion injury generally fall into one of three categories of severity. The first of these categories is found in very brief periods of ischemia and results in no identifiable functional deficit [13]. Essentially, cardiomyocytes are capable of tolerating limited periods of ischemia. The next level of injury is “myocardial stunning” and represents the mildest form of injury a cardiomyocyte can experience. While it has the potential to be severe, myocardial stunning represents systolic or diastolic dysfunction during normal perfusion following ischemia. Tissue that is experiencing myocardial stunning is not necrosed and will completely recover given sufficient time and absence from additional injury. Lastly, the most severe form of ischemia and reperfusion injury is

"tissue necrosis", where myocytes have experienced an irreversible injury. While these myocytes may be functional immediately following injury or reperfusion, they will eventually die despite the restoration of sufficient flow of oxygenated blood.

The severity of cardiomyocyte ischemia and reperfusion injury is dependent on several factors including the ratio of oxygen supply to demand, the presence of collateral blood flow, ambient energy demands, the role of vasoactive substances, the overall health of the tissue, and the duration of ischemia. Figure 6 illustrates the effect of ischemia duration on the severity of ischemia and reperfusion injury. Once a critical duration of ischemia is reached, cell dysfunction and death will occur. Murray and his associates discovered in 1986, however, that short bouts of ischemia and reperfusion to the heart before prolonged reductions in coronary blood flow were protective and made the tissue more resilient to ischemia and reperfusion injury [17]. "Preconditioning" the heart for ischemia became a cornerstone of intracardiac repair during the following decades as it increased the duration of tolerable cardiac ischemia, but it was eventually abandoned as simpler and safer methods were discovered [17].

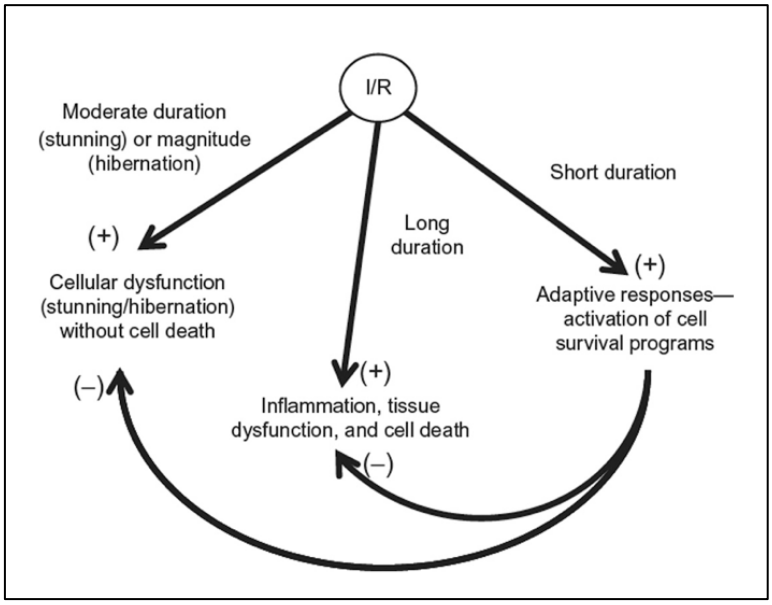

Figure 6. Effect of Ischemia Duration on Injury Severity [17]. While prolonged ischemia induces cell damage and death and reperfusion generally exacerbates the process, short bouts of ischemia (<5 min) followed by reperfusion have been shown to limit the severity of injury when cardiomyocytes later experience prolonged ischemia.

While early pioneers within the field of cardiac surgery likely did not understand the mechanisms by which they were able to safely operate for increasing lengths of time, their work provided the groundwork for the theoretical basis of cardioplegia. By reducing the energy demand of the heart through hypothermia, decompression of the heart using the heart-lung machine, and arrest utilizing hyperkalemic solutions, the window before necrosis occurred was sufficiently extended for major cardiac repairs to occur. Cardioplegia, and the mechanisms by which it offers myocardial protection, have continued to be reevaluated and refined in an attempt to establish a superior formulation for myocardial protection.

2.3 Myocardial Protection

The key to any myocardial protection strategy utilized during intracardiac surgery is reducing the energy demands of the heart to the furthest extent possible while oxygenated blood flow, needed to efficiently produce energy, is interrupted by the aortic cross-clamp. While the ability to enter the heart and make complex repairs necessitates ischemia, reduced myocardial energy demands allow for extended periods of ischemia before ischemic injury occurs. In other words, lower energy demands minimize the theoretical imbalance between energy supply and energy demand. As detailed in the previous sections, early pioneers within the field of cardiac surgery discovered three potential avenues for reducing energy demand – hypothermia, decompression of the heart utilizing the heart-lung machine, and arrest utilizing a hyperkalemic solution.

The first and most historic method of inducing myocardial hypothermia is profound systemic hypothermia. While systemic hypothermia can sufficiently reduce myocardial energy demand, the negative effects on coagulation and the extensive time required for cooling and rewarming of the patient have made its use impractical in modern-day operating rooms. Still, systemic hypothermia offers a benefit over other methods by reducing the potential for unintentional warming of the myocardium caused by contact with surrounding tissues and the return of warm blood to the heart through the venae cavae and noncoronary collaterals [13]. Cold cardioplegia administration is a second method of cooling the myocardium to reduce myocardial energy demand. A staple in cardiac surgery today due to its simplicity, cardioplegia is administered into the coronary circulation at temperatures between 3°C and 10°C and can lower the temperature of the

myocardium to approximately 15°C. The major drawback of cold cardioplegia administration for myocardial cooling is that its administration is generally intermittent based on the cardioplegia dosing strategy. Topical cooling utilizing cold saline or slush is the last common method of myocardial cooling. While topical cooling generally does not provide sufficient cooling to the deep myocardium and has the potential to injure the phrenic nerve with prolonged exposure, it is useful as an adjunct to cold cardioplegia for maintaining hypothermic temperatures between cardioplegia doses or in the presence of noncoronary collateral flow.

Myocardial hypothermia's benefit is most easily realized in the definition of the Q_{10} effect. Commonly associated with biological reactions, the Q_{10} effect can be defined within this context as a 50% reduction in myocardial energy demand for every 10°C that the myocardium decreases in temperature. If utilizing cold cardioplegia administration where the myocardium reaches temperatures of about 15°C, the myocardial energy demand is approximately 50% of 50%, or 25%, of normothermic myocardial energy demand. The Q_{10} effect is therefore a model with diminishing returns – the first 10°C that the myocardium decreases in temperature provides the largest reduction in myocardial energy demand with each additional 10°C temperature drop offering half the benefit of the previous. While hypothermia's major advantage is allowing blood flow to be interrupted for short periods, it should be noted that hypothermia is associated with cardiomyocyte injury, myocardial edema, and a resultant decrease in ventricular function [14].

To further reduce the energy demand of the heart, the heart can be decompressed, and the work of the heart can be offloaded utilizing the heart-lung machine. Ernest Starling, a British physiologist and one of the developers of the Frank-Starling law of the heart, stated, “The oxygen consumption of the isolated heart...is determined by its diastolic volume, and therefore by the initial length of its muscular fibers” [20]. The initial length of the fibers, better defined as the end-diastolic volume, would later become known as preload. Starling, therefore, was making the association that preload is directly related to myocardial oxygen consumption, which is directly related to myocardial energy demand. During cardiac surgery, this principle can be practically applied by adequately draining the heart with the heart-lung machine to minimize preload, specifically through the use of an intracardiac vent.

To understand this relationship, it becomes necessary to consider the definition of work. Within physics, work is defined as the product of force and distance. This equation can be simplified when talking about the heart to define work as the product of blood volume ejected (i.e., stroke volume) and the pressure required to eject it (i.e., systolic pressure), an association illustrated in Figure 7 [21]. By minimizing the preload through decompression of the heart, end-diastolic volume is reduced and the difference between end-diastolic volume and end-systolic volume (i.e., stroke volume) is minimized, reducing the work required to propel it. Work and energy have a direct linear relationship, so it can be concluded that decompression of the heart directly reduces myocardial energy demand. Additionally, after the aortic cross-clamp is applied and the

heart is arrested, decompression prevents the overextension of cardiac muscle fibers and the associated tissue damage caused by distension [22].

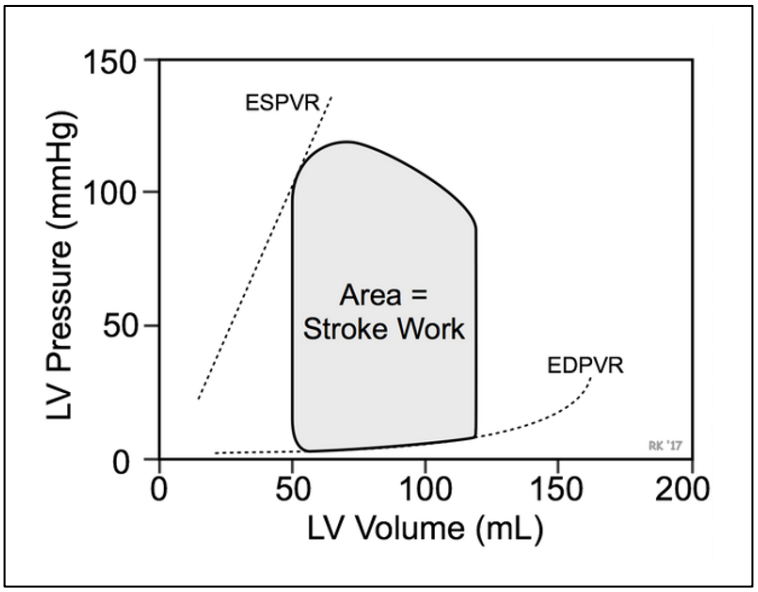

Figure 7. Ventricular Pressure-Volume Diagram [21]. Stroke work can be estimated as the product of stroke volume and systolic pressure. Decompressing the heart by properly draining the heart with the heart-lung machine and an intracardiac vent reduces the difference between end-diastolic volume and end-systolic volume (i.e., stroke volume). Therefore, reducing end-diastolic volume or "preload" directly reduces myocardial energy demand.

The last common mechanism utilized to reduce the imbalance between energy supply and demand is to arrest the heart – if the heart is not beating, it does not consume the energy required to propel blood. Most commonly, the heart is arrested utilizing hyperkalemic cardioplegia solutions, also called extracellular solutions because electrolyte concentrations are similar to extracellular fluid but with high potassium concentrations [13]. Intracellular cardioplegia solutions are discussed in the next section of this paper.

By administering cardioplegia with elevated potassium concentrations of 10-40 mEq/L, the resting cardiomyocyte membrane potentials become more positive, increasing from -85mV to between -65 and -40mV [23]. At these elevated membrane potentials, fast sodium channels are in their inactivated state and are unable to reset and return to their resting state as seen in Figure 8. With fast sodium channels unable to open, myocardial action potentials are blocked, cardiomyocytes become unexcitable, and the heart experiences a depolarized arrest. As the cardioplegia washes out from the extracellular space, the resting membrane potential returns to a more negative state where sodium channels can reset. As a result, redosing of cardioplegia is required in cases with extended ischemic times. It is important to remember that while redosing is needed to maintain arrest, redosing also has the potential to cause reperfusion injury by introducing oxygenated blood after periods of ischemia.

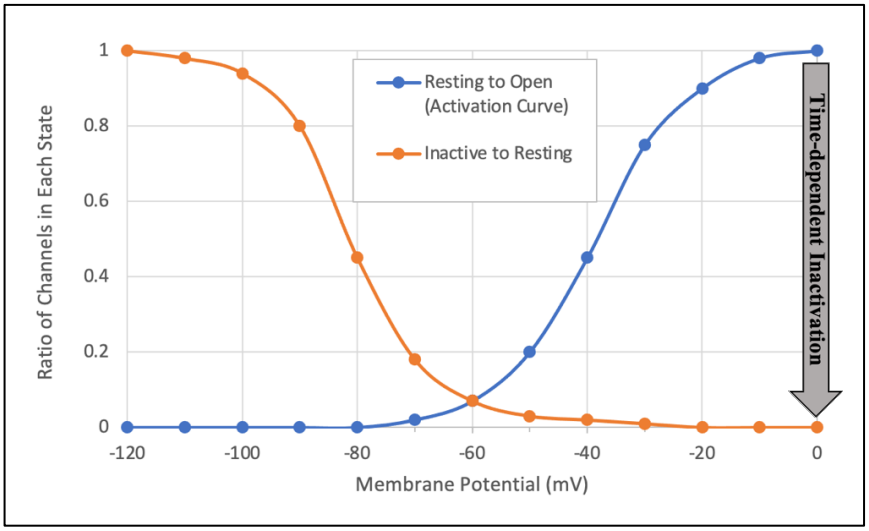

Figure 8. Activation and Inactivation Curves for Fast Sodium Channels [24]. The normal resting membrane potential of cardiomyocytes is -85mV, at which point most fast sodium channels can reset. By administering hyperkalemic cardioplegia solutions, the resting membrane potential rises to between -65 and -40 mV. This corresponds to 88 to 98% of fast sodium channels being stuck in a state of inactivation.

Decompression of the heart by adequately draining the heart through the proper utilization of vents reduces myocardial energy demand by 30 to 60% alone – a significant reduction in energy demand compared to a working heart under normal physiological conditions [25]. Arresting the heart reduces the myocardial demands by an additional 50% – a compounded reduction of about 90%. Despite the heart being arrested, there is residual energy demand to maintain electrolyte gradients. The remaining 10% of myocardial energy demand compared to normal physiological conditions can be further reduced by hypothermia with total myocardial energy demand being reduced by up to 98% as seen in Figure 9 [14]. In between doses of cardioplegia, myocardial energy demand will slowly rise as the myocardium warms up and the cardioplegia is washed out of the coronary circulation.

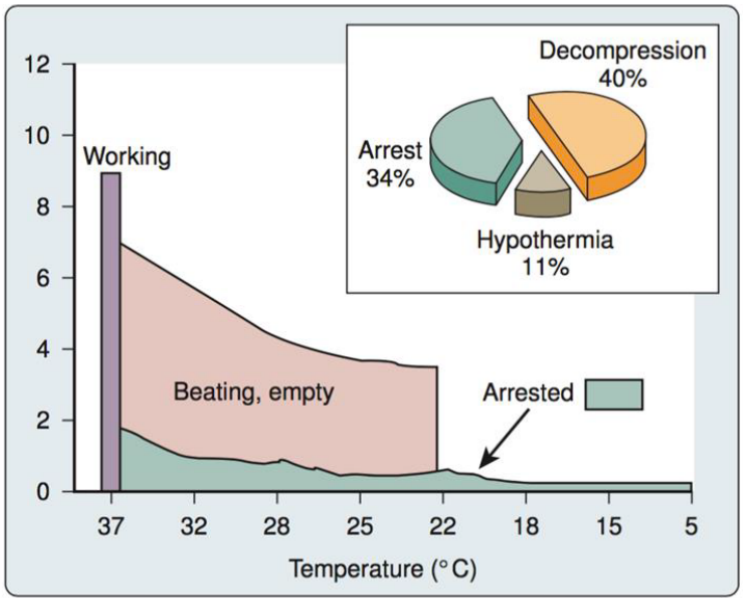

Figure 9. Myocardial Oxygen Consumption versus Temperature [25]. Given myocardial oxygen consumption and energy demand have a direct relationship, decompressing and arresting the heart significantly reduces myocardial energy demand (~90%). Hypothermia can be used to further supplement decompression and arrest to obtain up to a 98% reduction in myocardial energy demand. Note that the y-axis is missing in the above image and should be "O_2 Uptake (mL/min/100g of Tissue)".

2.4 Types of Arrest

While hyperkalemic (i.e., extracellular) arrest solutions predominate modern-day cardioplegia formulations, opposition to their use has persisted in the decades since Melrose's discovery. Early experimental studies showed that hyperkalemic solutions can lead to left ventricular dysfunction, refractory ventricular fibrillation, and cardiac myocyte death [1]. As cardiac surgery centers repeatedly encountered adverse outcomes from hyperkalemic arrest during the 1950s, the method was all but completely abandoned by 1960 in the wake of a report published by James McFarland in the *Journal of Thoracic and Cardiovascular Surgery* [10]. As the result of two patients who underwent potassium citrate arrest and were shown to have definitive patterns of unusual cardiac necrosis upon death, McFarland and his associates reevaluated the hearts of all patients who had undergone open-heart surgery at their institution. They found evidence of myocardial damage in 79% of patients subjected to potassium citrate arrest – a damning piece of evidence against hyperkalemic arrest [10].

The five commonly cited concerns with hyperkalemic arrest are as follows [2]:

1. Elevated resting membrane potentials and electrolyte imbalances lead to calcium overload and ischemia-reperfusion injuries.
2. Hyperkalemia is a potent vasoconstrictor, causing cardioplegia to be maldistributed and limiting myocardial protection. Vascular spasm is also common postoperatively, occurring in up to 8% of patients [1].
3. Vascular endothelium is activated by elevated potassium levels, resulting in edema, inflammation, and increased platelet aggregation.

4. The atria and ventricles have varied sensitivities to high potassium levels, which can cause postoperative arrhythmias and ECG changes.
5. The combination of calcium overload, vasoconstriction, arrhythmias, and endothelial dysfunction results in a high incidence of reduced cardiac output from myocardial stunning.

