

Review Article

Perfusion Preservation of the Donor Heart: Basic Science to Pre-Clinical

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Abstract: As a consequence of technology improvements and refinement, perfusion of the donor heart has moved from the research laboratory to clinical studies. Multiple investigators are currently leading pre-clinical trials of devices using perfusion preservation, and one device is now in European clinical trials. One major problem with the donor heart is the high metabolism relative to other organs, and depletion of ATP leads

rapidly to acidosis and necrosis of the myocardium. Two techniques in development to address the issue are normothermic and hypothermic perfusion. This review examines the current issues regarding donor heart preservation and techniques of pre-clinical evaluation necessary for regulatory approval. **Keywords:** cardiac transplant, donor heart, myocardial protection. *JECT. 2009;41:140–148*

Adequate donor heart preservation remains a difficult problem in cardiac transplantation. Immediately after cardiectomy, the donor heart undergoes necrosis from acidosis despite hypothermic conditions (Figure 1). Many techniques to prolong the preservation have been developed and tested; however, large-scale pre-clinical tests are limited by the need for specialized equipment and high cost to carry out clinical studies. Interestingly, in 1967, hypothermic donor heart perfusion was used in the first heart transplant by Christiaan Barnard at the Groote Schuur Hospital in South Africa (1). The first truly portable device for donor heart preservation was used by Wicomb et al. (2,3) in a series of experiments in the porcine model and subsequently in four human heart transplants in 1981. Although decades of research justify machine perfusion of the donor heart, only now is a strong effort being made to develop a clinically viable and portable preservation system (4,5).

There is no single problem of the donor heart preservation; rather, there are multiple inter-related pathophysiologic changes originating at the time of cardiectomy from global myocardial ischemia and hypoperfusion. The high metabolism of the heart leads to acidosis, necrosis, and edema. Thus, normothermic or hypothermic donor heart preservation techniques are directed to minimize the detrimental changes and prevent formation of edema. Evaluation of the donor heart quality after perfusion is important, and analytical techniques are described in detail below. For completeness, additional discussion regarding risk and benefit analysis of donor heart perfusion and pathway through regulatory approval is included in this review.

Myocardial Metabolism and Acidosis

Immediately after cardiectomy, the heart becomes ischemic, and ATP is depleted in catabolic action. As a product of ATP catabolism, adenine nucleotide metabolites such as adenosine, inosine, hypoxanthine, and xanthine are generated. These dephosphorylated purines permeate through the cell membrane, and without their prompt replenishment into the Cori cycle, the electron transport chain fails, causing lactic acidosis and cell necrosis (6). Hypoxia causes a switch from oxidative metabolism to glycolytic energy

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Dr. Rivard has a financial relationship with a healthcare-related entity whose product is discussed in this manuscript.

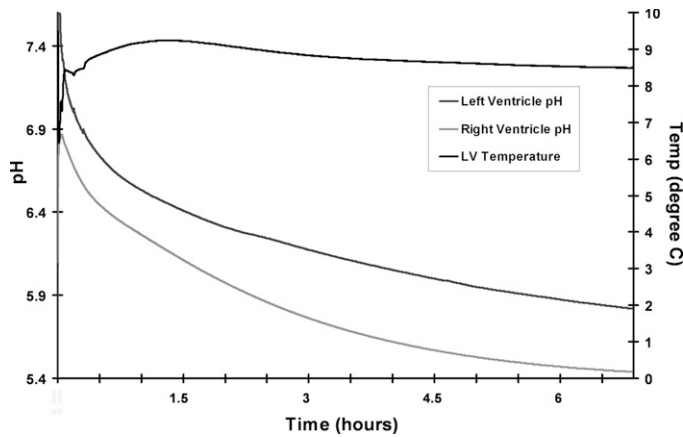


Figure 1. A rapid exponential decrease in pH occurs immediately after explantation of the donor heart. Approximately 50% of the pH drop occurs in the first 40 minutes from continuous production of lactic acid.

generation, leading to a 10-fold increased glucose consumption and a rapid production of lactic acid (7).

