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Preserving and evaluating hearts with ex vivo machine perfusion: an avenue to improve early graft performance and expand the donor pool⋆

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Summary

Cardiac transplantation remains the first choice for the surgical treatment of end stage heart failure. An inadequate supply of donor grafts that meet existing criteria has limited the application of this therapy to suitable candidates and increased interest in extended criteria donors. Although cold storage (CS) is a time-tested method for the preservation of hearts during the ex vivo transport interval, its disadvantages are highlighted in hearts from the extended criteria donor. In contrast, transport of high-risk hearts using hypothermic machine perfusion (MP) provides continuous support of aerobic metabolism and ongoing washout of metabolic byproducts. Perhaps more importantly, monitoring the organ's response to this intervention provides insight into the viability of a heart initially deemed as extended criteria. Obviously, ex vivo MP introduces challenges, such as ensuring homogeneous tissue perfusion and avoiding myocardial edema. Though numerous groups have experimented with this technology, the best perfusate and perfusion parameters needed to achieve optimal results remain unclear. In the present review, we outline the benefits of ex vivo MP with particular attention to how the challenges can be addressed in order to achieve the most consistent results in a large animal model of the ideal heart donor. We provide evidence that MP can be used to resuscitate and evaluate hearts from animal and human extended criteria donors, including the non-heart beating donor, which we feel is the most compelling argument for why this technology is likely to impact the donor pool.

Keywords

Non-heart beating donor; Myocardial viability; Machine perfusion; Heart transplantation; Myocardial preservation

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1. Introduction

Despite excellent outcomes for cardiac transplantation in the patients with severe heart failures, the number of cardiac transplantation procedures has reached a plateau over the last several years [1–3]. Currently in the United States, virtually all thoracic allografts are obtained from brain-dead, heart beating (i.e. cadaveric) donors maintained on life support systems. Non-heart beating donors (NHBD) currently represent a novel source of organs that has safely expanded the donor pool outside of cardiac transplantation [4]. In light of myocardial sensitivity to ischemia, the inability to evaluate organ viability or functional status prior to transplant has been a roadblock for the use of hearts from NHBD. Without novel approaches to this challenge, the justifiable concern about primary dysfunction after grafting is likely to continue excluding the NHBD as a source of organs for heart transplantation.

Cold static storage (CS) is a simple, inexpensive, and reliable method for preserving donor hearts for transplantation during the ex vivo transport interval. As a result of generally good outcomes, it is the current standard of care. However, CS is an imperfect method of preservation that is associated with a low-level but persistent anaerobic metabolism within the heart [5]. Primary graft dysfunction, evidenced by the need for significant inotropic or other mechanical support after cardiac transplantation, remains a pervasive problem that occurs in 10–25% of recipients even with the current system of procurement that is limited to donors designated as ideal [6,7]. This fact makes the need for better preservation techniques even more imperative when considering the use of hearts from extended criteria donors that are likely to be at even higher risk for developing primary graft dysfunction.

Continuous machine perfusion (MP) of donor hearts has been proposed as an alternative to CS. Prior studies using ex vivo MP have demonstrated physiologically important support of aerobic metabolism during the transport period [8–10]. A potential advantage of aerobic metabolism, even at low levels, is that it would be expected to maintain cell integrity and vital cell functions better than anaerobic metabolism. This benefit may be most relevant in an organ from an extended criteria donor with less available metabolic reserve to draw from, placing it at higher risk for postoperative dysfunction following an ischemic transport interval [11]. In addition, MP enables homogeneous myocardial cooling through the native coronary circulation and the ongoing washout of metabolic byproducts. A disadvantage of MP has been the development of myocardial edema during the preservation interval, a problem not seen with CS.