Despite the moratorium placed on hyperkalemic arrests during the 1960s and 1970s due to myocardial tissue damage and necrosis caused by the above mechanisms, hyperkalemic solutions were eventually reintroduced when alternative myocardial protection techniques used in the interim were insufficient [10]. These new hyperkalemic cardioplegia formulations generally contained moderate hyperkalemia in an attempt to limit the deleterious effects seen in previous decades. This resurgence of hyperkalemic cardioplegia solutions can be attributed to the work of William Gay and Paul Ebert who showed that a potassium chloride concentration of 25mEq/L allowed for hearts to be arrested for 60-minute periods with only a mild reduction in ventricular function [10]. Enthusiasm for Gay and Ebert's work only grew when a 1974 report examined Melrose's experiments and determined that he had used potassium citrate concentrations between 245mEq/L and 980mEq/L – an obvious contributor to why such adverse effects were experienced utilizing the Melrose technique [10]. The same report determined that potassium concentrations of 26mEq/L or below would reliably limit the damage caused by ischemia.

Despite the prominence of hyperkalemic cardioplegia solutions to this day, the concerns of the 1960s and 1970s sparked investigations into “safer” alternatives to hyperkalemic arrest – an objective still common in modern research studies. While hyperkalemic cardioplegia formulations are considered “extracellular” due to the presence of ion concentrations that are similar to extracellular fluid, the alternative looked to arrest the heart with cardioplegia that contained ion concentrations similar to intracellular fluid. Intracellular cardioplegia formulas maintain the membrane potential close to the physiological resting potential of cardiomyocytes and therefore induce a polarized arrest.

The mechanism through which intracellular cardioplegia formulations arrest the heart is simple – erase the concentration gradients of sodium and calcium between the extracellular fluid (delivered as crystalloid cardioplegia) and the intracellular fluid so that sodium and calcium do not cross the cell membrane and depolarize the cell. An added benefit is that the cell will not become overloaded with calcium, reducing a major source of ischemia-reperfusion injury [1]. The first intracellular cardioplegia formulation to gain clinical success was proposed by German pioneer, Hans Bretschneider, and contained a sodium concentration that was similar to intracellular sodium concentrations to prevent an excitation potential [10]. Additionally, procaine was added, which blocked fast sodium channels and provided membrane stability [10]. In 100 consecutive patients undergoing aortic valve replacement in 1975, the use of his cardioplegia was not attributed to any of the six deaths that occurred. The report published declared that Bretschneider’s solution was safe and effective in inducing arrest during cardiac surgery [10].

As intracellular cardioplegia formulations continued to be developed and trialed, new targets for maintaining the cell in a polarized state were identified and drugs that affected these targets were trialed in cardioplegia formulations. Common targets of drugs used in intracellular cardioplegia formulations can be seen in Figure 10. While intracellular cardioplegia formulations have shown promise and are still occasionally used today, clinical trials are limited, and adoption has been slow due to the simplicity and seeming effectiveness of moderate hyperkalemic cardioplegia formulations. Still, the lessons learned from intracellular cardioplegia formulations, specifically the inclusion of additives that reduce sodium and calcium influx, have been integrated into extracellular cardioplegia formulations to improve efficacy and safety.

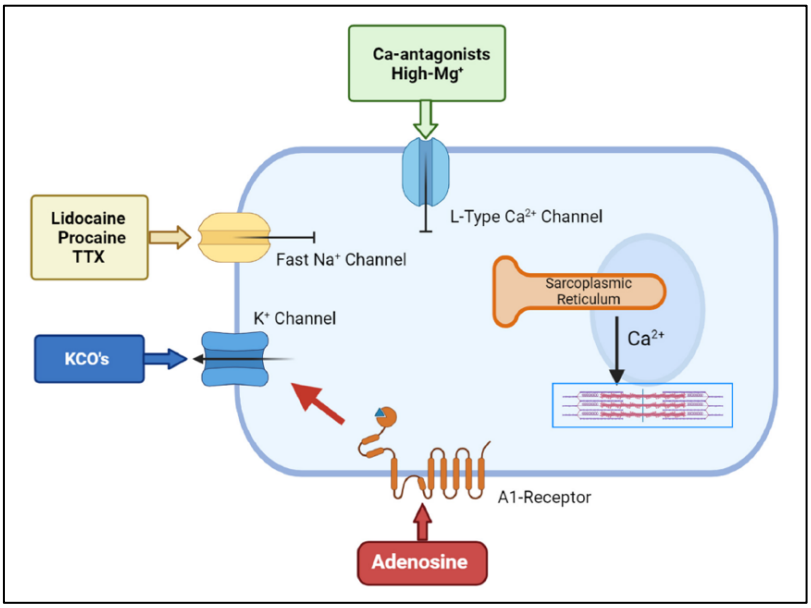

Figure 10. Common Cellular Targets for Intracellular Cardioplegia Formulations [1]. Common targets work to maintain physiological resting membrane potential near -85mV. Del Nido cardioplegia, addressed later in this paper, utilizes a hyperkalemic arrest but is classified as a modified depolarizing cardioplegia formulation due to the inclusion of lidocaine and magnesium. "KCOs" represent potassium channel openers and "TTX" represents tetrodotoxin, a sodium channel blocker.

2.5 Cardioplegia Additives

As discussed previously, mechanisms of ischemia and reperfusion injury are multifactorial and complex, making it difficult to prevent these injuries. While the simple answer for the prevention of ischemia and reperfusion injury during cardiac surgery is to never introduce ischemia, the complexity of an essential cardiac repair may dictate its necessity. Still, there are mechanisms that can minimize injury, such as limiting the imbalance between energy supply and demand through hypothermia, offloading the work of the heart, and cardiac arrest. Additionally, specific causes of ischemia and reperfusion injury can be addressed through the inclusion of additives to the cardioplegia formula. It should be noted that many potential additives possess functions similar to components found in the blood – a benefit of blood-based cardioplegia formulations.

During ischemia, calcium overloads the cardiomyocytes, leading to damage of cellular contractile proteins and contractile band necrosis. If intracellular cardioplegia formulations without supplemental calcium are used, there is also a subsequent influx of calcium upon reperfusion due to intracellular calcium depletion during ischemia [19]. Hypercontracture of the myocytes results from the excess calcium and induces cell death. The addition of calcium channel blockers, mitochondria uniporter antagonists, and sodium-hydrogen exchanger inhibitors to cardioplegia can, therefore, attenuate calcium overload and reduce cell death. In experimental studies, calcium channel blockers (CCBs) were capable of reducing myocardial infarct size by up to 50% [26, 27]. Figure 11 illustrates the ability of CCBs and calcium chelators to reduce infarct size in pigs.

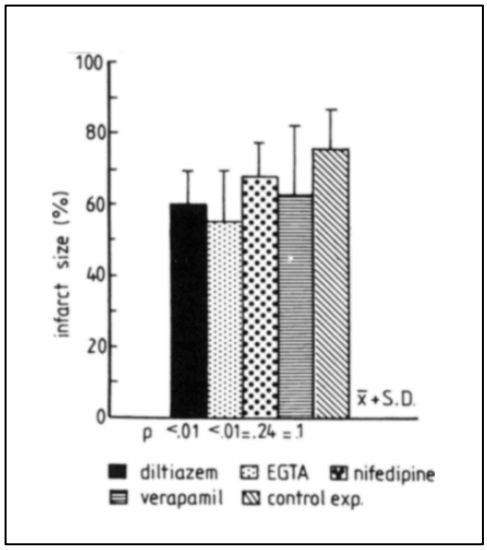

Figure 11. Effect of Calcium Antagonists on Infarct Size [26]. Five treatment groups were established and subjected to 45 minutes of left anterior descending (LAD) coronary artery occlusion. The above pharmacological agents were infused from 1 minute before reperfusion till 45 minutes after reperfusion. The infarct size was then measured after 3 days of reperfusion. EGTA (a calcium chelator) was found to be the most effective in reducing infarct size, with verapamil and diltiazem (cardiac CCBs) offering a significant reduction in infarct size as well. Nifedipine (a vascular CCB) offered the least improvement.

CCBs like verapamil and diltiazem that are specific to the heart can be added directly to cardioplegia formulations to limit calcium overload during ischemia and subsequent reperfusion. If CCBs are not utilized, low concentrations of calcium must be added to intracellular cardioplegia formulations to prevent intracellular calcium depletion. While the above CCBs are effective, the most commonly utilized additive for reducing calcium overload is magnesium. Magnesium works through two mechanisms: (1) it competitively binds to calcium channels and blocks the entry of calcium, reducing calcium overload and increasing coronary vasodilation and (2) it stabilizes cardiomyocyte cell membranes by inhibiting a myosin phosphorylase, preserving ATP for post-ischemic activity. One study found that the addition of 16 mmol/L of magnesium in cardioplegia reduced postoperative troponin-I release by 28% when compared with cardioplegia that only

contained 5 mmol/L of magnesium, as shown in Figure 12 [28]. However, other studies have indicated that there is a negligible benefit to the inclusion of magnesium [29].

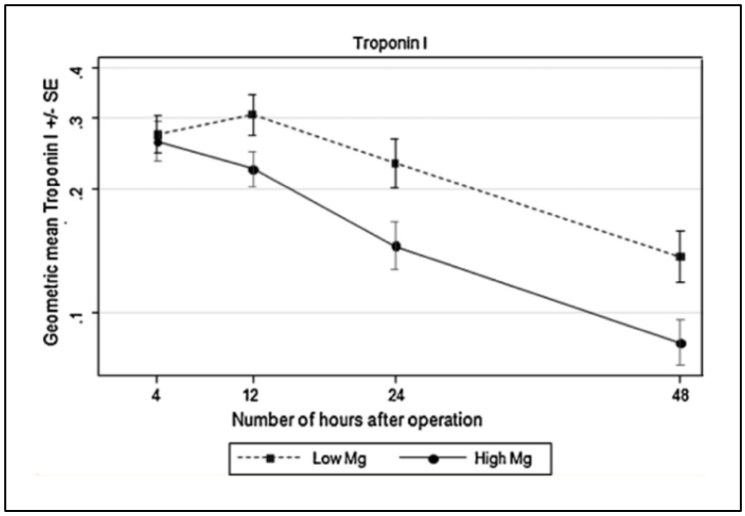

Figure 12. Effect of Magnesium on Postoperative Myocardial Injury [28]. To assess the efficacy of magnesium in cardioplegia, one group was administered cardioplegia with 16mmol/L of magnesium, and the other group was administered cardioplegia with 5mmol/L of magnesium. Within 48 hours of reperfusion, magnesium levels of both groups returned to normal, yet the group with elevated magnesium cardioplegia had a 28% reduction in troponin-I levels, an indication of reduced myocardial injury.

During ischemia, intracellular pH decreases and becomes more acidic due to the accumulation of lactate and hydrogen – byproducts of anaerobic respiration. While maintaining a physiological pH is critical to cardiac function, the rapid restoration of a normal pH during reperfusion contributes to reperfusion injury, creating a paradox. Experimental studies in rats have shown that reperfusion with a buffer is cardioprotective though, reducing the negative effects of returning to a physiological pH [30]. Additionally, the inclusion of a buffer prevents the cardiomyocytes from becoming excessively acidotic between redoses of cardioplegia [13].

Blood contains naturally occurring buffers, including the imidazole groups of proteins and histidines, reducing the need for adding buffers to blood-based cardioplegia formulations. Buffering additives are therefore more beneficial in crystalloid-based cardioplegia formulations. Two historically utilized additives for buffering are tromethamine (THAM) solution and sodium bicarbonate. THAM solution combines with hydrogen ions to form bicarbonate and retains more buffering ability at cold temperatures, while sodium bicarbonate directly creates alkalosis. Although THAM solution has been used extensively in humans, studies have shown a myocardial depressive effect in other animals [31, 32]. Further, THAM solution was removed from the market in 2016 for reasons unrelated to safety or efficacy and is not currently available in the United States [33]. Both THAM and sodium bicarbonate offer similar alkalizing effects, though, as seen in Figure 13 [34].

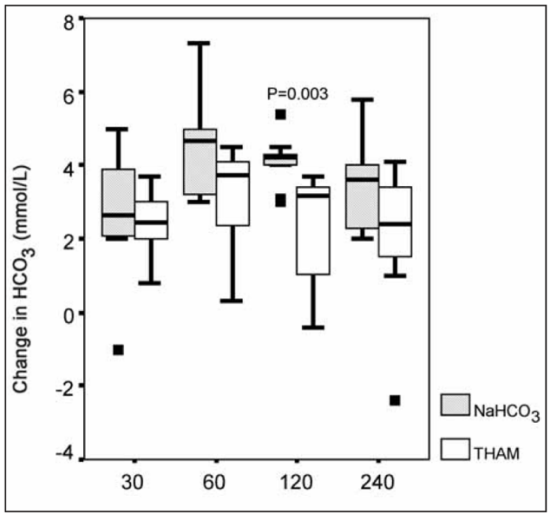

Figure 13. Change in Serum Bicarbonate after THAM or Sodium Bicarbonate [34]. After infusion, both THAM and sodium bicarbonate offered equal buffering, with sodium bicarbonate's alkalizing effects lasting 4 hours and THAM's alkalizing effects lasting 3 hours. Additionally, THAM decreased serum sodium with no effect on serum potassium. Sodium bicarbonate decreased serum potassium and increased serum sodium.

To limit the myocardial edema caused by the introduction of cardioplegia into the coronary circulation, the osmolarity of cardioplegia is an important consideration. Blood is isosmotic so the inclusion of additives into blood-based cardioplegia formulations generally serves to make the cardioplegia hyperosmotic. Blood-based cardioplegia formulations therefore offer inherent protection to myocardial edema even without additives. In contrast, crystalloid fluids are generally hypoosmotic and hypotonic, which drives fluid into the cardiomyocytes and causes cellular swelling. Mannitol and glucose are common additives used to increase the osmotic pressure of cardioplegia and reduce myocardial edema [13]. In a study done on rabbits, the inclusion of mannitol to make a slightly hyperosmotic cardioplegia formulation was found to be statistically significant in limiting right ventricle water content and improving postoperative left ventricle function [35].

Mannitol also acts as an oxygen free radical scavenger, which may warrant its inclusion in both blood- and crystalloid-based cardioplegia formulations. Oxygen free radicals are naturally occurring byproducts of biological reactions that possess an unpaired electron, making the molecules highly reactive to cellular components [36]. While blood offers sufficient protection from oxygen free radicals during normal physiological conditions, the production rate of oxygen free radicals dramatically increases during reperfusion and can become a significant source of reperfusion injury. Oxygen free radical scavengers are most effective if present at the moment of reperfusion, making it ideal to include them in cardioplegia formulations where every redose of cardioplegia is a period of reperfusion [13].

As discussed previously, hyperkalemic cardioplegia formulations cause the resting membrane potential of cardiomyocytes to become more positive, increasing from -85mV to between -65 and -40mV [23]. With the membrane potential being elevated, fast sodium channels are unable to reset or be activated, preventing the depolarization of cardiomyocytes. As an adjunct to hyperkalemic arrest, lidocaine can be used to further attenuate the availability of fast sodium channels, preventing reset at near-physiologic resting membrane potentials. Figure 14 illustrates that at resting membrane potentials that are more positive than physiologic but less positive than during hyperkalemic arrest, the percent of available fast sodium channels is a fraction of the maximum in the presence of lidocaine [37]. Lidocaine is therefore capable of preventing cardiomyocyte depolarization even in the absence of significantly elevated resting membrane potentials attained through hyperkalemic arrest.

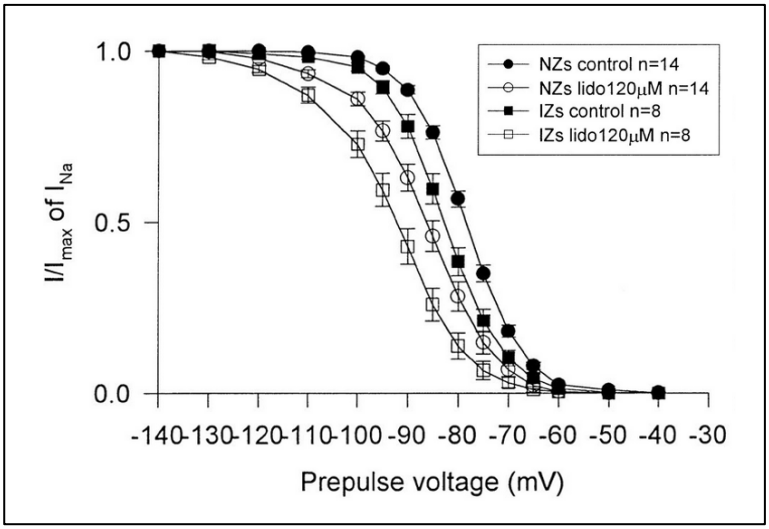

Figure 14. Effect of Lidocaine on Fast Sodium Channel Availability [37]. For noninfarcted hearts (NZs) in the above figure, the fraction of fast sodium channels available at a given voltage can be compared between the lidocaine group and the control group. 50% availability of fast sodium channels occurs at about -78mV in the control group and about -88mV in the lidocaine group, illustrating lidocaine's ability to prevent cardiomyocyte depolarization at near physiologic resting membrane potentials.

A further benefit is the ability to prevent depolarization of injured or necrosed cells in the postischemic heart. If significant damage or death occurs to a region of cardiac tissue, potassium will leech out of the cells, making the resting membrane potential more positive. In the range of resting membrane potentials that would result from this rise in extracellular potassium, lidocaine prevents depolarization of the injured cells without affecting the ability of healthy cells to depolarize. Given that these damaged cells are a significant source of abnormal ventricular rhythms after ischemia, lidocaine acts as a dysrhythmic when administered in cardioplegia or to the systemic circulation after cross-clamp removal [38].