A seminal study by Graham et al. (8) showed that even in the presence of glucose and oxygen, pH was the determining factor in cell survival. The group showed that massive cell death occurs in cultured myocytes when the pH falls below 6.5. On the other hand, myocyte survival in cell cultures and the donor heart is directly related to the removal of lactic acid. The decrease in pH from lactic acidosis and subsequent cell death is halted by cell washing or organ perfusion, respectively (8,10,11).

Myocytes are not the only cell type to be affected by acidosis. Using endothelial cell cultures, Bemanesh and Kempski (12) showed that maximal cell swelling occurred when extracellular dropped to pH = 6.0. Physiologically, the endothelial cell swelling affects myocardial perfusion likely from a reduction in capillary diameter. Supporting this concept, a study by Rivard et al. (13) showed that prolonged hypothermic storage causes a significant decrease in microvascular perfusion occurring in the acidotic donor heart.

Cardiac function is dramatically affected by lactic acidosis. Kumbhani et al. (14) proved in a large clinical trial that a pH of <6.3 is independently associated with twice as many deaths after cardiac surgery. Furthermore, a myocardial pH of 6.3 has 50% less ATP than myocardium with a normal pH of 7.2, which may explain the poor outcomes (9). Because of their study results; Kumbhani et al. now recommends that myocardial pH be maintained above 6.5 by intermittent reperfusion in cardiac surgical procedures.

Myocardial Necrosis

As ATP is depleted during prolonged ischemia, cellular necrosis ensues from the loss of trans-membrane ion gradients. Without ATP, outer cellular membrane pumps such as the Na⁺/K⁺ ATPase are paralyzed and failure of those

pumps allows Na⁺ to accumulate in the cytoplasm, causing increased cell osmolarity and phospholipid breakdown. Water follows the osmotic gradient and causes the cell membranes to swell and rupture. This is best represented by electron microscopy as nuclear blebbing and mitochondrial matrix disruption.

ATP is the primary energy source used by the sarcoplasmic reticulum to sequester Ca²⁺ through the Ca²⁺/ATPase membrane pump. Depletion of ATP leads to the release of the sequestered Ca²⁺ that binds to troponin-C, causing cross-bridge cycling of the myofilaments (muscle contraction). The unopposed sarcomere activation leads to contraction bands and Z-line disruption. In addition, uncontrolled release of calcium ions into the cytoplasm activates intracellular lipases such as phospholipase A₂ and lysophospholipids that subsequently initiates cell membrane lysis (15).

With a decrease in intracellular energy, there is a concomitant increase in intracellular calcium, which activates a cascade of enzymes culminating in the formation of superoxide anion (–O•), which oxidizes critical cellular enzymes, nucleic acids, and cellular membranes. Superoxide anion is linked to endothelial injury and is a known major contributor in ischemic-related vascular dysfunction (16,17).

Myocardial Edema

A problem with donor heart preservation is the formation of myocardial edema that causes left ventricular wall thickening and stiffness, characterized by pathologists and surgeons alike as a “stone heart.” Edema occurs whether or not the heart receives coronary artery perfusion during cold storage; however, it is thought to be exacerbated by higher flow rates (18). Myocardial edema occurs from two mechanisms. The first focuses on the generalized nutrient and energy depletion causing intracellular edema. The intracellular edema is directly related to the process of necrosis and likely has a minor overall contribution to myocardial stiffness. The second mechanism of myocardial edema is from the lack of lymphatic removal. The myocardium has an abundant supply of lymphatics that drain interstitial fluid from the subendocardium to the subepicardium during each contraction. The subepicardial lymphatics drain into the coronary lymphatics and eventually into the cardiac lymph node between the innominate artery and superior vena cava (19).