The translation of decades of preclinical investigations into the application of MP for clinical heart transplantation has been slow, in part because of the lack of commercial interest in the development of devices for this small market. Only limited case reports of hypothermic MP exist in the literature [12,13]. Recently, a multicenter clinical investigation of MP using continuous, sanguineous perfusion of the donor heart in the beating state has been initiated in Europe and the US. The perfusion apparatus used for this trial has proven to be well designed and effective, but is prohibitively expensive and requires the use of donor blood as the perfusate. In contrast, hypothermic MP allows the heart to remain in diastolic arrest. The difference in metabolism of a heart that is hypothermic and arrested versus warm and beating significantly influences the engineering demands of MP with a more complex device design increasing the risk of malfunction. The risk of organ injury should a device malfunction occur is reduced for hypothermic MP given that the default mode is a nonperfused but cold and arrested organ, the current standard of care. In this review, we will summarize the development of techniques that have achieved the most consistent results and discuss how this technology could be exploited for the safe adoption of hearts from extended criteria and NHBDs.

2. Development of the machine perfusion protocol

Because cold static storage of the heart during ex vivo transport is clinically successful at preserving most hearts, the degree of subclinical injury that occurs as a result of anaerobic metabolism is generally underappreciated. Our group and others [9,14] have observed significant intramyocardial acidosis and depletion of ATP stores in hearts preserved with CS as early as 4 h. While MP provides a means to address this concern, this technology carries additional challenges not encountered with CS. In particular, the optimal perfusate and pump parameters (e.g. pulsatile vs nonpulsatile, low vs high pressure) remain unclear. In addition, myocardial edema, and resulting diastolic dysfunction, has been a significant concern that limits enthusiasm about MP [15]. Using a modification of a device that is currently FDA approved for kidney preservation, our group has developed a protocol to address these challenges and verified our results in a large animal model of the ideal standard heart donor and NHBD.

Unlike donor heart MP using warm sanguinous perfusion, an important objective of cold MP is to maintain full cardiac arrest during the preservation period. Clinically available solutions for creating cardiac arrest include the intracellular type with high potassium concentration (e.g. University of Wisconsin solution) and extracelluar type with lower potassium levels (e.g. Celsior). Importantly, these solutions have been developed for static storage and not perfusion of the heart. Hypothermic MP with donor blood has been reported [16], but is more complicated to perform. Hence, the best perfusate to maintain arrest while still supporting metabolism is unclear. Our own initial preservation studies were performed at a constant temperature of 4–6 °C utilized a modified University of Wisconsin perfusate with a potassium concentration of 115 mEq/l. We found that a majority, but not all of the hearts preserved for 24 h with this MP strategy were able to recover >75% of their baseline left ventricular function [8]. These variable results, consistent with other MP studies utilizing a high potassium perfusate [17,18], were likely the result of inhomogeneous tissue perfusion [10]. High potassium solutions have been documented to create an effective cardiac arrest but are associated with endothelial dysfunction and vasoconstriction during coronary perfusion [9,19,20]. In addition, cold temperatures are known to increase the basal tone of coronary vessels and decrease their response to acetylcholine [21]. Using gadolinium-DPTA enhanced MRI to define tissue perfusion during MP, we established that our initial protocol resulted in inhomogeneous perfusion. First pass imaging demonstrated large areas of myocardium with poor signal intensity while delayed imaging showed contrast enhancement in these areas at later time points, hallmarks of inhomogeneous tissue perfusion [22,23] (Fig. 1A). Using tissue perfusion as the end point, we tested a series of modifications to our protocol with two changes making the most difference in terms of providing for homogeneous tissue perfusion. First, a perfusate solution with a similar overall composition but lower final potassium concentration (5 mEq/l) was adopted. Second, a brief midthermic interval (i.e. 25 °C for 30 min) was added to the protocol. These changes were tested based on studies that have demonstrated that physiologic potassium concentration and midthermic temperatures reduce the tendency for vasospasm and allow for endothelial dependant vasodilation in perfused hearts [24]. With this modified protocol, both homogeneous perfusion and full functional recovery have been consistently observed [10] (Fig. 1B).