The last common cardioplegia additives worth detailing are glutamate and aspartate. Glutamate and aspartate are intermediates in the Krebs cycle and serve to quickly restore ATP levels after depletion during ischemia. Given the Krebs cycle's reliance on enzymatic reactions, glutamate and aspartate must be administered warm during warm induction of cardioplegia or during a cardioplegic "hot shot" before aortic cross-clamp removal. In rat hearts arrested for five hours, the combination of glutamate and aspartate recovered 53.7% of prearrest cardiac function compared to only 31.7% of function recovered in the control group [39]. While the above cardioplegia additives are some of the most prominent and noteworthy, they are far from an exhaustive list of potential additives; Table 1 provides a more complete summation.

Table 1. Common Cardioplegia Additives. While not exhaustive, Table 1 provides commonly used historic and modern cardioplegia additives.

Additive	Mechanism of Action	Purpose
Adenosine	Opens ATP-sensitive potassium channels (K_{ATP}).	Enhances arrest. Increases ATP stores.
Albumin	Increases oncotic pressure of the intravascular fluid.	Prevents tissue swelling and reduces myocardial edema.
Aspartate and Glutamate	High-energy intermediates of the Krebs Cycle.	Helps replenish myocardial ATP stores.
Bicarbonate (Commonly $NaHCO_3$)	Binds with hydrogen ions to form carbonic acid.	Buffers the acidity created during ischemia.
Citrate Phosphate Dextrose (CPD)	Citrate binds free calcium.	Reduces injury caused by reperfusion calcium overload.
Diltiazem	Blocks cardiac calcium channels.	Prevents calcium overload during ischemia and reperfusion.
Esmolol	Blocks beta-1 adrenergic receptors.	Reduces myocardial oxygen demands [40].
Glucose	Increases osmotic pressure. Basic unit for glycolysis.	Reduces myocardial edema. Helps replenish ATP stores.
Lidocaine	Blocks fast sodium channels.	Helps prevent depolarization and reduces ventricular rhythms.
Magnesium (Commonly $MgSO_4$)	Blocks calcium channels	Prevents calcium overload and increases coronary vasodilation.
Mannitol	Increases osmotic pressure. Scavenges oxygen radicals.	Reduces myocardial edema. Prevents damage by radicals.
Nicorandil	Opens ATP-sensitive potassium channels (K_{ATP}).	Allows for arrest at lower doses of potassium.
Procaine	Blocks fast sodium channels.	Helps prevent depolarization and reduces ventricular rhythms.
Sodium (Commonly NaCl)	Decreases sodium outflow caused by the Na/H exchanger.	Prevents intracellular calcium overload by supporting the Na/Ca exchanger.
Steroids	Suppresses multiple inflammatory genes.	Reduces inflammation and associated edema.
Tromethamine (THAM)	Combines with hydrogen to form bicarbonate (See above).	Buffers the acidity created during ischemia.
Verapamil	Blocks cardiac calcium channels.	Prevents calcium overload during ischemia and reperfusion.

2.6 The Invention of del Nido Cardioplegia

In the early development of cardiac surgery, myocardial protection strategies remained similar in both pediatric and adult cardiac surgery despite fundamental differences in physiology. While the volume, flow, and pressure of cardioplegia delivery were adjusted to accommodate for the variance in size between adults and children, St. Thomas Solution (i.e., Plegisol) was commonly used for both patient populations [41]. The composition of Plegisol can be seen in Table 2. Its contents are similar to those found in extracellular fluid, with negligible differences in electrolyte concentrations as it pertains to cardioplegia additives except for elevated levels of magnesium. To specifically cater to the needs of immature hearts, Pedro del Nido and his associates at the University of Pittsburgh set out to identify an ideal pediatric cardioplegia solution during the 1990s.

Table 2. Extracellular Electrolyte Composition Compared to Plegisol [42]. Plegisol cardioplegia utilizes a hyperkalemic arrest like many common cardioplegias utilized today but offers minimal additional additives. Two notable benefits of Plegisol are elevated magnesium and hyperosmolarity.

Component	Extracellular Fluid Range	Plegisol Concentration
Potassium (K^+)	3.5 – 5.2 mEq/L	16 mEq/L
Magnesium (Mg^{2+})	1.3 – 2.1 mEq/L	32 mEq/L
Bicarbonate (HCO_3^-)	22 – 29 mEq/L	10 mEq/L
Sodium (Na^+)	135 – 145 mEq/L	120 mEq/L
Chloride (Cl^-)	96 – 106 mEq/L	160 mEq/L
Calcium (Ca^{2+})	4.3 – 5.3 mEq/L	2.4 mEq/L
Osmolarity	275 – 295 mOsm/L	324 mOsm/L
pH	7.35 – 7.45	7.8

One of the challenges posed by the development of a pediatric-specific cardioplegia formulation was that the requirements of the pediatric heart were often described in contradictory terms. Studies during this time described the pediatric heart as both more tolerant and less tolerant of ischemia, Plegisol as being both effective and ineffective for pediatric patients, and the pediatric heart as both preferring single-dose cardioplegia and being indifferent to redosing [41]. Pedro del Nido and his colleagues persisted despite the contradictions by focusing on targets of cardioplegia additives previously discussed – regulation of intracellular calcium, preservation and production of ATP, reduction in lactate production, and buffering of intracellular acidosis that persists after ischemia [41].

As the base for their cardioplegia, del Nido and his team at the University of Pittsburgh chose Plasma-Lyte A, which has an electrolyte concentration similar to extracellular fluid. High-dose potassium was utilized to provide cardiac arrest due to its simplicity and rapid induction of arrest, but lidocaine was also added to block fast sodium channels and help maintain arrest as washout of the potassium-containing solution occurred. By this point, it was known that intracellular calcium overload was a significant source of ischemia and reperfusion injury, so magnesium was added as a calcium channel blocker. Mannitol was included because of both its abilities to reduce myocardial edema and act as an oxygen free radical scavenger. Lastly, sodium bicarbonate's ability to buffer the acidic conditions created by ischemia-induced anaerobic respiration made it useful for returning the myocardial tissue to a physiologic pH. The final formulation of what would colloquially become known as "1:4 del Nido cardioplegia" can be seen in Table 2 with PlasmaLyte-A contents included.

Table 3. Contents of 1 Bag of 1:4 del Nido Cardioplegia [41]. Note that the formulation shown is mixed with whole blood from the cardiopulmonary bypass circuit at a ratio of 1 part blood to 4 parts crystalloid.

Component	Contents of 1 del Nido Bag
Potassium (K^+)	31 mEq
Magnesium (Mg^{2+})	19.24 mEq
Bicarbonate (HCO_3^-)	13 mEq
Lidocaine	130 mg
Mannitol	3.26 g
Sodium (Na^+)	140 mEq
Chloride (Cl^-)	98 mEq
Acetate	27 mEq
Gluconate	23 mEq

The complete cardioplegia solution was delivered to a patient's coronary arteries in a 1:4 (blood:crystalloid) ratio with oxygenated blood from the cardiopulmonary bypass circuit to provide support for aerobic respiration, increase buffering abilities, and improve coronary perfusion [41]. Additionally, the formulation was delivered at temperatures below 10°C to obtain the benefit of reduced energy demand of hypothermic myocardial tissue. In the pediatric applications of del Nido and his team, 1:4 del Nido cardioplegia was given as a single 20 mL/kg dose, with a maximum dose of one liter for patients larger than 50 kilograms. Subsequent doses were not given unless electrical activity was observed or aortic cross-clamp time was expected to be greater than 3 hours.

While 1:4 del Nido cardioplegia was originally developed and patented by the University of Pittsburgh, Pedro del Nido relocated to Boston Children's Hospital where 1:4 del Nido cardioplegia has been utilized for the last 30 years. In their opinion, "excellent myocardial outcomes" have been observed when utilizing del Nido cardioplegia in

pediatric applications. This assertation has been affirmed through numerous studies, including observations of lower intracellular calcium levels and decreased spontaneous contractions [43], lower troponin T release in pediatric patients compared with an alternative adult cardioplegia formula [44], and no statistically significant difference in postoperative complications with a cross-clamp time greater than 90 minutes when compared to a previous myocardial protection strategy [45].

2.8 del Nido Cardioplegia in Adults

During the early 2000s, the use of 1:4 del Nido cardioplegia in adults was rare, limited to adult congenital surgeries at pediatric centers [41]. The simplicity of delivery and increased surgical efficiency due to less frequent redosing made del Nido cardioplegia appealing to adult cardiac surgery centers though, and by the early 2010s, its use in noncongenital adult patients was becoming more common. Much of the expansion of del Nido cardioplegia in adult cardiac surgery was based on anecdotal evidence spread between cardiac surgery centers, with limited support from scientific studies on adult humans [46]. The process of obtaining Institutional Review Board (IRB) approval for prospective studies can be an excessively tedious process, leading to the use and abuse of the ethical gray area known as “surgical innovation” to institute change like the adoption of 1:4 del Nido cardioplegia [47]. Surgical innovation is the concept that a surgical team can make modifications to their practice based on the belief that the application of a given idea will improve outcomes [47]. While surgical innovation has led to the continued advancement of cardiac surgery despite bureaucratic red tape, its application has limited studies about the use of del Nido cardioplegia in adults.

The earliest narrative detailing the use of del Nido cardioplegia in adult noncongenital cardiac surgery was from the Cleveland Clinic – a top center for cardiac surgery in the United States [48]. Cleveland Clinic first became interested in the use of del Nido cardioplegia around 2010 and began utilizing it in 2012 on minimally invasive and robotic mitral valve surgeries, following a similar protocol to that described by Matte and del Nido for use in pediatric patients [46]. As their experience grew, they began utilizing it in more complex cases like double-valve, aortic valve, and atrial septal defect (ASD) repairs. One of their early observations was that the use of 1:4 del Nido cardioplegia led to significant hemodilution of the patient – a result commonly cited by opponents of 1:4 del Nido cardioplegia and discussed in a later section. For an initial arresting dose of one liter of 1:4 del Nido cardioplegia, 800 milliliters of crystalloid would be added to the circulating volume of the cardiopulmonary bypass circuit. While not significant in large patients with high hematocrits, 800 milliliters of crystalloid volume could have a significant impact on small patients or those with marginal hematocrits.

Despite concerns associated with the hemodilution of 1:4 del Nido cardioplegia, the Cleveland Clinic achieved early success in its application. Before 2012, Cleveland Clinic had been using Buckberg solution as their preferred cardioplegia solution. Buckberg cardioplegia is a hyperkalemic, blood-based solution given at a ratio of 4:1 (blood:crystalloid) and is redosed every 15 to 20 minutes. Cleveland Clinic found that in adult valve surgeries, postoperative troponin-T levels and left ventricular ejection fraction were similar between the two cardioplegia methods, indicating similar ventricular recovery after ischemia. Traditional 1:4 del Nido also provided a statistically significant

reduction in cross-clamp, bypass, and operating room times, as well as a reduction in intraoperative glucose and postoperative insulin drips due to the presence of glucose in Buckberg solution [49]. The authors of the Cleveland Clinic study concluded that 1:4 del Nido was a safe and effective alternative to Buckberg solution in isolated valve surgery in adults.

A 2014 study done at Columbia University looked to draw similar conclusions to the Cleveland Clinic study above by comparing their 4:1 (blood:crystalloid) cardioplegia formulation with 1:4 del Nido cardioplegia in redo aortic valve surgery [50]. The study subjects were retrospectively reviewed, and no statistically significant differences in cross-clamp time or cardiopulmonary bypass time were observed. Primary outcomes included common postoperative complications, specifically infection, acute renal injury (ARI), atrial fibrillation (AFib), arrhythmias, permanent pacemaker (PPM) implantation, stroke, and transient ischemic attack (TIA). No statistically significant differences were found between 1:4 del Nido and their 4:1 blood cardioplegia, as seen in Figure 15. Again, del Nido cardioplegia was declared effective when compared with primarily blood-based cardioplegia.

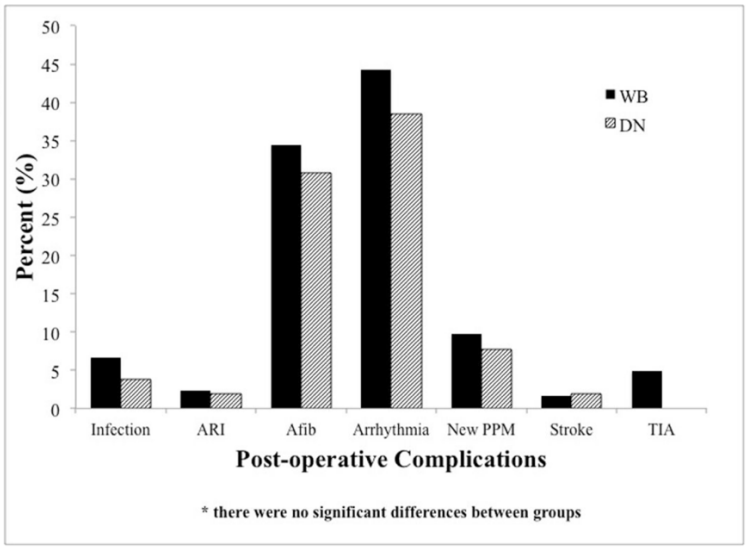

Figure 15. Postoperative Complications by Cardioplegia Formulation [50]. In all primary outcomes, no statistically significant differences were found between the University of Columbia's 4:1 cardioplegia formulation and 1:4 del Nido cardioplegia. “WB” in the key represents their 4:1 cardioplegia formulation and “DN” represents 1:4 del Nido cardioplegia.

Microplegia, as mentioned previously, is a recent innovation within cardiac surgery that removes the crystalloid component from a cardioplegia formulation and injects the arrest agent and additives directly into whole blood from the cardiopulmonary bypass circuit. Microplegia cardioplegia delivery generally has a higher associated cost than cardioplegia delivered using a ratio system (e.g., 1:4, 4:1, etc.) due to more costly disposables and more complex equipment like the MPS Quest, seen in Figure 16. Proponents of microplegia are encouraged by theoretical advantages, including superior oxygen-carrying capacity and natural buffering abilities, as well as superior osmotic properties and decreased hemodilution – both perceived as significant contributors to reducing myocardial edema [51]. Opponents of microplegia argue that hemodilution is a benefit of crystalloid-based cardioplegia as it reduces viscosity and increases the coronary distribution of the cardioplegia [52]. It should be noted that the Quest MPS

device allows for the arrest agent and additives to be adjusted separately, allowing potassium levels to be reduced for redoses. This may be beneficial in reducing systemic hyperkalemia and postoperative arrhythmia, but it has not been studied.

Figure 16. Quest MPS 3 Cardioplegia Delivery System [53]. The Quest MPS 3 is capable of delivering the arrest agent and additives directly into whole blood from the cardiopulmonary bypass machine. It is also capable of delivering crystalloid-based cardioplegias at any desired ratio. Compared to other cardioplegia delivery methods, the Quest is the most versatile, but the machine and disposables come at an added cost that must be justified in patient outcomes.

A 2021 study in Turkey looked to compare the efficacy of 1:4 del Nido with a true microplegia formulation delivered via the MPS Quest [54]. Patients who underwent coronary artery bypass graft (CABG) surgery between January 2017 and January 2020 were randomized into two groups – one group that received a magnesium-based microplegia and one group that received 1:4 del Nido cardioplegia. Overall patient demographics and preoperative characteristics were not statistically different between the

two groups. While no significant differences were observed in length of ICU stay, extubation time, length of hospital stay, or cardiopulmonary bypass time, average cross-clamp time was significantly shorter in the 1:4 del Nido cardioplegia group due to a longer acceptable ischemic period between cardioplegia doses [54]. Myocardial protection via 1:4 del Nido cardioplegia was also shown to better preserve both the left and right ventricular ejection fraction as measured in the second month postoperatively.

A similar prospective study done in the United States yielded similar results to the study done in Turkey [55]. In the study of adult patients undergoing CABG and/or valve surgery, primary outcomes were all related to myocardial protection. Again, 1:4 del Nido cardioplegia offered superior support, resulting in a higher return to spontaneous rhythm after reperfusion, decreased need for postoperative inotropic support, and lower postoperative troponin release [55]. The troponin-I levels of the study can be seen in Figure 17. Systematic meta-analyses of 1:4 del Nido cardioplegia studies frequently find a handful of benefits to 1:4 del Nido over alternative cardioplegia formulations, with benefits of the alternative cardioplegia over 1:4 del Nido being rare [56, 57, 58]. While the theoretical advantages of microplegia are common arguments used to push hospitals toward the adoption of microplegia formulations, studies comparing 1:4 del Nido cardioplegia to alternative cardioplegia formulations have rarely provided the evidence needed to back their claims.