Myocardial edema is exacerbated by factors that increase microvascular permeability, a decrease in colloid oncotic pressure, prolonged diastole, or an increase in coronary perfusion pressure. Crystalloid cardioplegia is associated with significant accumulation of edema (20). In an attempt to prevent the problem of edema formation, impermeable solutes have been added to some cardioplegic solutions such as mannitol, albumen, or lactobionate. Also a coronary perfusion pressure >50 mmHg causes a rapid accumulation of

edema during asystole, causing subsequent poor left ventricular contractility (21–23). Interestingly, using mannitol and starch fortified kidney preservation solution (KPS-1), continuous preservation at 15 mmHg for 24 hours using a modified Lifeport kidney transporter (Organ Recovery Systems, Chicago, IL) results in an 18–30% wet weight gain (24,25).

Edema within the myocardium is commonly determined by lyophilization. Lyophilization is the process of drying samples and can be used with sample weighing to determine the total moisture content (edema). The first step is to determine the “wet weight” and then the “dry weight” of the myocardium after drying. Lyophilization requires that all the water is removed from the sample—which is easiest and least expensive by oven heating—but also can be assisted by vacuum, a moisture balance, or chemical drying. The temperature must be above the boiling point of water (100°C) and not too high to decompose organic components (180°C). Finally, two weighings of the dried sample (at separate time points) are necessary to evaluate for weight stability. The typical water weight of hearts perfused for 4 hours using the LifeCradle (Organ Transport Systems, Frisco, TX) is close to 79% (26,27).

DONOR HEART PRESERVATION TECHNIQUES

Three commercially viable methods have evolved for donor heart preservation (Table 1). These include two

Table 1. A systematic review of English language Medline literature from 1966 to the 2008 used the key words donor, heart, and perfusion.

	Number of Studies
Animal model	
Canine	17
Porcine	11
Baboon	3
Rabbit	3
Perfusion method	
Oxygenated	19
UW	5
Blood	4
Perfusion	3
Cardioplegia	
Continuous	28
Intermittent	6
Functional evaluation	
Transplantation	27
Working heart	7
Histologic evaluation	
Light microscopy	12
Electron microscopy	8

Potentially relevant citations were screened, and studies using only a single flush of cardioplegia and duplicate data were excluded, leaving 34 studies. Journal articles were retrieved reviewed either online or in print. The average perfusion pressure was 35 cm H₂O (range, 9–82 cm H₂O; *n* = 19) and temperature of 11°C (range, 1–21°C; *n* = 26) for a preservation time between 3 and 24 hours. Functional data obtained included HR, CVP, LVEDP, CO, and dP/dt. Most studies used some type of a biochemical method (*n* = 26) for evaluation including % water weight, lactate, ATP, or pNMR.

hypothermic methods, oxygenated and non-oxygenated, using two perfusion types: continuous and intermittent. The third method is a derivation of the Langendorff preparation using a warm blood perfusion and an isolated working heart.

Oxygenated hypothermic continuous donor heart perfusion is used by groups led by Rosenbaum et al. (27) (LifeCradle) and Poston et al. (25) (LifePort). Each investigator has had success in the pre-clinical transplantation studies. Both devices use a membrane oxygenator and compressed oxygen to provide elevated oxygen tension within the cardioplegia solution, which is re-circulated through a cannula secured to the aortic root. The LifeCradle uses a thermoelectric Peltier type device to maintain hypothermia, whereas the LifePort uses ice as the cooling medium. Rivard et al. (13) have used a simple portable approach (Asporto) without oxygenation using intermittently pumped non-re-circulated cardioplegia, also cooled with a Peltier device.

Transmedics, lead by Dr. Hassanein, has developed the Organ Care System (formerly the Portable Organ Preservation System) (28). The device has had success in clinical trials in Germany and received CE approval for European use. The device is based on the Langendorff method whereby the heart is perfused with a normothermic oxygenated blood-based mixture enhanced with various metabolites. The donor heart is placed into a chamber; connected to arterial and venous cannulae, EKG leads, as well as an external pacemaker. The heart is continuously monitored by sensors for cardiac output, heart rate, temperature, pressure, and lactic acid production by an advanced (wireless) microprocessor system.

ANALYTICAL METHODS

Multiple methods are available for the analysis of the donor heart. In general, functional evaluation precedes histologic evaluation. The latter exam involves a biopsy of the heart and can interfere with functional evaluations. A biopsy of the right heart can be made using a percutaneous approach through the right jugular vein with a bioprobe inserted into the right ventricle, or alternatively, surgical core biopsies of the myocardium are possible after sternotomy or thoracotomy. Functional evaluation of the heart is dependent on arterial and venous catheterization for pressure monitoring and may be obtained in conjunction with an imaging exam [sonography, computed tomography (CT), magnetic resonance imaging (MRI), or nuclear] for anatomic measurements.

Isolated Heart Preparations

The most frequently used methods to functionally examine the heart are Langendorff perfusion and the isolated working heart model. In 1895, Oscar Langendorff developed a method whereby the aorta is cannulated and blood

flows into the coronary arteries and arterial and venous capillaries and out the coronary sinus (29). The heart beats in sinus rhythm, but the atrial and ventricular chambers are without pre-load. In contrast, a four-chamber isolated working heart simulated the normal physiologic systemic and pulmonary flow in a human (30).

The isolated working heart setup is considered ideal for the study of pressure and flow in the aorta as well as the left and right ventricles and can provide data similar to that obtained with transplantation (Table 2). The perfusate of the isolated heart has a direct effect on the contractility of the donor heart. Saline-based perfusate compounds typically lack effective osmotic colloids, which results in poor long-term function and profound interstitial edema formation. Whole blood is the optimal perfusate; however, it is prone to clotting and hemolysis, necessitating anticoagulation and filtration.

Transplantation Models

Lower and Shumway's (31) original work in 1960 involving orthotopic transplantation in the canine model paved the way for future success with clinical heart transplantation. Orthotopic transplantation is the gold standard for the study of cardiac transplantation and is technically feasible with cardiopulmonary bypass circuits in the canine, porcine, and non-human primate animal models. The major disadvantage of orthotopic transplantation is the surgical knowledge, immunosuppression, and post-operative care found typically only at a limited number of university surgical laboratories. With appropriate instrumentation, transplantation studies can provide important data regarding donor heart performance including left and right ventricular pressures, heart rate, cardiac output, and blood flow.

The goal in transplantation studies is to definitively show normal cardiac function—ideally improved from the current standard of preserving the donor heart. The problem of pre-clinical transplantation studies are twofold: (i) the numerous post-transplant complications and (ii) the high cost to complete a well-designed study. The combination clearly is responsible for the lack of progress in the last 50 years, and the ice-filled insulated container still is the most widely used method to transport the donor heart.

Table 2. Calculated functional evaluation parameters of the working or transplanted donor heart.

EDV-ESV =	SV (mL)
SV/EDV =	EF (%)
SV × HR =	CO (mL/min)
EDV index =	EDV/BSA (l/m ²)
ESV index =	ESV/BSA (mL/m ²)
CO/BSA =	CI (l/min/m ²)

BSA, body surface area; CI, cardiac index; CO, cardiac output; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; HR, heart rate; SV, stroke volume.

In the early days of transplantation research, studies with 10–20 animals in each cohort were done comparing perfusion vs. no perfusion. Nowadays, most studies have 5–10 animals in each cohort (32–35). Of those survival studies, the strongest include not only post-transplant functional data but also pathological data. Interestingly, some of the early transplantation studies reported only morbidity and mortality with limited or no functional data. A complete pathologic evaluation is necessary to exclude complications related to rejection or technical reasons. The evaluation transplanted donor heart should include functional and anatomic measurements, preferably with a baseline measurement before transplantation. For example, an echocardiogram is done of the donor before explanation and repeated in the recipient after transplantation. Similarly, pressure measurements of the recipient can be made before transplantation and afterward with routine catheterization.

Comparative Anatomy

Whether or not the donor heart is studied ex vivo using an isolated setup or transplanted in an animal, the anatomic differences in the animal model can affect the study outcomes. Because studies can be very expensive to complete, a thorough understanding of the different animal models is important to ensure the hypothesis is not negatively influenced by the “human surrogate.” Most frequently, the canine or porcine animal model is used in donor heart studies.