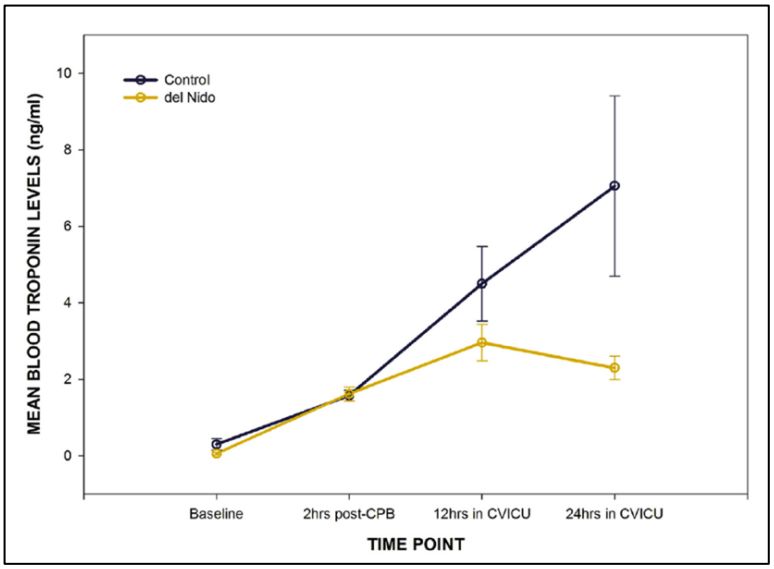

Figure 17. Troponin-I Levels between Microplegia and 1:4 del Nido Cardioplegia [55]. While postoperative troponin-I levels were similar between the two groups shortly after surgery, troponin-I levels continued to increase in the microplegia group while the troponin-I levels in the 1:4 del Nido group plateaued – an indication of increased myocardial injury in the microplegia group.

2.8 del Nido Cardioplegia on a Cellular Level

To assess the safety and efficacy of del Nido cardioplegia, three animal studies have been conducted to date, all done at Dalhousie University in Nova Scotia, Canada [43, 44, 60]. While animal studies regularly face criticism from activists for the harm they cause animal test subjects, animals offer an unequivocal representation of humans in instances where it would be unethical to experiment on human subjects. There is simply no artificial substitute for the complex functions of living beings with circulatory and respiratory systems similar to those found in humans [59]. In the studies done at Dalhousie University, researchers isolated rat cardiomyocytes and looked to evaluate del Nido cardioplegia on a cellular level.

The first of these studies exposed isolated cardiomyocytes of young rats (~3 months old) to two different cardioplegia formulations, modified Buckberg and 1:4 del Nido, and measured intracellular calcium, contractions, and membrane potentials [44]. The cardioplegia formulas were delivered for 30 minutes in a hypoxic environment to simulate the ischemia experienced during aortic cross-clamping. During the hypoxic arrest period, the cardiomyocytes were briefly stimulated to assess the robustness of arrest. Electrical stimulation yielded cardiomyocyte contraction in the modified Buckberg solution group, but not in the del Nido cardioplegia group – an indication of the myocardial protection offered by del Nido cardioplegia [44]. The results of their electrical stimulation experiment can be seen in Figure 18. Cardiomyocytes exposed to modified Buckberg solution also exhibited excessive contractility during reperfusion. In contrast, cardiomyocytes exposed to del Nido recovered more slowly but did not exhibit a contractility overshoot.

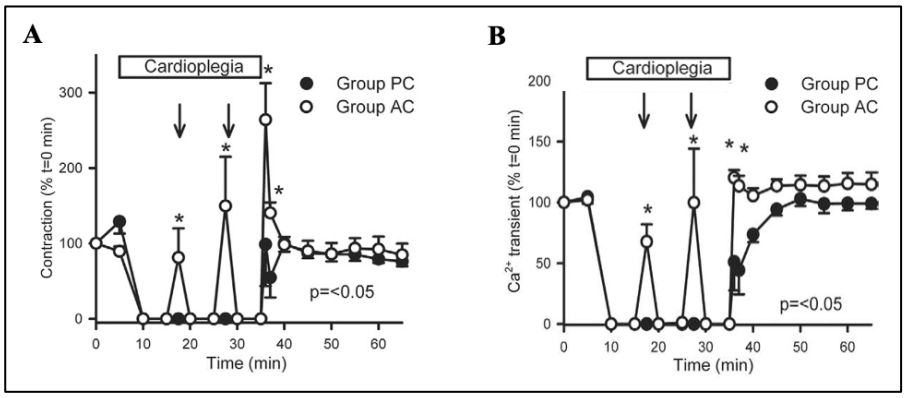

Figure 18. Contractions and Calcium Changes between Cardioplegia Formulations [44]. (A) The modified Buckberg group (group AC) but not the 1:4 del Nido group (group PC) exhibited contractility when stimulated. It can also be seen that the modified Buckberg group exhibited excess contractility upon reperfusion before returning to a physiologically normal condition. (B) The modified Buckberg group also exhibited a rapid calcium influx upon reperfusion that correlated to excess contractility. Given calcium's role in ischemia and reperfusion injury, this influx is likely a contributing factor. Arrows indicate field stimulation during cardioplegia.

In addition to measuring the contractility of the cardiomyocytes, the researchers at Dalhousie University measured the transient calcium fluctuations. It can be seen in Figure 18 that stimulation led to a calcium influx into the myocytes protected by modified Buckberg solution. Upon reperfusion, there was also a rapid influx of calcium that correlated to the excessive contractility observed [44]. Given calcium's role in ischemia and reperfusion injury, 1:4 del Nido cardioplegia's slower recovery and slower calcium influx is a contributing factor to why del Nido cardioplegia results in reduced myocardial injury following reperfusion. Lastly, this study looked to evaluate differences in membrane potentials between the two cardioplegia formulas. During cardioplegia administration, the modified Buckberg solution group had a resting membrane potential of about -55mV, and the 1:4 del Nido cardioplegia group had a resting membrane potential of about -45mV – a predictable outcome since del Nido cardioplegia contains more potassium than their modified Buckberg solution [44].

The second study conducted at Dalhousie University possessed many similarities to the first study discussed, with many experiments being repeated and similar results being attained, with the notable exception that the rats in this study were old (~24 months) [60]. One metric for evaluating the efficacy of 1:4 del Nido cardioplegia that was not presented in the previous paper was the ability of cardiomyocytes to spontaneously contract. In this study, 11 rat cardiomyocytes were included in each cardioplegia group. Of the cardiomyocytes in the modified Buckberg solution group, 9 exhibited spontaneous contractile activity during the hypoxic arrest period. In contrast, only 3 of the 11 cardiomyocytes in the 1:4 del Nido cardioplegia group exhibited spontaneous contractile

activity – further evidence of the robust arrest provided by del Nido cardioplegia [60]. As the study details, a reduction in spontaneous activity reduces energy requirements, leading to a reduction in intracellular acidosis, subsequent calcium influx, and the injuries associated with ischemia and reperfusion. Additionally, while young rat cardiomyocytes in the previous study experienced large transient calcium fluctuations and hypercontracture upon reperfusion, old rat cardiomyocytes had a dampened response, providing evidence that aged hearts are more tolerant of elevated calcium levels.

The third and final study conducted at Dalhousie University looked to evaluate the effect of lidocaine on sodium channel kinetics in cardiomyocytes of old rats (~24 months old), as well as to assess recovery of cardiac function in rat hearts after myocardial ischemia protected by the two aforementioned cardioplegia formulations [43]. To determine the efficacy of lidocaine found in 1:4 del Nido cardioplegia, isolated cardiomyocytes were added to a solution that contained lidocaine at concentrations found in 1:4 del Nido cardioplegia. Sodium channel activation and inactivation curves were then experimentally determined through measurements of sodium currents and can be seen in Figure 19. The presence of lidocaine shifted the inactivation curve to the left by 12.3mV, indicating lower sodium channel availability in the presence of lidocaine at a given membrane potential [43]. Lidocaine also shifted the activation curve to the right by 9mV, indicating a reduction in open sodium channels at a given membrane potential. This illustrates lidocaine as a metaphorical double-edged sword in the prevention of myocyte depolarization – its presence both requires more negative membrane potentials to reset sodium channels and less negative membrane potentials to open sodium channels.

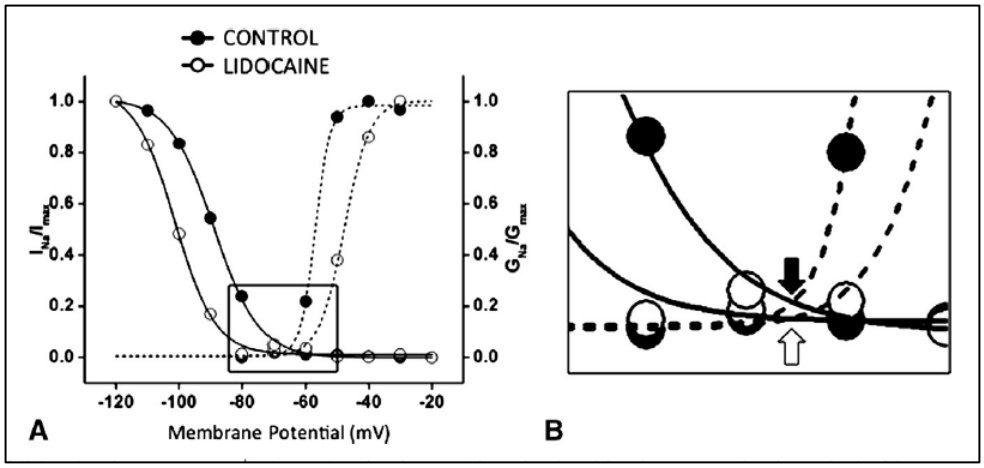

Figure 19. Impact of Lidocaine on Sodium Channel Activation and Inactivation [43]. (A) Lidocaine shifts the activation curve (dashed line) to the right and the inactivation curve (solid line) to the left, illustrating lidocaine's ability to maintain arrest even as potassium is washed out between cardioplegia doses. (B) Magnified view of the highlighted panel on the left, illustrating how lidocaine minimizes the sodium window current.

The third study at Dalhousie University continued by evaluating complete rat hearts that were arrested and protected by modified Buckberg solution and 1:4 del Nido cardioplegia using protocols similar to those utilized in adult patients [43]. Echoing conclusions of their second study, 1:4 del Nido cardioplegia provided a greater reduction in spontaneous contraction during arrest and a slower return to spontaneous rhythm upon reperfusion – contributors to reducing overall myocardial energy requirements and limiting ischemia and reperfusion injury [43]. After a 20-minute reperfusion period, the rat hearts were switched into "working mode" for 60 minutes, and hemodynamic pressures were monitored. In their study, the 1:4 del Nido cardioplegia group had higher systolic pressures, left ventricular pressures, and more appropriate heart rates, leading to the conclusion that 1:4 del Nido offered superior cardiac recovery after arrest over modified Buckberg solution. Aortic pressure and cardiac output after reperfusion can be seen in Figure 20.

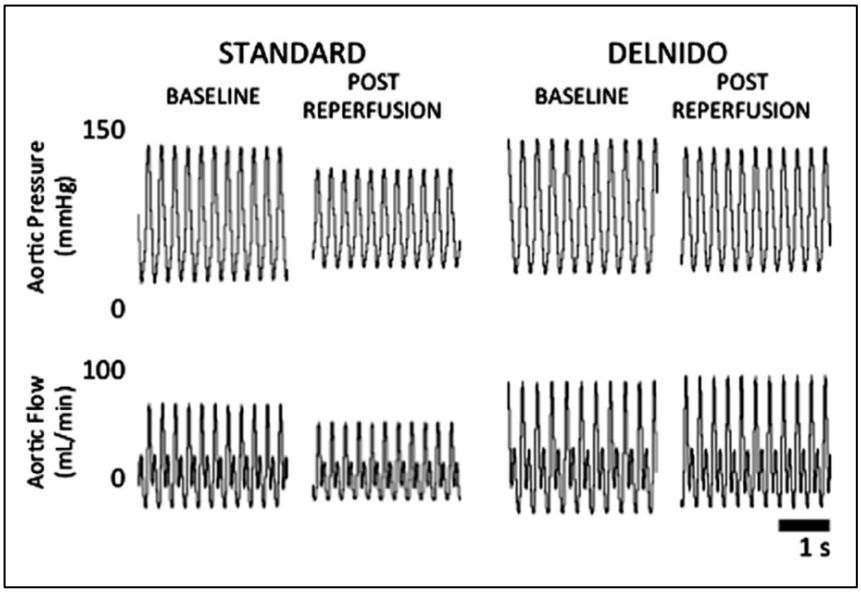

Figure 10. Cardiac Function Before and After Arrest [43]. Compared to their standard modified Buckberg cardioplegia solution, 1:4 del Nido solution offered a superior return to baseline conditions, demonstrating a minimally impacted aortic pressure and cardiac output after reperfusion.

One noteworthy detail of their study that could be easily overlooked is the inclusion of coronary vascular resistance measurements during retrograde reperfusion after arrest. In hearts arrested with del Nido cardioplegia, coronary vascular resistance was almost identical from baseline to reperfusion, while vascular resistance increased significantly from baseline in the modified Buckberg solution group [43]. The next section discusses this finding in depth, but proponents of 1:4 del Nido cardioplegia argue that the hemodilution of 1:4 del Nido increases coronary distribution, exhibiting a cardioprotective effect. The authors of this study acknowledge that argument's viability, stating "increased microvasculature obstruction related to hypothermia-induced sludging with the higher hematocrit level" could be the cause of the increased vascular resistance [42]. However, they offer an alternative explanation that lidocaine's inclusion in 1:4 del Nido cardioplegia is the cause of decreased coronary vascular resistance– a property of

the additive formulation over its hemodilutional effects. It is worth noting that despite the hemodilution of 1:4 del Nido cardioplegia, the study found similar levels of myocardial edema between the two groups.

2.9 Hemodilution and 1:4 del Nido Cardioplegia

As the Cleveland Clinic detailed in early observations of its use, 1:4 del Nido cardioplegia results in significant hemodilution – a property of the cardioplegia being mixed at a ratio of one part blood to four parts crystalloid. A common argument among proponents of 1:4 del Nido cardioplegia is that hemodilution offers a cardioprotective effect through improved distribution of cardioplegia in the coronary arteries [61]. While coronary distribution is a complex and poorly understood function, the cardioplegia formulation's viscosity and associated flow rate is undoubtedly a contributing factor. Proponents of microplegia formulations argue in response that the hemodilution associated with 1:4 del Nido cardioplegia is not worth the drawbacks, namely increased myocardial edema, increased need for blood transfusions due to reduced hematocrits, and increased incidence of postoperative acute kidney injury (AKI) associated with the use of ultrafiltration dictated by hemodilution [62].

Prior to the initiation of cardiopulmonary bypass during cardiac surgery, a patient will have whole blood at physiologic temperatures flowing in their coronary circulation with a hematocrit equal to that of their systemic circulation. The patient's systemic circulation will then be integrated with the cardiopulmonary bypass circuit upon initiation of bypass, and the patient's blood will be diluted by the priming volume of the cardiopulmonary

bypass circuit. Assuming a standard 1500mL prime volume and a 38% hematocrit, a patient's dilutional hematocrit can be calculated as approximately 30% [63]. Microplegia cardioplegia formulations have minimized arrest and additive volumes, so the hematocrit of microplegia will be close to the calculated dilutional hematocrit. In contrast, the hematocrit of 1:4 del Nido cardioplegia delivered to the coronary circulation will be 20% of the dilutional hematocrit, or 6% for the example above.

In a study conducted at the University of Nebraska, expired blood and fresh frozen plasma (FFP) were mixed to obtain a starting hematocrit of 38% – a common physiologic hematocrit [63]. At 37°C, the viscosity of this reconstituted blood solution was measured as 2.44 centistokes and represents the viscosity of blood in a patient's circulation before cardiopulmonary bypass initiation [63]. The University of Nebraska study continued by measuring the viscosity of 38% hematocrit blood, 20% dilutional hematocrit blood, and pure crystalloid at temperatures of 37°C, 24°C, and 6°C. While the study left much to be desired in terms of hematocrits considered and data points measured, specifically because 20% is an extreme dilutional hematocrit, useful inferences can be drawn through regression calculations. At a common cardioplegia delivery temperature of 6°C, the viscosity of microplegia (i.e., blood with a 30% hematocrit) can be estimated as 4.41 centistokes – an 81% increase in viscosity from that of physiological coronary blood. In contrast, the viscosity of 1:4 del Nido cardioplegia (i.e., blood with a 6% hematocrit) can be estimated as 1.73 – a 29% decrease in viscosity from that of physiological coronary blood. Figure 21 illustrates the trendline used to estimate viscosities of dilutional hematocrits not measured in the University of Nebraska study. Note that the presence of

only three data points limits the ability to assess model accuracy, so the calculated data points should only be used as rough estimates.

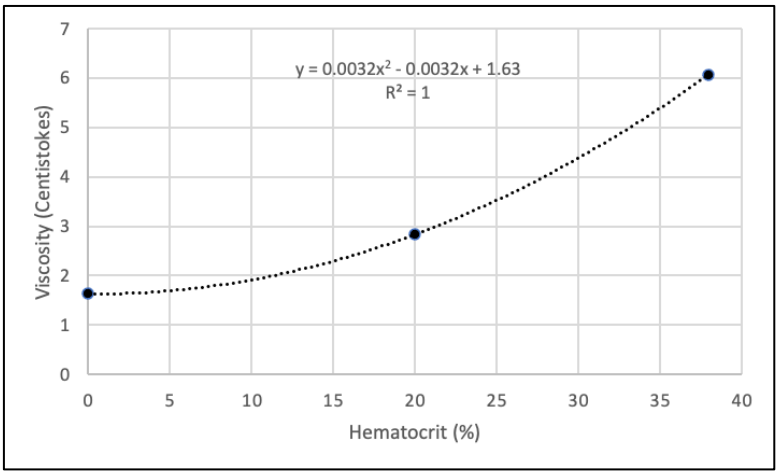

Figure 21. Effect of Hematocrit on Viscosity at 6°C [63]. Using data from the University of Nebraska study, the viscosity of various hematocrits at 6°C could be estimated, allowing the viscosity of microplegia and 1:4 del Nido cardioplegia to be compared to physiological coronary blood flow.

To understand the impact of viscosity on coronary distribution, Poiseuille's Law can be applied as an estimate of coronary flow [14]. For this example, Poiseuille's Law is as follows:

$$Q = \frac{\pi * \Delta P * r^4}{8 * \eta * l},$$

where $Q =$ flow rate,
$\Delta P =$ pressure drop,
$r =$ vessel radius,
$\eta =$ fluid viscosity,
and $l =$ vessel length.

If the assumption is made that only viscosity is changing as hematocrit and temperature change, it can be calculated that an 81% increase in viscosity correlates to an

approximate 44.7% decrease in coronary flow, i.e., microplegia at a common cardioplegia delivery temperature of 6°C will flow at a rate 44.7% lower than physiological coronary blood flow if coronary pressures are the same. In contrast, 1:4 del Nido cardioplegia can be estimated to flow at a rate 41.0% higher than physiological coronary blood flow – a significant benefit when trying to ensure adequate distribution of cardioplegia especially in narrow, stenosed vessels.

Using the equation in Figure 21, the hematocrit at which viscosity at 6°C is equal to that of undiluted blood at physiological temperatures can be calculated to be 16.4%. Assuming undiluted blood has a hematocrit of 38% and the dilutional hematocrit is about 30%, this indicates that for cardioplegia to be delivered at physiologic flow rates to the coronary circulation, cardioplegia should be administered at a 1:1 ratio of blood to crystalloid. Without further studies that look to determine whether physiological flow rates are necessary for sufficient myocardial protection, it becomes challenging for opponents of 1:4 del Nido cardioplegia to entirely dismiss the potential benefits of improved coronary flows in protecting the myocardium during ischemic periods. The effect of cardioplegia formulation on other variables represented in Poiseuille's equation, like vessel radius, should also be investigated before conclusions can be drawn.

While crystalloid-based cardioplegia formulas appear to offer a theoretical benefit of improved coronary distribution, the associated hemodilution has been a common point of opposition by proponents of microplegia due to a perceived increase in myocardial edema, blood transfusions, and postoperative AKI incidence. As discussed previously,

the study at Dalhousie University found no statistically significant difference between the water content of ventricular myocardium in rat hearts protected by their 4:1 modified Buckberg cardioplegia and 1:4 del Nido cardioplegia [43]. In their opinion, this is attributable to both cardioplegia formulations having similar osmotic pressures that are hypertonic relative to the myocardium [43].

At the State University of New York (SUNY), an alternative study hypothesized that microplegia may benefit an injured heart over cardioplegia formulations with a crystalloid component through a reduction in myocardial edema [64]. To test this hypothesis, 20 Yorkshire pigs were randomized into two groups to receive either 4:1 cardioplegia or microplegia for myocardial protection. The pigs were then placed on cardiopulmonary bypass and their aortas were cross-clamped for 30 minutes of unprotected normothermic ischemia. After 30 minutes, cardioplegia of the specified formulation was delivered following specific protocols for 90 minutes before the aortic cross-clamp was removed. To measure the myocardial edema, histologic morphometrics obtained through echocardiography were used to assess left ventricular mass.

In the SUNY study, preoperative left ventricular mass for both groups was ~97g. Postoperatively, left ventricular mass increased to 130g in the microplegia group and 168g in the 4:1 cardioplegia group, a statistically significant difference [64]. Further, sections of the myocardium were analyzed, and the percentage of edema was statistically higher in the 4:1 cardioplegia group than in the microplegia group. Representative cross-sections of the myocardium can be seen in Figure 22.

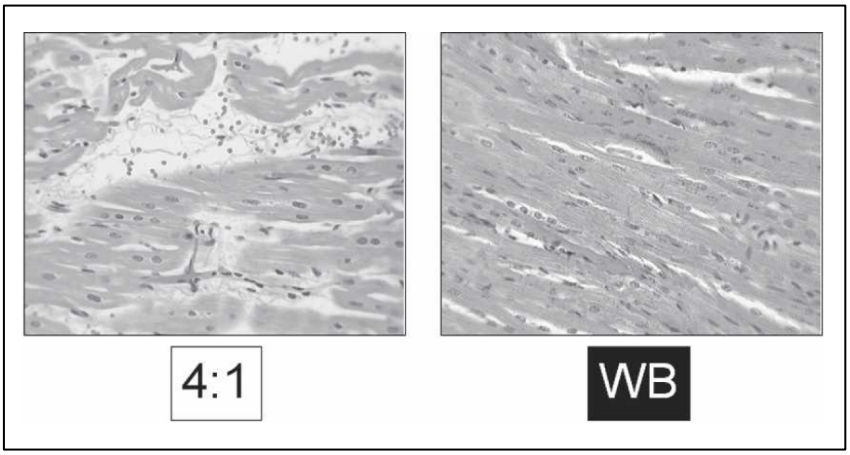

Figure 22. Histology of Pig Myocardium after Ischemia and Reperfusion [64]. On the left, the 4:1 cardioplegia group is shown to have a significant occurrence of myocardial edema when compared with the microplegia group (WB) on the right.

While the SUNY study seems like damning evidence against the use of 1:4 del Nido cardioplegia given its significantly elevated crystalloid content over that of 4:1 cardioplegia, several questions arise about the study that cast doubt over the results:

1. What was the formulation of the 4:1 cardioplegia, and specifically, what was its osmotic pressure? Based on the results of the Dalhousie University study [43], it seems reasonable to question if the 4:1 cardioplegia was hypotonic as that would be an obvious cause of myocardial edema.
2. Why were the pigs subjected to 30 minutes of unprotected myocardial ischemia before cardioplegia administration was initiated, and why was cardioplegia administered continuously during the following 90 minutes? Without an explanation from the authors, it seems plausible that the use of this nonstandard practice may have contributed to the attained results.
3. In the study, all of the microplegia pigs were weaned from bypass successfully while only 40% of the 4:1 cardioplegia pigs were successfully weaned. Two hours

after weaning, the survival rate in both groups was 40%. Why was the success rate of weaning significantly dissimilar from that seen in human cardiac surgery? This may be a result of the nonstandard protocol questioned in Question 2.

The SUNY study raises significant questions to be answered before the results of their study should be applied in practice. Using nonstandard techniques introduces confounding factors that are impossible to account for as the results could be specifically related to the technique utilized. Given that limited alternative studies exist that discuss the relationship between cardioplegia formulations and myocardial edema, the theory that crystalloid cardioplegia formulations result in increased myocardial edema does not possess sufficient supporting evidence without further investigation.

In patients with marginal hematocrits, the hemodilutional effects of 1:4 del Nido cardioplegia have the potential to lower the hematocrit to detrimental levels, increasing the risk of postoperative complications. While the hematocrit value at which these complications occur is poorly defined, various studies indicate that maintaining a hematocrit greater than 25% during cardiopulmonary bypass is sufficient. In one study conducted within six medical centers of northern New England, the mortality rate of patients with a hematocrit less than 19% was over double the mortality rate of patients with a hematocrit greater than or equal to 25% [65]. In a separate study done at the Medical College of Ohio, the risk of AKI increases as the hematocrit falls below 24% [66]. In a final study, also done at the Medical College of Ohio, hematocrits less than 22% were found to be a statistically significant contributor to postoperative stroke, myocardial infarction, low cardiac output, and pulmonary edema [66].

To avoid the aforementioned postoperative complications, reduced hematocrits caused by hemodilution may necessitate blood transfusions during cardiopulmonary bypass – a problem exacerbated by the use of crystalloid-based cardioplegia formulations. Blood transfusions are accompanied by their own list of associated risks, including infectious disease transmission, hemolytic reactions, allergic reactions, increased infection risk, acute lung injury, and immunomodulation [67]. It is therefore ideal to avoid blood transfusions during cardiopulmonary bypass, but it is generally understood that the risks of blood transfusions are preferable to the risks associated with hematocrits maintained below 25% [68].

In a prospective study conducted in Turkey to assess whether crystalloid cardioplegia was a predictor of intraoperative hemodilution, 100 participants undergoing cardiac surgery were randomized into two groups and received either 100% crystalloid cardioplegia (Plegisol) or microplegia for myocardial protection during ischemia [69]. If hematocrit levels fell below 22% during cardiopulmonary bypass, packed red blood cells (PRBCs) were transfused into the cardiopulmonary bypass circuit. It should be noted that ultrafiltration, a method of removing excess fluid from circulation, was not utilized in this study. Average intraoperative hematocrit was found to be 18.4% in the crystalloid cardioplegia group and 24.2% percent in the microplegia group – an outcome predicted by the hemodilution caused by crystalloid cardioplegia formulations [69]. Further, the crystalloid cardioplegia group averaged 2.7 units of PRBCs transfused intraoperatively, while the microplegia group averaged 0.9 units of PRBCs transfused intraoperatively. This study confirmed a statistically significant association between the use of crystalloid

cardioplegia formulations and blood transfusions in the absence of ultrafiltration – a predictable but noteworthy outcome given the risks associated with blood transfusions.

The use of ultrafiltration during cardiopulmonary bypass was first reported during the late 1970s and was found to improve several aspects of patient care during a handful of studies conducted during the 1980s [70]. Ultrafiltration is achieved through the use of a specially designed semipermeable membrane, called a hemoconcentrator, installed in the cardiopulmonary bypass circuit. The pressure within the bypass circuit creates a hydrostatic pressure differential across the membrane that drives water across the membrane. As water is driven across the membrane from the blood, a solute concentration gradient is created between the blood and water, removing solutes that are smaller than the pore size of the semipermeable membrane in addition to the water. Cellular elements, including red blood cells, are too large to cross the semipermeable membrane and become concentrated during ultrafiltration. Ultrafiltration therefore provides an alternative method for combating the hemodilution seen during cardiopulmonary bypass.

In a study conducted by SpecialtyCare to determine whether cardioplegia formulation influences hemodilution and transfusion requirements, records from 16,670 adult patients undergoing cardiac surgery with cardiopulmonary bypass were reviewed [61]. The patients were classified into four groups based on cardioplegia type: microplegia, 4:1 cardioplegia, del Nido cardioplegia, and HTK (i.e., a 100% crystalloid) cardioplegia. As expected, the highest average volume of crystalloid infused during cardioplegia

administration was found to be 2,000mL in the HTK group, while the microplegia group was found to be the lowest with an average crystalloid infusion of 50mL [61]. In contrast to the Turkish study [69], no statistically significant difference was found in the risk of intraoperative PRBC transfusion between the four cardioplegia groups as seen in Figure 23. While potentially a surprising outcome, it can be attributed to the use of ultrafiltration when justified by excessive hemodilution. Ultrafiltration was used in 85% of HTK cardioplegia cases with an average volume of 2,900mL removed, while it was used in 34% of cases in the microplegia group with an average volume of 1,500mL removed. In other words, crystalloid cardioplegia formulations lead to increased hemodilution, but the deleterious effects of hemodilution can be combatted through the judicious use of ultrafiltration.

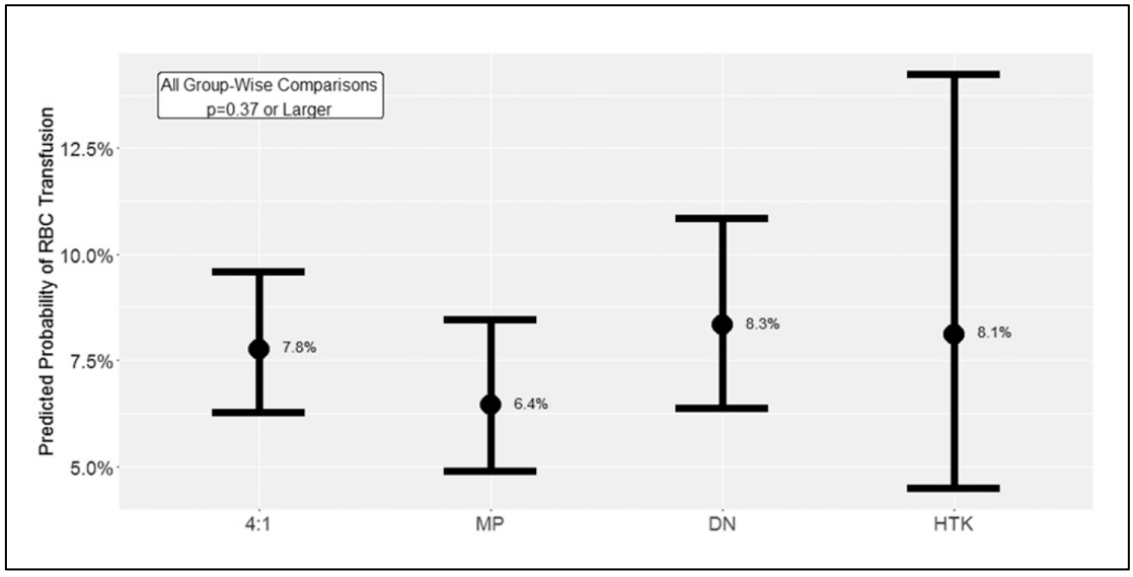

Figure 23. Risk of PRBC Transfusion Based on Cardioplegia Type [61]. After confounding factors were isolated, no statistically significant differences were found between cardioplegia types in terms of blood transfusion rate. It should be noted that in the study, del Nido (DN) represented both 1:4 del Nido and Micro del Nido, which went unrealized until the review of the statistical analysis. Study results are still significant, though, as the comparison between crystalloid cardioplegia (HTK) versus microplegia (MP) is the most relevant.

While ultrafiltration has been shown to be statistically significant in reducing blood transfusions by counteracting the hemodilutional effects of crystalloid-based cardioplegia formulations, the use of ultrafiltration during cardiopulmonary bypass has been scrutinized in recent years as the result of a perceived association between ultrafiltration and acute kidney injury (AKI) [71]. Postoperative AKI occurs in up to 50% of cardiopulmonary bypass patients and is associated with a significantly increased risk of morbidity [72]. Given the kidneys' role in fluid balance within the circulatory system, the belief is that ultrafiltration during cardiopulmonary bypass removes the workload of the kidneys, resulting in postoperative dysfunction when the work of fluid balance shifts back to the kidneys after cardiopulmonary bypass.

One recent study that looked to address the association between ultrafiltration and AKI was conducted on the PERFusion Measures and Outcomes (PERForm) registry [73]. Between 2010 and 2013, 6,407 consecutive patients underwent on-pump coronary artery bypass graft (CABG) surgery and were included in this retrospective study. The patients in the ultrafiltration group were more likely to have diabetes, vascular disease, congestive heart failure, and an intra-aortic balloon pump (IABP). They also had lower preoperative hematocrits, creatinine clearance, and a reduced ejection fraction. Congestive heart failure diagnosis, reduced creatinine clearance, and decreased hematocrit are all potential signs of fluid overload and decreased kidney performance so it is reasonable that this group would be more likely to need ultrafiltration during cardiopulmonary bypass. After adjustment for preoperative kidney function, the study found that patients exposed to ultrafiltration were 36% more likely to be diagnosed with postoperative AKI than their

counterparts who were not exposed to ultrafiltration [73]. This study did not consider the fluid volume administered to the patient or the volume of ultrafiltrate removed, nor did it define what the fluid balance goals were after completion of ultrafiltration, making it challenging to assess whether excessive fluid was removed through ultrafiltration.

The next study that looked to evaluate the effects of ultrafiltration on kidney performance was a prospective study that included 100 adults undergoing CABG or valve replacement surgery by the same surgical and perfusion team [71]. In this study, perfusionists used ultrafiltration during the duration of cardiopulmonary bypass, adding additional fluid volume to the reservoir if necessary to maintain a safe operating level in their venous reservoir. While 14 of the patients in their ultrafiltration group had a negative fluid balance upon leaving the operating room, the group overall had an average positive fluid balance of 9.2mL/kg [71]. In contrast, the group where ultrafiltration was not used had an average positive fluid balance of 34.5 mL/kg. This study found no statistically significant differences in bypass urine rate, intraoperative urine rate, or 24-hour urine rate, as well as no statistically significant differences between preoperative and 24-hour postoperative creatinine levels. The study concluded that aggressive ultrafiltration did not affect renal performance and could be used safely during cardiopulmonary bypass to “reduce fluid accumulation and elevate bypass hematocrit” [71].

One of the most commonly referenced studies by advocates of limiting ultrafiltration to reduce postoperative AKI is a study completed at Duke University School of Medicine [62]. In their retrospective study, postoperative AKI as defined by Kidney Disease:

Improving Global Outcomes (KDIGO) was assumed to be a function of weight-indexed ultrafiltration volume, allowing the researchers to perform regression analysis and assess potential relationships. It was determined that ultrafiltration volumes greater than 32.6mL/kg lead to a higher incidence of AKI and a higher incidence of KDIGO stage-2 and stage-3 AKI when compared to ultrafiltration volumes less than 32.6mL/kg [62]. A graphical summary of the Duke University study can be seen in Figure 23. The study also found that ultrafiltration volumes greater than 32.6mL/kg resulted in a statistically significant increase in blood transfusions and hospital length of stay. It was therefore the authors' recommendation that ultrafiltration volumes greater than 32mL/kg should be avoided.