The canine heart has an extensive collateral circulation connecting the left and right coronary circulation. This collateral circulation in the dog may protect marginal areas of the heart from ischemia. Furthermore, the dog's relatively large thorax and mediastinum allow for clear visualization of the heart and great vessels. The canine model for heart transplantation is generally considered the most appropriate and thus is the most frequently used pre-clinical model.

The porcine heart has few collateral vessels, and an end-artery coronary anatomy predominates, making it similar to human coronary circulation. Cannulation for cardiopulmonary bypass is difficult because of the short length of the great vessels and friable right atrial tissue (36). Unfortunately, pigs are prone to inter-operative arrhythmias, post-operative wound infections, and are sensitive creatures who are easily startled. The swine model can be used for evaluation of donor heart preservation and is appropriate for acute or short-term survival studies (37).

Heart transplantation using the primate model has had a long history; however, there is a difficulty with handling, and experienced veterinary care is a prerequisite (38,39). Specialized care facilities are necessary, and pressure from animal care groups has made primate studies difficult to initiate. Non-human primates are also susceptible to zoonoses such as *Mycobacterium tuberculosis* and are susceptible to

gastroenteritis and bacteremia after surgery. Furthermore, handling of the baboon typically requires sedation. The baboon heart differs from humans; the baboon heart has only two aortic arch vessels. However, the primate heart is less friable and arrhythmogenic than the porcine heart. Of course, the phylogenetic similarities of primates and humans have been extensively studied.

Donor Heart Perfusion

The human heart requires 5% of the cardiac output to maintain normal cardiac function. The coronary blood flow in the resting state is ~250 mL/min (50–150 mL/min/100 g). The arrested asystolic heart (electromechanical dissociation) uses as little as 10% of the energy requirements as a normally functioning heart (40). Furthermore, with mild hypothermia (27°C), the heart consumes only 50% of the energy of an arrested heart. Feemster (41) showed an improvement in end diastolic pressures and ATP levels with moderate hypothermia (12°C) over the standard ice-chest hypothermia (0–4°C). The optimal temperature of the donor heart preservation remains unclear; however, the use of hypothermia allowing a low level of metabolism has been the scientific basis donor heart preservation since the 1960s (41).

There has been an active ongoing debate whether or not warm blood cardioplegic perfusion conveys a benefit during off and on cardiac bypass pump surgical procedures (42,43). Multiple randomized controlled trials examining the various combinations have definitively shown that blood cardioplegia is optimal over crystalloid-based solutions (44). Landymore et al. (40) and Biagioli et al. (45) showed that carefully administered warm blood either continuously or intermittent can be safely used. However, the problem is that interruption of warm blood cardioplegia rapidly leads to warm ischemia, which is essentially the precursor to myocardial infarction (46,47).

A minimum aortic pressure is necessary for satisfactory valve leaflet coaptation during machine perfusion, which is >10 mL/min/100 g (18). A closed aortic valve closure is critical for appropriate flow into the coronary arteries, and low flow systems are susceptible to valve incompetence. The end result may be variable perfusion gradient in the myocardium from left ventricle (LV) distention from increased subendocardial wall pressure similar to dilated cardiomyopathy. Intermittent cardioplegic administration is one method proposed to provide a physiologic flow pattern to the donor heart facilitating valve closure (48).

Myocardial Blood Flow

Since its introduction in the 1960s, the gold standard for myocardial blood flow measurement is the radio-labeled microsphere. Basically, the invasive technique involves a catheter placed in the left atrium for injection of the microspheres, which essentially flow into the coronary arteries and obstruction of capillary level microvessels. Blood is collected from the femoral artery at a constant rate during the

time the microspheres are injected. The myocardial blood flow is calculated by measuring the counts of radioactivity in a heart sample by the following formula compared with that in the blood aliquot (Figure 2).

Derivations of this standard method have been developed and validated that use colored or fluorescent microspheres (49). Domenech et al. (50) used colored microspheres to examine donor heart perfusion characteristics after preservation using the LifeCradle. Unfortunately the microsphere technique is limited to animals where the entire heart can be examined ex vivo. Another invasive technique to estimate global myocardial blood flow uses the difference of arterial and coronary sinus concentrations during inhalation of inert argon (51). Non-invasive techniques with nitrogen-13 positron emission tomography (PET) using a various tissue compartmental models have been developed that rely on both a nuclear medicine laboratory and an available PET scanner (51). Another widely used invasive method of measuring coronary artery flow is achieved using a small ultrasonic Doppler probe (Transonic Systems, Ithaca, NY) (Table 3).