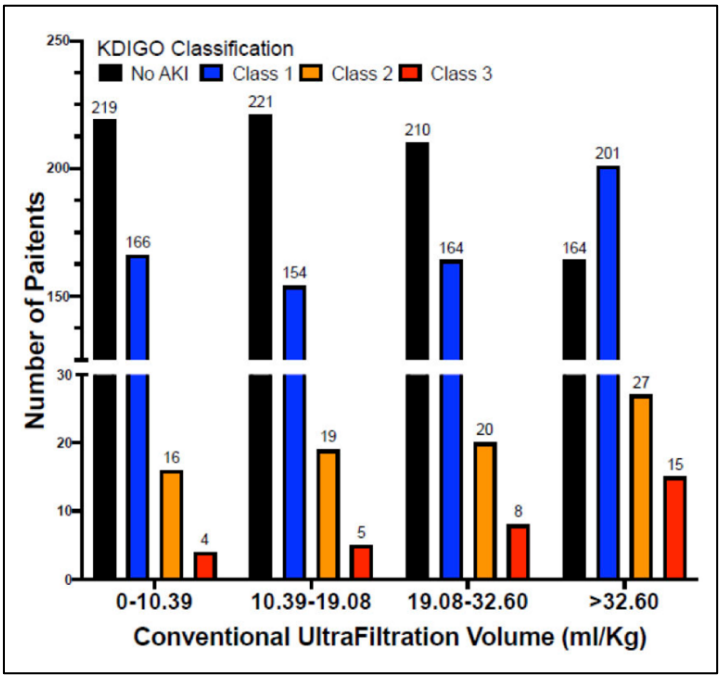

Figure 24. Effect of Weight-Indexed Ultrafiltration Volume on AKI Development [62]. KDIGO classification increases in severity from Class 1 to Class 3. As ultrafiltration volume increases above 32.6mL/kg, AKI occurrence and severity increase significantly. Notice how the lower three quartiles have very similar graphical representations.

While the Duke University study provides a good reference value for ultrafiltration volume, the study does not consider ultrafiltration volume relative to volume status, so it is challenging to assess if the incidence of AKI is related to the net fluid balance. This becomes particularly important when discussing the use of ultrafiltration to prevent excess hemodilution caused by crystalloid cardioplegia formulations. The question that remains is, "Does using ultrafiltration to maintain preoperative fluid status result in AKI?" This question is significant to the debate between crystalloid-based cardioplegia formulations and microplegia and should be considered in future studies. It is worth noting that the 32mL/kg threshold referenced in the Duke University study is an extreme amount of ultrafiltrate volume, rarely necessitated by the hemodilution associated with crystalloid-based cardioplegia formulations [74].

A 2023 AKI joint consensus report drew similar conclusions to the above studies, stating that postoperative AKI could be reduced by not using "excessive ultrafiltration during cardiopulmonary bypass" and citing the PERForm and Duke University studies [75]. They acknowledged, though, that alternative studies provided conflicting results and found no association between ultrafiltration volume and AKI. The joint consensus report was therefore purposefully vague by recommending the avoidance of excessive ultrafiltration but choosing not to define the volume of ultrafiltration that qualifies as "excessive" [75]. The report concluded that it is known that hypovolemia is deleterious to kidney function, leaving it open to the reader as to whether it is permissible to use ultrafiltration for preoperative fluid balance maintenance, i.e., discontinuing ultrafiltration during bypass before the patient reaches a hypovolemic state.

2.10 The Development of Micro del Nido

If improved coronary distribution cannot be entirely credited for 1:4 del Nido cardioplegia's benefit over alternative cardioplegia formulations, its success must be attributed to its additive profile, specifically the inclusion of mannitol, lidocaine, sodium bicarbonate, and magnesium sulfate in its formulation. While the debate over the effects of hemodilution will remain unsettled without further studies, anecdotal evidence spread between hospitals, first-hand experiences, and selective use of studies led to the desire for a cardioplegia formulation that contained the additives of 1:4 del Nido cardioplegia without the perceived negative effects of hemodilution. This new formulation would become known as "Micro del Nido" cardioplegia by researchers as they sought to reformat 1:4 del Nido cardioplegia into a microplegia cardioplegia formulation by adding potassium chloride and del Nido's proven additives directly into whole blood from the cardiopulmonary bypass circuit for delivery to the coronary circulation.

Micro del Nido cardioplegia, also referred to as "Modified del Nido" or "Whole Blood del Nido" cardioplegia, was first referenced in a 2014 study from Brigham and Women's Hospital in Boston [76]. At Brigham and Women's Hospital, their experimental Micro del Nido cardioplegia was mixed at a ratio of eight parts blood to one part crystalloid that contained the major additives of 1:4 del Nido cardioplegia. Due to the natural buffering and free-radical scavenging abilities of blood, mannitol and sodium bicarbonate were not included in their formulation's additives. While they admit that it was purely speculative, the authors of this study believed that their new cardioplegia formulation would minimize myocardial edema when compared to 1:4 del Nido cardioplegia [76].

To assess the efficacy of their Micro del Nido cardioplegia formulation, researchers at Brigham and Women's Hospital conducted a prospective study in late 2013 [76]. In 92 consecutive operations, a single dose of Micro del Nido delivered to the coronary circulation was used as the myocardial protection strategy. The study participants were then propensity-matched with 79 patients who received Brigham's conventional whole-blood cardioplegia formulation. No statistically significant differences were found in primary outcomes, including inotrope use, ventilation time, hospital length of stay, and intraoperative mortality [76]. Creatine kinase-MB levels were statistically higher in the Micro del Nido group, but the authors noted that it did not correlate to negative clinical outcomes. The study concluded that the safety and efficacy of the researchers' Micro del Nido cardioplegia was comparable to conventional whole-blood cardioplegia, but a multicenter, prospective, randomized trial was required to determine best practices.

In the Milwaukee area, Froedtert Memorial Hospital was the first hospital to experiment with Micro del Nido cardioplegia. According to Kate Herrmann, a perfusionist at Froedtert Hospital, they transitioned in 2016 due to a perceived rise in postoperative AKI attributed to the hemodilution and associated ultrafiltration of 1:4 del Nido cardioplegia. While studies were limited at the time, the Brigham and Women's Hospital study aided in Froedtert's quest to find an alternative to 1:4 del Nido cardioplegia that offered similar benefits without the dilution of a crystalloid-based cardioplegia formulation. Based on correspondence with an institution in Georgia and slight modification, the Micro del Nido additive formula seen in Table 4 was eventually established and is currently utilized at Froedtert Hospital for non-CABG surgeries. Froedtert's Micro del Nido cardioplegia is

delivered to the coronary circulation using the MPS Quest 2, with an additive setting of 35mL/L and an arrest setting of 24mEq/L for induction doses and ~6mEq/L for maintenance doses – reducing the potential for systemic hyperkalemia after aortic cross-clamp removal.

Table 4. Composition of Froedtert Hospital's Micro del Nido Additive Syringe. "Dose per Liter" refers to the amount of additive in one liter of cardioplegia delivered. "% Difference" refers to the excess or deficit of the additive relative to 1:4 del Nido cardioplegia. Note that the arrest agent was not included as Micro del Nido cardioplegia uses separate sources and settings for additive and arrest.

Component	Contents per Syringe	Dose per Liter	% Difference
Lidocaine (2%)	160 mg	122.4 mg	+24.68%
Magnesium Sulfate (50%)	2.3 g	1.76 g	+16.49%
Mannitol (20%)	3.75 g	2.87 g	+16.53%
Sodium Bicarbonate (8.4%)	14.4 mEq	11.02 mEq	+12.21%

Another hospital in the Milwaukee area, Ascension Columbia St. Mary's Hospital, transitioned to Micro del Nido in 2019 to also reduce the perceived negative effects of hemodilution and ultrafiltration associated with 1:4 del Nido cardioplegia. While St. Mary's was coincidentally following in the footsteps of Froedtert, their transition was inspired by an alternative study conducted at JFK Medical Center in Florida that was published in early 2019 [77]. This study looked to compare the outcomes of low-risk and high-risk patients where Micro del Nido was used for myocardial protection during cardiac surgery. The high-risk group was comprised of patients who underwent combined CABG and valve surgery, double-valve surgery, triple-valve surgery, and patients with an ejection fraction <40%. Despite several significant statistical differences between the

low-risk and high-risk groups, the study concluded that Micro del Nido is a safe strategy for a diverse spectrum of cardiac surgery types when compared with operative mortality risks published by the Society of Thoracic Surgeons (STS) for similar surgery types [77].

According to Holly Estill, a perfusionist at St. Mary's Hospital, the authors of this study were contacted to determine their Micro del Nido formulation, which St. Mary's adopted and is shown in Table 5. St Mary's Micro del Nido cardioplegia is also delivered to the coronary circulation utilizing the MPS Quest 2, with an additive setting of 32mL/L and an arrest setting of 24mEq/L for induction doses and ~7mEq/L for maintenance doses. It should be noted that when the perfusionists at St. Mary's Hospital calculated the Quest additive setting in 2019, they concluded it should be ~30mEq/L to match the additive concentrations given in one liter of 1:4 del Nido cardioplegia. Given JFK Medical Center's success, the perfusionists at St. Mary's chose to adopt their additive setting of 32mL/L so as to not introduce potential sources of differences in clinical outcomes. St. Mary's current Micro del Nido protocol can be found in Appendix A.

Table 5. Composition of St Mary's Hospital's Micro del Nido Additive Syringe. "Dose per Liter" refers to the amount of additive in one liter of cardioplegia delivered. "% Difference" refers to the excess or deficit of the additive relative to 1:4 del Nido cardioplegia. Note that the arrest agent was not included as Micro del Nido uses separate sources and settings for additive and arrest. To include the arrest agent would have introduced confusion.

Component	Contents per Syringe	Dose per Liter	% Difference
Lidocaine (2%)	130 mg	104.52 mg	+6.46%
Magnesium Sulfate (50%)	2 g	1.61 g	+6.46%
Mannitol (20%)	3.26 g	2.62 g	+6.46%
Sodium Bicarbonate (8.4%)	13 mEq	10.45 mEq	+6.46%

2.11 Additional Micro del Nido Studies

The JFK Medical Center study was followed up in 2019 by a second study at the same institution [78]. While the previous study looked to compare outcomes between low-risk and high-risk patients, this study looked to compare the outcomes of Micro del Nido cardioplegia as seen in Table 5 with the Brigham and Women's Hospital Micro del Nido cardioplegia. As discussed previously, the Brigham and Women's Hospital Micro del Nido cardioplegia formulation is the original microplegia adaptation of 1:4 del Nido cardioplegia, lacking mannitol or sodium bicarbonate as additives due to the natural ability of blood to act as a buffer and oxygen free radical scavenger. For this study, the primary endpoint was operative mortality. Secondary outcomes included postoperative outcomes, like reoperation for bleeding, infection, arrhythmias, stroke, length of stay, IABP insertion, and prolonged ventilation [78].

The study originally attempted to retrospectively compare all patients receiving either cardioplegia formula but found significant differences in age, sex, ejection fraction, prior MI, and hypertension. The data were then propensity-matched to provide a better comparison, which yielded no significant differences in preoperative characteristics. The study found no significant difference in the primary outcome of operative mortality. Statistically significant differences were found in hospital length of stay and IABP insertion, though, with JFK's Micro del Nido offering favorable results for both outcomes [78]. The study concluded that the Micro del Nido protocol used at JFK Medical Center yielded comparable outcomes to the cardioplegia formulation used at Brigham and Women's Hospital, indicating its safety and efficacy.

More recent studies have looked to directly compare Micro del Nido to 1:4 del Nido cardioplegia – a particularly useful objective in assessing the effect of hemodilution and ultrafiltration associated with 1:4 del Nido cardioplegia. In a retrospective Pakistani study of patients who underwent CABG surgery between January 2016 and March 2020, the Micro del Nido cardioplegia group was found to have better clinical outcomes, including lower inotropic support requirements, shorter hospital length of stay, and improved renal function [79]. The Micro del Nido group also had lower peak troponin-T and creatine kinase-MB levels – a measure of protection against ischemia and reperfusion injury. While it should be noted that lactated ringer was used as the crystalloid base for this study's 1:4 del Nido cardioplegia due to the limited availability of PlasmaLyte outside the United States, the study appears to show an association between better myocardial protection and the use of Micro del Nido cardioplegia over 1:4 del Nido cardioplegia. The authors conclude that a multicenter, prospective study should be completed before further conclusions are drawn about the benefits of Micro del Nido.

The last study published to date about Micro del Nido was based on retrospective data of CABG surgeries done at a research hospital in Turkey during early 2021 [80]. This study used a crystalloid base similar to PlasmaLyte but delivered their Micro del Nido formula at a ratio of 4:1 – a significant reduction in crystalloid volume compared to 1:4 del Nido cardioplegia. Preoperative patient characteristics contained no statistical differences. While the Pakistani study found several statistically significant clinical outcomes, this Turkish study found relatively negligible differences between the two cardioplegia formulations. Between the two groups, cardiac markers like troponin-t and creatine

kinase-MB were statistically similar, offering no apparent improvement in myocardial protection from ischemia and reperfusion injury. The most noteworthy statistically significant difference is that their Micro del Nido formula resulted in decreased fibrillation upon aortic cross-clamp removal and reperfusion [80]. Due to a relatively limited number of studies that assess the safety and efficacy of Micro del Nido, further investigation is needed before conclusions can be drawn about which del Nido variant offers superior myocardial protection.

3.0 Project Statement

Since 1:4 del Nido cardioplegia has generally been shown to offer superior myocardial protection over traditional blood-based cardioplegia formulations, studies like those by Haider et al. [79] and Karaarslan and Erdinc [80] are particularly useful in assessing whether 1:4 del Nido's additives or associated hemodilution are the primary contributor to improved outcomes. While both studies are valuable in the cardioplegia debate, they only looked to assess the safety and efficacy of Micro del Nido in CABG surgeries. In the Milwaukee area, CABG surgeries are primarily done with an alternative magnesium-based microplegia formulation, while Micro del Nido is almost exclusively used for non-CABG surgeries like valve repair and replacement surgeries at cardiac surgery centers that have adopted Micro del Nido. Additionally, the Pakistani [79] and Turkish [80] studies provided conflicting results, so additional studies may help guide the debate between the two discussed del Nido cardioplegia variants. To better assess whether Micro del Nido cardioplegia is safe and effective in valve repair or replacement surgeries, it is therefore necessary to conduct a study that compares outcomes between Micro del Nido and 1:4 del Nido cardioplegia in those types of surgeries.

Ascension Columbia St. Mary's Hospital began using 1:4 del Nido for their single valve repair or replacement surgeries in 2015 before transitioning to Micro del Nido for these same types of surgeries in 2019. A retrospective study at this facility therefore serves two purposes: (1) to assess the efficacy and safety of Micro del Nido and (2) to compare the efficacy of a blood-based cardioplegia to a crystalloid-based cardioplegia without being skewed by varying additive formulations. Additionally, confounding factors like surgeon,

facility, and procedure can be reduced or eliminated. The assumed hypothesis for this study is that there are no statistically significant differences in clinical outcomes between myocardial protection strategies that use 1:4 del Nido cardioplegia and Micro del Nido cardioplegia. One potential benefit of this study could be the alleviation of the negative stigma associated with crystalloid-based cardioplegia formulations and ultrafiltration.

4.0 Methods

To assess a patient's eligibility for study inclusion per the preliminary study protocol contained in Appendix B, paper cardiopulmonary bypass records (past and current method of charting done by perfusionists at Ascension Columbia St. Mary's Hospital) were to be reviewed for identification of potential study subjects. Following approval by Ascension's Institutional Review Board, it became apparent that the retention period of paper records was insufficient for the specified task, necessitating an alternative method for the assessment of patient eligibility. Fortunately, the quality management department at Ascension Columbia St. Mary's Hospital was able to generate a patient list based on surgeon, surgery type, and timeframe using the Society of Thoracic Surgeons (STS) database. Further exclusion criteria could then be applied to establish a complete list of eligible patients for study inclusion.

Eligible subjects included patients who underwent single valve repair or replacement surgery by one specified surgeon (hereafter referred to as "Surgeon X") with either 1:4 del Nido cardioplegia or Micro del Nido cardioplegia used for myocardial protection during ischemia. Since mitral valve repair ("MV Repair"), mitral valve replacement ("Isolated MVR"), and aortic valve replacement ("Isolated AVR") are the only specified categories used by the STS for single valve repair and replacement surgeries, tricuspid valve repair and replacement surgeries were inherently excluded from this study. Additional exclusion criteria were established to eliminate high mortality risk patients from influencing results, including patients with redo sternotomies, ejection fractions of less than 30%, uncontrolled type II diabetes, and uncontrolled hypertension. For both

groups, the desired minimum number of eligible patients was 40 – determined by St. Mary's perfusionists as a plausible number of patients that would meet the inclusion criteria during the specified timeframe. If more than 40 subjects were identified in either group, they were also to be included to limit the influence of bias on patient inclusion.

Once adult patients who underwent single valve repair or replacement surgery with Surgeon X between 2015 and the present were identified, the patients' medical record numbers (MRNs) were utilized to access their medical records in Epic where further exclusion criteria could be applied. The data collection forms found in Appendix C were used to record the specified parameters of eligible patients. Patient demographics, including age, gender, BMI, preoperative hypertension diagnosis, and preoperative diabetes diagnosis, were to be recorded to ensure statistically similar patient populations between the two groups. Clinical outcomes and operative data that were recorded for analysis included pre- and postoperative ejection fraction, pre- and postoperative hemoglobin and hematocrit, cardioplegia type, valve surgery type, CPB time, cross-clamp time, ultrafiltration use, postoperative inotropic support, postoperative balloon pump insertion, blood transfusions, AKI development, time to extubation, length of ICU stay, and length of hospital stay. A summary of the data collection parameters can be seen in Table 6.