Cardiac perfusion MRI is a well-established non-invasive clinical imaging modality (52). This type of analysis, originally used by Rivard et al. (53–55) in evaluating the donor heart, can identify regional perfusion abnormalities and can be used to quantify microvascular perfusion (Table 4). This technique, subsequently used by Ozeki et al. (56), showed myocardial perfusion homogeneity of the perfused isolated heart using the LifePort.

Histopathology

Microscopic evaluation of the donor heart is generally done for documentation of the preservation methods' ability to retain normal microscopic structure. The donor heart undergoes specific chronotropic changes visible in both light

$$\text{Myocardial Blood Flow (ml/min/gm)} = \frac{\text{Reference Flow (ml/min)} \times \text{Sample Counts (min}^{-1}\text{)}}{\text{Reference Counts (min}^{-1}\text{)} \times \text{Sample Weight (gm)}}$$

Figure 2. This simple calculation for myocardial blood flow for use with radio-labeled or colored microbead techniques uses a proportional fraction and a known reference flow.

Table 3. Quantitative analysis of right and left ventricular hemodynamic parameters by functional and perfusion MRI.

Global function
Ejection fraction
Myocardial mass
End diastolic volume
End systolic volume
Stroke volume
Cardiac output
Wall motion
Myocardial tagging
Systolic and diastolic motion
Viability imaging
Rest and stress perfusion
Delayed contrast enhancement imaging

Table 4. Techniques to determine cardiac function and perfusion for evaluation of the donor heart preservation.

Modality	Technique
Right and left heart pressures	Millar catheter
Pulmonary capillary wedge pressure	Swan Ganz catheter
Microvascular perfusion	Microbeads SPECT and PET imaging MRI
End systolic and diastolic volume	Balloon tipped catheter Sonocardiography MUGA scan Cardiac CT and MRI

SPECT, single photon emission computed tomography; PET, positron emission tomography, MRI, magnetic resonance imaging; MUGA, multi-gated acquisition scan.

and electron microscopy (Table 5). Histologic discrimination of ischemic-related injury is possible using a combination of staining techniques. The two stains principally used are: hematoxylin-eosin and Luxol fast blue. Both stains can be used to evaluate for extracellular edema, intracytoplasmic vacuoles, contraction bands, and coagulation necrosis. Although electron microscopy is extremely expensive and labor intensive, it is an excellent method to assess myocellular integrity, i.e., contraction bands, separation of myofibrils, myofibril abnormality, nuclear and sarcolemmal membrane disruption, amount of glycogen granules, mitochondrial calcium depositions, chromatin margination within the nucleus, and mitochondrial swelling (57,58).

Gross examination of the heart is also important and includes a visual inspection of the myocardium and great vessels for any areas of injury or abnormalities. Weighing the heart is important to assess for any mass differences between animals and weight change caused by the preservation. The aortic valve is examined for leaflet body appearance and coaptation. The coronary ostia are examined for location. In the early days of transplantation, a donor heart firm to palpation (a stone heart) by the transplant surgeon was uniformly associated with a poor post-transplant prognosis and was attributed to a poor preservation (59).

Biochemical

The use of biochemical methods to evaluate the transplanted donor or isolated heart can augment and provide additional evidence of support of the preservation technique. However, in planning a study of donor heart preservation, the investigator should take into account all the basic science supporting the mechanism for preservation and build on prior methodology rather than exploring new and innovative analytical methods. Except for whole blood preparations, all preservation solutions are inadequate for the preservation of the donor heart and have been extensively studied as intra-operative cardioplegic solutions. The initial studies focused on electrolytes and sugars and quickly moved to examine oncotic substitutes for albumen.