Table 6. Summary of Data Collection Parameters.

Parameter	Notes
Acute Kidney Injury (AKI)	Postoperative Development → 0 = No, 1 = Yes
Age	Years
Blood Transfusions	Intraoperative Administration → 0 = No, 1 = Yes
Body Mass Index (BMI)	Kilograms/m^2
Cardiopulmonary Bypass (CPB) Time	Minutes
Cardioplegia Type	1:4 del Nido or Micro del Nido
Cross-Clamp (XC) Time	Minutes
Diabetes Mellitus (DM)	Preoperative Diagnosis → 0 = No, 1 = Yes
Ejection Fraction (EF)	Pre- and Postoperative Measurement → %
Extubation Time	End of Surgery to Initial Extubation → Hours
Gender	0 = Male, 1 = Female
Hematocrit (HCT)	Pre- and Postoperative Measurement → %
Hemoglobin (HGB)	Pre- and Postoperative Measurement → g/dL
Hospital Length of Stay (LOS)	Surgery Date to Discharge Date → Days
Hypertension (HTN)	Preoperative Diagnosis → 0 = No, 1 = Yes
Inotropic Support	w/in 48 Hours Postoperatively → 0 = No, 1 = Yes
Intensive Care (ICU) Stay	ICU Admission to ICU Transfer → Hours
Intra-Aortic Balloon Pump (IABP)	Postoperative Insertion → 0 = No, 1 = Yes
Ultrafiltration	Intraoperative Use → 0 = No, 1 = Yes
Valve Surgery Type	Mitral Repair (MVr), Mitral Replacement (MVR), or Aortic Replacement (AVR)

All surgeries included in this study used the Terumo System 1 Heart-Lung Machine with the Terumo FX25 oxygenator. Cardioplegia was administered with the Quest MPS 2 for both 1:4 del Nido cardioplegia and Micro del Nido cardioplegia. The protocol for Micro del Nido can be seen in Appendix C. While the protocol used for 1:4 del Nido cardioplegia from 2015 to the Micro del Nido transition is no longer accessible, it was similar to the Micro del Nido protocol, with the notable exception that 1:4 del Nido

cardioplegia delivery was done by putting the Quest MPS 2 in 1:4 cardioplegia delivery mode and turning the arrest and additive delivery settings off since 1:4 del Nido cardioplegia contains both the arrest and additive elements in the crystalloid component.

5.0 Results

To adequately assess whether Micro del Nido cardioplegia is a safe and effective alternative to 1:4 del Nido cardioplegia, this study sought to review the records of patients who had undergone single valve repair or replacement surgery at Columbia St. Mary's Hospital with either cardioplegia formulation – a task that required approximately eight years of complete and thorough patient records. The medical records of recent patients were generally found to contain all the desired operative data and clinical outcomes. The only notable exception was that pre- and postoperative ejection fraction values were not recorded in a standardized fashion by the cardiac anesthesiologist, commonly resulting in days to months between an ejection fraction measurement and surgery. While this does present the ability to evaluate long-term ventricular recovery, the ability to compare ejection fractions from before and after cross-clamp removal offers a superior and more useful assessment of the cardioplegia formulation's impact on the return of cardiac function after ischemia and reperfusion. Any future studies should therefore look to include operating room measurements of both pre- and postoperative ejection fraction.

Complete medical records containing the desired study parameters were found for patients who had undergone surgery after October 2021. Before this date, cardiopulmonary bypass records that had been scanned into Epic were absent, as well as point of care (POC) measurements for pre- and postoperative hemoglobin and hematocrit. Lacking a suitable alternative to the cardiopulmonary bypass record, cardiopulmonary bypass time, aortic cross-clamp time, blood transfusions, and ultrafiltration use became

unattainable parameters. Additionally, these patients' medical records did not contain an alternative method for verification of cardioplegia formulation – a necessity for producing a reliable, reproducible, and transparent research study. Upon investigation and conversations with St. Mary's perfusionists, October of 2021 coincided with a hospital-wide conversion from Cerner to Epic for the management of electronic medical records, which likely contributed to the absence of desired documents. After exhaustion of any alternative resources, it was concluded that the needed data could not be obtained and that the study could not be conducted as intended. It is therefore recommended that future retrospective studies be conducted nearer to the clinical change being investigated as the evolution of technology and modification of retention policies may limit the preservation of desired parameters.

After a review of all patient charts where cardioplegia formulation could be verified, the clinical outcomes of 14 patients were recorded and can be seen in Appendix D. Given all included patients had surgery after the conversion to Micro del Nido cardioplegia, a comparison of outcomes between the two del Nido variants is not possible. Of the Micro del Nido cardioplegia outcomes, acute kidney injury (AKI) incidence is the only outcome that warrants further analysis without the ability for direct comparison. Postoperatively, two of the 14 analyzed patients developed AKI as defined by the RIFLE (Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease) classification used by the STS [81]. Per the RIFLE classification, AKI is defined as a 1.5x increase in postoperative serum creatinine, a reduction in glomerular filtration rate (GFR) greater than 25%, or urine output less than 0.5mL/kg/hr for 6 hours. While studies that look to assess

postoperative AKI find varied incidences of AKI occurrence, a study done at Mayo Clinic found that postoperative AKI as defined by RIFLE classification occurred in 18.9% of patients undergoing cardiac surgery with cardiopulmonary bypass [82]. Using Fischer's Exact Test, it can then be concluded that there is not a statistically significant difference between AKI incidence when using Micro del Nido at St. Mary's and the AKI incidence observed at Mayo Clinic ($p > 0.999$).

6.0 Discussion

Due to the recency of Micro del Nido cardioplegia's development and the limited availability of comparative studies, Micro del Nido's overall safety and efficacy remain unanswered questions. Given its similar additive profile to 1:4 del Nido cardioplegia, it seems plausible to conclude that it is at least as safe and effective while avoiding the perceived negative effects of hemodilution inherent to crystalloid-based cardioplegia formulations. A question arises, though – is there conclusive evidence that the hemodilution associated with crystalloid-based cardioplegia formulations is detrimental, or are studies selectively applied to support that viewpoint simply because hemodilution feels unnatural and unphysiological to clinicians?

As detailed above, proponents of microplegia cardioplegia formulations argue that crystalloid-based cardioplegia formulations cause myocardial edema, increase blood transfusions, and lead to the development of postoperative AKI through the use of ultrafiltration. While the SpecialtyCare study [61] effectively concludes that the crystalloid content of cardioplegia formulations does not increase blood transfusions with the judicious use of ultrafiltration, suggested future research topics include the following:

1. A research study that repeats the Yorkshire pig study conducted at SUNY [64] but adheres to standard myocardial protection practices seen in human cardiac surgery, i.e., the pigs should not be subjected to 30 minutes of unprotected myocardial ischemia before cardioplegia administration. Additionally, this study should look to evaluate the relationship between cardioplegia's osmolarity and the development of myocardial edema.

2. A study that assesses the association between AKI and ultrafiltration but considers the patient's volume status relative to the volume of ultrafiltration removed. Ideally, this study would be prospective to allow for the definition of standard practices, as well as the specification of required variables that may not be commonly recorded during cardiac surgery. Further, this study could be divided into two parts: (1) the effect of ultrafiltration on AKI development when volume status is considered and (2) the effect of ultrafiltration on AKI development when only the volume of crystalloid administered in cardioplegia is removed via ultrafiltration.

In contrast to the perceived negative effects of hemodilution associated with crystalloid-based cardioplegia formulations, hemodilution may offer a cardioprotective effect through reduced viscosity and improved coronary flows at hypothermic delivery temperatures. It is therefore advantageous to develop a study that evaluates whether there is a benefit to these elevated flows in terms of cardioplegia distribution and myocardial protection as it may broadly outweigh the benefits of adopting microplegia cardioplegia formulations. While the reduction of hemodilution associated with crystalloid-based cardioplegia formulations, like 1:4 del Nido, seems like a simple and beneficial proposition, further studies that directly assess the benefits and drawbacks of hemodilution are needed to conclusively settle this debate. Lessons learned from these studies can also be used more broadly to refine cardioplegia formulations not discussed in this paper.

In addition to the need for studies that evaluate the hemodilution associated with crystalloid-based cardioplegia formulations, it is advantageous to concurrently conduct studies that assess the safety and efficacy of Micro del Nido, specifically in relation to the more proven formulation of 1:4 del Nido cardioplegia. At Dalhousie University in Canada, the results of their studies that evaluated the efficacy of two different cardioplegia formulas in rats were invaluable in concluding that 1:4 del Nido offered a more robust arrest and superior ventricular recovery over modified Buckberg solution [43, 44, 60]. A similar rat study conducted with 1:4 del Nido and Micro del Nido could isolate the hemodilution debate from the debate over cardioplegia additives – if a del Nido cardioplegia variant offers a statistical benefit over the other, it becomes apparent that the hemodilution or lack thereof is a contributing factor. This benefit could also be the result of factors not yet considered, like lower calcium concentrations in 1:4 del Nido cardioplegia than in Micro del Nido due to the absence of calcium in the crystalloid component and the limited use of blood from the cardiopulmonary bypass circuit.

Lastly, it is important to continue comparative studies in humans between the two discussed del Nido cardioplegia variants for single valve repair and replacement surgeries. The concept of surgical innovation allows cardiac surgery teams to adapt their practice in response to the perceived benefit of making a given change. While this allows for the continued adoption of new concepts, it often does so with limited conclusive evidence supporting the change, possibly to the detriment of beneficial progress. Retrospective studies at cardiac surgery centers that have switched from 1:4 del Nido cardioplegia to Micro del Nido should be conducted as confirmation that the adopted

change was beneficial. To avoid the limitations encountered by the retrospective study conducted in this paper, special consideration should be paid to the timeframe at which the retrospective study is conducted. Optimally, the study should be timed long enough after the change to ensure sufficient study subjects for statistical analysis but short enough after the change to ensure the desired parameters will be accessible. It should be considered that this balance may only be achievable at a high-volume surgery center. Further, the challenge of retrospective studies is the lack of control over protocol and practices, like the lack of a standard practice for the recording of pre- and postoperative ejection fraction at Ascension Columbia St. Mary's Hospital, which may limit the conclusiveness of these studies.

The limitations of retrospective studies illustrate the need for a prospective study, specifically a study that divides subjects undergoing single valve repair or replacement surgery between two cardioplegia groups: 1:4 del Nido cardioplegia and Micro del Nido cardioplegia. By conducting a prospective study, desired parameters can be defined before the study is conducted, allowing for the creation of standard protocols for attaining those parameters. Additionally, confounding factors can be reduced to the furthest extent possible, allowing an intimate comparison of the two previously discussed cardioplegia variants and their associated hemodilutional properties. While IRB approval processes for prospective studies are thorough and lengthy, the role cardioplegia plays in myocardial protection during cardiac surgery justifies the effort, specifically because myocardial protection translates to myocardial recovery and continued myocardial function through the remainder of a patient's life.

7.0 A Novel del Nido Cardioplegia Formulation

While the need for further research studies that assess cardioplegia-associated hemodilution and directly compare 1:4 del Nido cardioplegia and Micro del Nido cardioplegia cannot be overshadowed, it is advantageous to address a commonly held reservation toward the adoption of microplegia cardioplegia formulations before the reservation is used to disregard this paper. In hospitals throughout the United States, fixed 1:4 ratio roller pumps are still commonly used for cardioplegia delivery, with microplegia-capable delivery systems not yet being predominant. Benefits of roller pump cardioplegia systems over microplegia-capable delivery systems include reduced initial cost, significantly cheaper disposable cost, and maintenance of the status quo for surgeons, perfusionists, and hospitals. Future conclusive evidence that Micro del Nido is superior to 1:4 del Nido may therefore warrant the development of a del Nido cardioplegia variant that contains less crystalloid than 1:4 del Nido but can be delivered using a fixed ratio cardioplegia delivery system for adoption by hospitals without a microplegia-capable delivery system.

The formula seen in Table 7 details a new del Nido concept formula, 4:1 del Nido cardioplegia, that contains similar additive concentrations as 1:4 del Nido cardioplegia but is designed to be delivered at a 4:1 ratio since most fixed 1:4 ratio roller pump cardioplegia delivery systems can quickly be altered to a 4:1 ratio using Luer lock connections. By reversing the ratio, the volume of crystalloid being delivered within the cardioplegia formulation is significantly reduced – from 800mL to 200mL of crystalloid per one liter of cardioplegia delivered. To aid in the adoption of 4:1 del Nido cardioplegia

by hospitals and their pharmacies, particular care was paid to using a commercially available PlasmaLyte bag size (i.e., 500mL), as well as to use additive volumes that align with commercially available products. Given that 1:4 del Nido cardioplegia has been shown to be safe and effective, further studies that show the same for Micro del Nido will illustrate the safety and efficacy of this hybrid 4:1 del Nido cardioplegia, while reducing any deleterious effects associated with the larger volume of crystalloid delivered by 1:4 del Nido cardioplegia. Adoption should therefore be a relatively seamless endeavor for hospitals without microplegia-capable cardioplegia delivery systems. It is important to reiterate the desire for further studies that assess the effects of cardioplegia-associated hemodilution and compare the safety and efficacy of 1:4 del Nido cardioplegia to Micro del Nido cardioplegia before 4:1 del Nido cardioplegia is adopted.

Table 7. Novel 4:1 del Nido Cardioplegia Formulation. Designed to be delivered at a 4:1 ratio of blood to crystalloid, one 501mL bag of crystalloid delivered corresponds to over 2.5 liters of 4:1 del Nido cardioplegia delivered, significantly reducing the amount of crystalloid from 1:4 del Nido cardioplegia. "Dose per Liter" refers to the amount of additive and arrest in one liter of cardioplegia delivered when delivered at a 4:1 ratio of blood to crystalloid. "% Difference" refers to the excess or deficit of the additive relative to 1:4 del Nido cardioplegia.

Component	Contents per Bag	Dose per Liter	% Difference
Plasma-Lyte A	390mL	N/A	N/A
Lidocaine (1%)	25mL	99.80mg	+1.65
Magnesium Sulfate (50%)	7.5mL	1.50g	-0.89%
Mannitol (25%)	25mL	2.5g	+1.34%
Sodium Bicarbonate (8.4%)	25mL	9.98mEq	+1.65%
Potassium Chloride (2mEq/mL)	28.5mL	23.53mEq	+0.52%
Total Volume	**501mL**		

8.0 Conclusion

During cardiac surgery, planned myocardial ischemia is often necessary to allow the surgeon to enter the heart, as well as to provide a quiet, bloodless field for ease of repair. While the heart is generally resilient to brief periods of myocardial ischemia, the extended durations necessitated by complex intracardiac repairs would be detrimental to the heart without a method of protection. It has also been shown that the restoration of blood flow to ischemic tissue upon aortic cross-clamp removal has the potential to cause damage to the heart. To address the need for a sufficient protection mechanism against both ischemia and reperfusion injury, cardioplegia has been continuously developed and refined since the 1950s. In its present form, cardioplegia generally consists of three main components – hypothermia to reduce myocardial energy demand, an arrest agent to stop the heart from beating and further reduce energy demand, and additives to support the arrest and help the heart resume normal function after arrest.

During the 1990s, the quest for a cardioplegia formula that catered to the specific needs of immature hearts led Pedro del Nido to the development of a pediatric-specific cardioplegia formula, termed 1:4 del Nido cardioplegia. Mixed one part blood from the cardiopulmonary bypass circuit to four parts crystalloid, the crystalloid component of 1:4 del Nido cardioplegia contains potassium for cardiac arrest, as well as additives with favorable targets – mannitol for increasing osmotic pressure and oxygen free radicals scavenging, magnesium as a calcium channel blocker, bicarbonate as an acidic buffer, and lidocaine as a fast sodium channel blocker. While each of these additives is generally viewed as beneficial when added to a cardioplegia formulation, the most noteworthy and

novel inclusion was that of lidocaine, which reduces myocardial energy demand and helps maintain a robust arrest through a reduction in cardiomyocyte excitability [43].

In both animal and human studies, 1:4 del Nido cardioplegia has been shown to be advantageous over other common cardioplegia formulations leading to the eventual, and generally successful, adoption of 1:4 del Nido cardioplegia in many adult cardiac surgery centers. With the rise of microplegia cardioplegia formulations and the notable hemodilution associated with 1:4 del Nido cardioplegia, a new cardioplegia formulation was more recently developed to combat the perceived negative effects of hemodilution – Micro del Nido. In early Micro del Nido studies, it has been shown to be safe and effective compared to 1:4 del Nido cardioplegia despite lacking sufficient evidence to support the perceived negative effects of hemodilution. As a result, further studies are needed that assess the effects of hemodilution and ultrafiltration, evaluate the safety and efficacy of Micro del Nido, and use animal models to compare ventricular recovery and robustness of arrest between the two common del Nido variants.

While it is common within the field of cardiac surgery to have a preference toward a particular cardioplegia formulation due to personal experiences and instilled beliefs, studies, particularly retrospective studies, rarely provide a unified voice that supports the superiority of a specific cardioplegia formulation over other variants. While this allows clinicians to pick and choose the studies that support their preference, it speaks to the larger need for a change within the field of clinical research. If cardioplegia formulations continue to be developed and adopted because a clinician thought it would work, it

worked, and the institutional review board process is too tedious to justify prospective human studies, a disservice is being done to patients that could have been better served by the development of an “ideal” cardioplegia formulation for their specific physiological needs. While it will be ethically challenging to both protect human subjects and remove the associated bureaucratic red tape, operative changes continue to be made under the guise of surgical innovation, and the opportunities to gain valuable knowledge from these changes and advance the field of cardiac surgery are being missed.

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Appendix A – Ascension Columbia St. Mary's Micro del Nido Protocol

Ascension Milwaukee Wi: Micro Del Nido Protocol with Quest MPS

Additive Cassette:
Micro Del Nido consists of the following
Premixed in a 60ml syringe:

Mannitol 20% (use filter needle)	16.3mL
Sodium Bicarbonate 8.4%	13mL
Magnesium Sulfate 4.06 mEq/mL	4mL
Lidocaine 2% (preservative free)	6.5mL
Total	39.8mL

Arrest Cassette:
KCl - concentration of 2mEq/ml
Load cassette with 40mEq(20ml) of KCl. With the MPS arrest setting at 24mEq, 24mEq of KCl will be given for every 1000ml of blood cardioplegia delivered.

General Guidelines:
Target Delivery Pressures:
Aortic Root Pressure: 200-250 mmHg
MPS System Pressure for Antegrade Delivery: 250-300mmHg
Retrograde/coronary sinus Pressure: 35-45 mmHg
MPS system Pressure for Ostial Delivery: 150mmHg

- **Initial Dose: Cold, Antegrade Dose**
  - Additive Setting: 32 ml/L. Arrest Setting: 24mEq/L
  - Dose: 1500ml typically given antegrade. If using retrograde for initial arrest, deliver 1000ml Antegrade and 500ml Retrograde.

- **Maintenance Doses:**
  - Notify the surgeon at 30 min. Remind every 15 min. thereafter. Redose at or before 60min.
  - Additive Setting: 32 ml/L. Arrest Setting: 7-8mEq/L
  - 400 ml Cold
  - If K is greater than 5.5mmol/L, arrest settings should be 2 for the next dose.

- **Hot Shot:**
  - Surgeon dependent.
  - Set warm temp to 36 C (warm cardioplegia several minutes before cross clamp removal)
  - A minimum of 4 ml of additive in the cassette is required for hot shot.
  - Deliver 400 ml volume total.
  - Arrest setting: 0 mEq/L
  - Additive setting: 0 mEq/L

Updated: 5/17/2023

Appendix B – Preliminary Study Protocol

1. PROJECT TITLE

A Comparison of the Efficacy of 1:4 del Nido and Micro del Nido in Single Valve Repair or Replacement Surgery: A Single-Center Retrospective Analysis

2. OBJECTIVES OF THE PROJECT

During cardiac valve repair and replacement surgeries, cardioplegia is used to provide a quiet, bloodless field for the surgeon to operate. Using high dose potassium and varying additives, cardioplegia arrests the heart and protects it from ischemic injury during the arrest period. In 2015, surgeons at Columbia St. Mary's Hospital in Milwaukee began using 1:4 (blood:crystalloid) del Nido in valve surgeries as their preferred cardioplegia formulation. In 2019, the surgeons at this hospital transitioned to micro del Nido in these same types of valve surgeries. Micro del Nido contains the same concentrations of arrest agent and additives as 1:4 del Nido but uses 100% whole blood from the cardiopulmonary bypass (CPB) in place of crystalloid (generally PlasmaLyte).

In order to assess and compare the efficacy of 1:4 del Nido and micro del Nido, confounding factors can be reduced by doing a single-center retrospective study where a surgeon or surgeons transitioned between given cardioplegia formulations. The goal of this study is to assess whether there is a significant difference in clinical outcomes following valve repair or replacement surgery between the two aforementioned cardioplegia formulations. The assumed hypothesis is that there are no significant differences in clinical outcomes. Patient demographics that will be compared include age, gender, BMI, hypertension diagnosis, and diabetes. Clinical outcomes/operative data that will be analyzed include pre- and postoperative ejection fraction, pre- and postoperative hemoglobin and hematocrit, cardioplegia type, valve surgery type, CPB time, cross-clamp time, hemoconcentrator use, postoperative inotropic support, postoperative balloon pump insertion, blood transfusions, AKI development, time to extubation, length of ICU stay, and length of hospital stay.

If no significant outcomes are found or if there is found to be a benefit to micro del Nido, this study will likely have no impact on policy other than to confirm that the transition to micro del Nido was safe and effective. If there is found to be a significant difference or differences in favor of 1:4 del Nido, this study has the potential to inform policy and guide a transition back to 1:4 del Nido for valve repair and replacement surgeries. The decision to transition back to 1:4 del Nido will ultimately be a joint decision between the surgeons and perfusionists, aided by this study.

3. **BACKGROUND AND SIGNIFICANCE**

One mainstay of the cardioplegia debate is whether crystalloid cardioplegia, blood cardioplegia (i.e., microplegia), or a combination of crystalloid and blood is superior. Crystalloid cardioplegia adds the arrest agent and additives to a crystalloid base to be delivered at hypothermic temperatures into the coronary circulation for cardiac arrest. Microplegia is generally also delivered at hypothermic temperatures and can contain arrest agents and additives similar to crystalloid cardioplegia but replaces the crystalloid base with whole blood from the cardiopulmonary bypass circuit. Cardioplegia formulations with a crystalloid base have been historically used since the development of cardioplegia, while microplegia is a more recent development. Proponents of both cardioplegia types offer arguments for their cardioplegia's superiority, but continued studies must be done to eliminate confounding factors and isolate the debate between microplegia and crystalloid from secondary debates about delivery methods, arrest agents, and additives.

One prominent example of a cardioplegia formulation that primarily possesses a crystalloid base is 1:4 del Nido, which is delivered at a ratio of one part blood to four parts crystalloid. The crystalloid, PlasmaLyte, is supplemented with potassium chloride to provide cardiac arrest, as well as additional additives – lidocaine, mannitol, sodium bicarbonate, and magnesium sulfate. In recent years, to reduce the perceived negative effects of crystalloid-based cardioplegia formulations, Micro del Nido was developed. Micro del Nido possesses near identical drug concentrations as 1:4 del Nido but uses a specialized cardioplegia delivery system to inject the arrest agent and additives directly into whole blood from the cardiopulmonary bypass circuit for delivery to the coronary circulation.

Although both methods of cardioplegia have been used in the Milwaukee area and appear to be safe and effective, limited studies exist about Micro del Nido cardioplegia's overall performance in comparison to 1:4 del Nido. Ascension Columbia St. Mary's began using 1:4 del Nido in 2015 for their single valve repair or replacement surgeries in 2015 before transitioning to Micro del Nido for these same types of surgeries in 2019. A retrospective study at this facility therefore serves two purposes: (1) to assess the efficacy and safety of Micro del Nido and (2) to compare the efficacy of a blood-based cardioplegia to crystalloid-based cardioplegia without the confounding factor of varying additive formulations. Additionally, confounding factors like surgeon, facility, and procedure can be reduced or eliminated. Therefore, this project aims to discuss myocardial injury and protection strategies and compare the safety and efficacy of 1:4 del Nido and Micro del Nido following valve repair or replacement surgeries at Ascension Columbia St. Mary's in Milwaukee, WI.

Investigators' prior experience within the debate between 1:4 del Nido and micro del Nido is limited, other than anecdotal evidence based on personal experiences with the aforementioned formulations. While the investigators possess a clear understanding of the mechanisms of action of cardioplegia and cardioplegia additives, a study as described would look to close gaps in current knowledge as to whether microplegia formulas are superior or beneficial over crystalloid-based formulas. Relevant preliminary data is purely anecdotal – no investigations or studies have been conducted previously at Columbia St. Mary's. Two recent studies exist that found either no significant benefit or minor benefit to micro del Nido over modified del Nido in coronary artery bypass graft surgery. Modified del Nido is only commonly used on valve repair or replacement surgeries at St. Mary's in Milwaukee so there is benefit in reviewing and analyzing St. Mary's data [1, 2].

4. PROJECT METHODS

This study will be retrospective in nature and will assess data from January 1, 2015, through the present (September 31, 2023). To assess a patient's ability to be included in this study, paper records (past and current method of charting done by perfusionists at Columbia St. Mary's) will be reviewed and patients who underwent single valve repair or replacement surgery will be identified.

Once study subjects are identified through paper charts, their Epic charts will be accessed by the investigators utilizing patient MRN numbers from the paper charts, and necessary data will be recorded in "del Nido Study – Data Collection.xlsx". The demographics and outcomes recorded in the aforementioned spreadsheet directly correlate to the defined objectives of this study.

The data recorded will be recorded and maintained on the Ascension server with the PI – Brittany Finger. Again, no PHI will be recorded, and Matthew will be working under the direct supervision of Ascension employees to ensure compliance. The data will be maintained for 3 years after the completion of this study (June 1, 2024). Brittany will delete the data from the file location at that time. The data will not be disclosed to anyone outside of Ascension other than Matthew, but the statistical analysis will be presented as part of the defense of his thesis to his proctors at Milwaukee School of Engineering (MSOE). No hard copy documents will be made during the acquisition of this data. Paper charts that will be accessed are stored in the perfusion room at Columbia St. Mary's, where they will stay throughout the duration of this project. These charts will not be destroyed at the completion of this project; they will instead be destroyed per their retention policy.

The project data variables can be found in the Excel spreadsheet found within the IRB project called "del Nido Study – Data Collection.xlsx". The measurements are also available within that spreadsheet. "Post-op inotrope support" will be defined as patients who receive inotropic support from the time they leave the operating room till 48 hours after leaving the operating room. The only other data variables that may need clarification are LOS and age. These two variables will be computed when reviewing the medical record to prevent the need to record PHI, specifically admission date, discharge date, surgery date, and DOB.

5. STATISTICAL ANALYSIS

Any missing detail will depend on the extent of missing data: (1) if an entire category of data is unattainable, the category will be removed and the study will continue without accessing that outcome and (2) if data is missing for just one patient, the patient will be removed from the study. Outliers will not pose an issue as medians for the data will be compared rather than means.

Obtained data will be assessed for normality using the Anderson-Darling normality test. If data is normal, it will be compared between the 1:4 del Nido group and the micro del Nido group using the unpaired T-Test. If data is not normal as assessed by the Anderson-Darling test, the data will be compared between the two groups using the Mann-Whitney U-Test as the data is ordinal. A p-value of less than 0.05 will be considered significant for all tests.

In this study, given its retrospective nature, confounding factors are inherently reduced by the project proposal to do a single-center study where surgeons and their outcomes will only be compared to themselves. As a consequence of the retrospective nature, any additional confounding factors will not be able to be controlled – the hope is that they have been minimized to the furthest extent possible though.

6. PRESENTATION OF RESULTS

This data is only planned to be presented by Matthew Drexler for the defense of his thesis as this project is of his design.

7. RESULTS INFORMING/INSTRUCTING OTHER PRACTICE SETTINGS?

We do not anticipate the results of our project being used to instruct or inform practice settings outside of our local Ascension Ministry.

8. LONG-TERM PLANS

There are no long-term plans for quality improvements. If the analysis comes back in favor of 1:4 del Nido, the decision to make alterations to procedure will ultimately be the joint decision of perfusionists and surgeons.

9. REFERENCES

[1] A. Haider *et al.*, "Efficacy of Whole-Blood Del Nido Cardioplegia Compared with Diluted Del Nido Cardioplegia in Coronary Artery Bypass Grafting: A Retrospective Monocentric Analysis of Pakistan," *Medicina*, vol. 57, no. 9, Art. no. 9, Sep. 2021, doi: 10.3390/medicina57090918.

[2] K. Karaarslan and I. Erdinc, "Is Modified Del Nido Cardioplegia as Effective as Del Nido Cardioplegia in Patients With Isolated Coronary Artery Bypass Surgery?," *Heart Surg Forum*, vol. 25, no. 1, pp. E163–E167, Feb. 2022, doi: 10.1532/hsf.4491.

Note: Matthew Drexler, while not an employee of Ascension, is an actively rotating student at Ascension Columbia St. Mary's and has signed necessary NDA disclosures.

Appendix C – Data Collection Forms

1:4 del Nido Data

Note: Micro del Nido Data can be found on the "Micro del Nido" Sheet

	Pre-Op Characteristics							Operative Data					Post-Op Characteristics						
Subject	Age	Gender	BMI	HTN	DM	EF	HGB	HCT	Valve	CPB	XC	UF	Blood	EF	HGB	HCT	Inotrope	IABP	AKI	EXT	ICU	LOS
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Age = Patient Age (Years) - computed when reviewing patient's medical record so PHI (i.e., DOB) is not recorded.
Gender = Patient Gender (0 = Male, 1 = Female)
BMI = Body Mass Index (Kilograms/m^2)
HTN = Hypertension (0 = No, 1 = Yes)
DM = Type 2 Diabetes Mellitus (0 = No, 1 = Yes)
EF = Ejection Fraction (%)
HGB = Hemoglobin (g/dL)
HCT = Hematocrit (%)
Valve = Valve Type (MVr = Mitral Valve Repair, MVR = Mitral Valve Replacement, AVR = Aortic Valve Replacement, TVR = Tricuspid Valve Replacement)
CBP = Cardiopulmonary Bypass Time (Minutes)
XC = Cross-Clamp Time (Minutes)
UF = Ultrafiltration Used (0 = No, 1 = Yes)
Blood = Blood Transfusions (0 = No, 1 = Yes)
Inotrope = Post-Op Inotropic Support (0 = No, 1 = Yes)
IABP = Post-Op Balloon Pump Insertion (0 = No, 1 = Yes)
AKI = Post-Op Acute Kidney Injury (0 = No, 1 = Yes)
EXT = Time to Extubation (Hours)
ICU = Length of ICU Stay (Hours)
LOS = Length of Hospital Stay (Days) - computed when reviewing patient's medical record so PHI (i.e., admission date, surgery date, and discharge date) is not recorded.

Micro del Nido Data

Note: 1:4 del Nido Data can be found on the "1 to 4 del Nido" Sheet

	Pre-Op Characteristics								Operative Data					Post-Op Characteristics								
Subject	Age	Gender	BMI	HTN	DM	EF	HGB	HCT	Valve	CPB	XC	UF	Blood	EF	HGB	HCT	Inotrope	IABP	AKI	EXT	ICU	LOS
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Appendix D – Collected Data

Micro del Nido Data

Note: 1:4 del Nido Data can be found on the "1 to 4 del Nido" Sheet

	Pre-Op Characteristics							Operative Data						Post-Op Characteristics						
Subject	**Age**	**Gender**	**BMI**	**HTN**	**DM**	**EF**	**HGB**	**HCT**	**Valve**	**CPB**	**XC**	**UF**	**Blood**	**EF**	**HGB**	**HCT**	**Inotrope**	**IABP**	**AKI**	**EXT**	**ICU**	**LOS**
1	62	0	28.8	1	0	64	12.2	36	AVR	133	120	0	0	51	10.2	30	1	0	0	1.83	125.86	5
2	68	0	33.7	1	0	36	12.9	38	MVR	73	60	0	0	35	8.5	25	1	0	0	2.45	77.83	5
3	63	0	37.9	0	0	45	13.3	39	AVR	120	99	0	0	47	11.2	33	0	0	1	3.33	106.75	6
4	68	0	30.2	1	0	62	12.2	36	AVR	82	72	0	0	?	9.9	29	1	0	0	3.12	123.63	5
5	57	1	27.3	1	0	46	10.9	32	AVR	73	56	0	0	52	8.5	25	1	0	0	3.56	47.00	4
6	77	0	25.2	1	0	60	9.9	29	MVR	86	67	0	0	53	8.2	24	0	0	0	2.75	99.75	4
7	59	0	35.9	1	0	60	12.2	36	AVR	95	85	0	0	?	10.2	30	0	0	0	1.60	34.70	3
8	62	0	29.6	1	0	52	11.9	35	AVR	101	93	0	0	?	9.2	27	0	0	0	1.87	55.92	5
9	55	0	50.4	1	0	60	12.6	37	AVR	116	98	0	0	58	11.6	34	0	0	0	22.03	97.27	5
10	54	1	33.7	1	0	58	9.9	29	AVR	108	97	0	0	58	8.8	26	0	0	0	19.43	25.90	4
11	60	1	41.7	0	0	59	8.5	25	AVR	101	91	0	1	62	9.2	27	1	0	0	5.13	43.68	5
12	54	0	31.0	1	0	56	12.9	38	AVR	134	120	0	0	56	11.9	35	1	0	1	5.13	74.75	3
13	55	0	27.9	0	0	45	11.9	35	AVR	104	94	0	0	60	10.2	30	0	0	0	5.85	58.25	3
14	67	1	29.7	1	1	57	12.2	36	MVR	76	62	0	1	57	8.8	26	0	0	0	23.12	170.72	8
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Perfusion Thesis Approval Form

Master of Science in Perfusion -- MSP

Milwaukee School of Engineering

This thesis, entitled "A Comparison of the Efficacy of 1:4 del Nido Cardioplegia and Micro del Nido Cardioplegia in Adult Single Valve Repair or Replacement Surgery: A Single-Center Retrospective Analysis" submitted by Matthew C. Drexler, has been approved by the following committee:

Faculty Advisor: Ron Gerrits Ron Gerrits (Dec 6, 2023 19:57 CST) Date: Dec 6, 2023

Dr. Ron Gerrits, Ph.D.

Faculty Member: Brittany Finger Brittany Finger (Dec 7, 2023 09:45 CST) Date: Dec 7, 2023

Brittany Finger, MSP, CCP

Faculty Member: Katelyn Herrmann, CCP Katelyn Herrmann, CCP (Dec 6, 2023 19:39 CST) Date: Dec 6, 2023

Katelyn Herrmann, MSP, CCP