The FDA does not require testing of a specific biochemical marker in pre-clinical applications for clinical use. Many pre-clinical studies have used various analytical methods focused on cellular energy utilization and metabolites (60). Energy sources such as ATP, phosphocreatine, O₂, and glucose all have been measured from biopsies of the donor or isolated heart. Likewise, metabolites such as lactate, myoglobin, troponin, CO₂, and pyruvate have been analyzed directly or indirectly. A major problem with most analytical methods is that a biopsy of the heart must be obtained for tissue. This biopsy typically leaves a substantial defect in the myocardium or is taken from a non-representative location such as the left atrial auricle.

Genetic

The metabolic changes in the donor heart have distinct effects on the myocardial nucleus. The most apparent are the changes after transplantation that likely have an origin at some point during the procurement process and preservation of the donor heart. Multiple studies have examined cardiomyocyte apoptosis after transplantation; however, apoptosis is not identifiable during ongoing hypothermic donor heart preservation (unpublished data). Presumably, this is from a markedly decreased metabolic rate of the myocyte from the low temperature. In their transplant study, Rosenbaum et al. (27) showed that continuous perfusion of the donor heart with the LifePort device significantly reduces apoptosis in the donor heart after reperfusion.

Hypoxia inducible factor (HIF-1 α) is a nuclear transcription factor expressed in response to non-lethal low oxygen levels. The RNA transcription of HIF-1 α activates a broad array of nuclear genes and initiates coding for proteins involved in angiogenesis, energy metabolism, erythropoiesis, cell proliferation and viability, vascular remodeling, and vasomotor responses. As expected, the stresses of donor management and procurement activates HIF-1 α and is associated with long-term graft dysfunction (61).

REGULATORY APPROVAL

The modern approach for European (CE) and United States (FDA) clinical approval of medical devices is based on the claims made and a risk/benefit analysis. The FDA uses two pre-clinical pathways for regulatory approval: a pre-market notification (510k; the 510k is named after the section contained in the Food, Drug, and Cosmetic Act section containing the provisions for clearance of class II medical devices) and a Pre-Market Approval (PMA) application. Any device that proceeds through 510k notification must be "substantially equivalent" to a device on the market before May 28, 1976. A PMA approval process is typically used for devices that are life sustaining or have insufficient information regarding their safety or efficacy. Organ recovery system's kidney transporter is one such

Table 5. Myocardium undergoes a slow process of changes from time of donation or cardiectomy. The process is typified by cellular death resulting in necrosis of the myocardium. Donor hearts that have passed the 4- to 6-hour window of viability have distinct ultra-structural changes associated with an oncotic pathway of degradation.

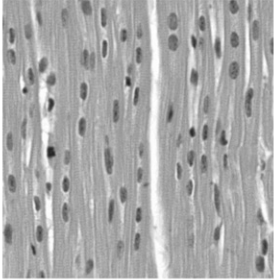
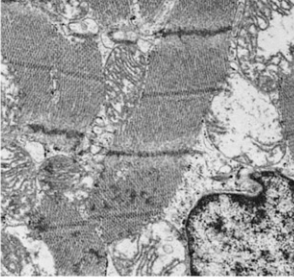
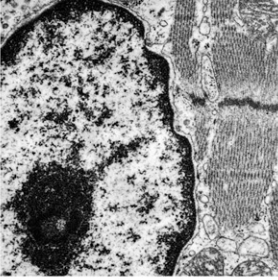
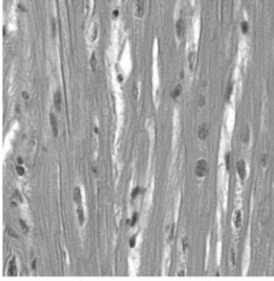
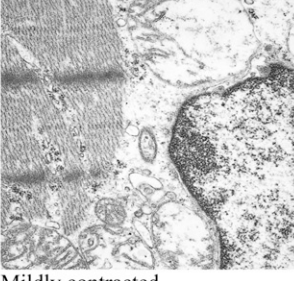
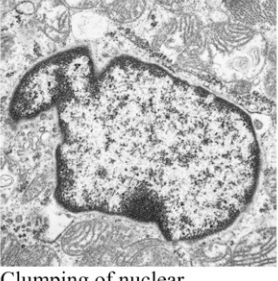
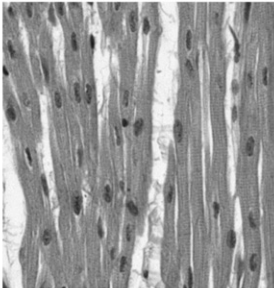
Myofibrils 40x	Sarcomere 15,000x	Nucleus 15,000x	Mitochondria 30,000x
4 Hours  Linear organization Regular cross-striations, dark intercalated discs	 Relaxed Thin Z disk Clear H band	 Nuclear envelope intact Slight dispersion of nucleolar chromatin Clumping of nuclear chromatin	 Intact membrane Mild edema with a few widely spaced cristae
6 Hours  Muscle fibers are not linear with increasing separation due to intracytoplasmic vacuoles and increased extracellular material, due to membrane loss. Moderate contraction band formation	 Mildly contracted Myofilaments visible w/ slight disruptions	 Clumping of nuclear chromatin Irregular nuclear shape	 Intact membrane Obvious edema with widely spaced cristae Cristae still present
9 Hours  Myofibril waviness Extreme contraction band formation and diffuse staining. Plasma membrane loss Prominent extracellular edematous changes	 Highly contracted Separation of H bands Myosin / Actin myofibrillar disruption I band elimination	 Clearing of nuclear chromatin clumping but with some nuclear clearing. Irregular nuclear shape with obvious membrane disruption and nuclear blebbing	 Matrix fragmentation Obvious membrane disruption and blebbing Large amounts of edema with cristae disruption. Some distinct cristae still visible Many flocculent granules

Table 6. FDA device classifications.

Class I	Devices that do not require premarket approval and have only minimal risk associated with their use.
Class II	Devices in which special controls are necessary to mitigate their risk (labeling requirements, mandatory performance standards, and postmarket surveillance).
Class III	Devices in which insufficient information exists to provide reasonable assurance of its safety and effectiveness, i.e., the device is life supporting or life sustaining.

device approved for clinical use through the 510k pathway. On the other hand, Transmedic's Organ Care System is a device proceeding down the PMA pathway.

The European Union grants device approval when a minimum set of regulatory, technical, and safety requirements are met. Conformance to these requirements is validated by an independent organization (a Notified Body) with a Certificate of Conformity. The Council Directive 93/42/EEC of 14 June 1993 concerning medical devices defines the classification of the device based on Article 9 and a series of rules based on the risk listed in the Annex IX.

The FDA considers the insulated container the predicate device for 510k approvals. The insulated container has been the industry standard since the advent of transplantation and until recently has been the only preservation and transport device available for donor organs. From a risk standpoint, any catastrophic failure of a hypothermic preservation process has the advantage of a time-proven preservation method as a backup. On the other hand, normothermic preservation of the organ is novel, and insufficient information currently exists regarding donor heart preservation using this technique. In simple terms, if a warm preservation technique were to fail, the organ remains at a temperature known to cause ischemia and irreversible organ damage. Thus, specific controls are necessary to prevent and mitigate a catastrophic failure (Table 6).

Perfusion preservation of the donor heart provides a unique setting whereby the device functions as a drug delivery device. As a design, the FDA considers both the mechanical device and the cardioplegic solution to be a single device or combination product rather than an analog of a cardioplegia pump. Despite a long history of cardioplegic delivery devices being evaluated in the FDA's Division of Cardiovascular Devices, a perfusion heart preservation device is evaluated in the Division of Gastroenterology and Renal Devices branch because the group has extensive knowledge regarding renal preservation devices.

CONCLUSION

Effective implementation of donor heart preservation devices in clinical trials is dependent on well-structured pre-clinical studies with supportive results. Ideally these studies contain functional and histologic data permitting a direct comparison of different machine preservation

techniques. Despite a long history of research supporting perfusion preservation, the time has come to use the technology to improve cardiac transplant patient's quality of life and survival.

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