Though myocardial edema has long been associated with MP, the impact of myocardial weight gain during preservation is unclear. In our initial studies, hearts preserved with MP showed a five-fold higher degree of weight gain during the preservation interval compared with hearts preserved with CS despite the use of a perfusate with high oncotic pressure [5]. However, this weight gain did not affect myocardial function following restoration of whole blood flow, a finding consistent with other studies [14,15]. While some have suggested intermittent periods of MP during the preservation interval as a means of reducing weight gain [19,25], edema in

the setting of MP is a complex phenomenon affected by hydrostatic pressure created by MP and colloid oncotic pressure of the perfusate. A lack of lymphatic flow in the arrested heart further exacerbates myocardial edema [26]. While multiple different perfusate solutions with differing compositions and oncotic pressures have been described [27], none have significantly reduced myocardial edema in the setting of MP compared with hearts preserved with CS. Edema may also result from delivery of the perfusate to the capillaries using a nonphysiologic pattern of flow. Unlike conditions existing during conventional MP, normal physiologic pressure and flow in the coronary vasculature are not constant but rather show normal variations over the course of the cardiac cycle. Recent studies have mathematically modeled these normal variations in coronary perfusion pressure and have developed software programs that continuously alter perfusion pressure and are thus capable of delivering perfusion in a biologically variable manner [28,29]. Since the addition of physiologic biologically variable perfusion to our preservation protocol, mean weight gain during MP has been reduced from 17% of baseline weight to less than 5% in hearts perfused in a biologically variable manner [30] ($p < 0.05$ on two-tailed *t*-test, Fig. 2).

3. Machine perfusion of standard ideal heart

Although hypothermic arrest significantly reduces myocardial oxygen demand, low-level metabolism continues in the absence of perfusion characterized by a continuous gradual decline in tissue pH [10]. MP of the warm heart provides tangible evidence of metabolic support in the form of a functioning, beating heart [31] whereas the support of aerobic metabolism in the hypothermic heart is less obvious. A series of observations suggest that hypothermic MP is able to support metabolism at levels that are adequate for maintaining cell integrity and vital cell functions during the transport interval. First, hearts that were preserved with 4 h of MP demonstrated superior ATP stores and maintained a tissue pH that was closer to baseline compared to CS [8–10,17]. These changes were associated with reduced markers of oxidative damage, DNA damage and apoptosis for the MP versus CS group [32,33] (Fig. 3). Second, a comparison of coronary sinus and aortic perfusate samples demonstrates a reduction in oxygen and lactate levels across the heart during MP, suggesting the consumption of substrates necessary for aerobic metabolism [34]. Finally, the MP hearts recover function after blood reperfusion quicker than the CS control hearts. Using a Langendorff model, MP hearts were noted to perform significantly better than CS hearts on all measured parameters of systolic and diastolic function, including peak systolic pressure, developed pressure, rate of pressure generation ($+dP/dt$) and the rate of relaxation ($-dP/dt$) [10]. These results are supported by numerous other studies that have likewise demonstrated improved hemodynamic recovery of MP hearts even after preservation for periods in which CS hearts do not recover [8,9,14,35].

This technology provides additional opportunities that can be exploited to the advantage of the organ. MP provides the chance to deliver a high concentration of antioxidants and other pharmaceutical agents targeting pathways involved in ischemia-reperfusion injury. Delivery of these agents directly to the heart minimizes the chance for systemic side effects [36–38]. Pharmacologic inhibition of post-transplant inflammation using a perfusate supplemented with mitomycin C [37] or a pyrazolotriazine derivative [38] has been shown to improve post-transplant graft survival and histologic appearance. As ex vivo tissue acidosis activates the Na/H^+ exchange system causing intracellular sodium accumulation and calcium overload, inhibition of this system using cariporide resulted in less tissue injury, reduced troponin release after whole blood reperfusion, and significantly improved functional recovery [39].

It is well know that endothelial cell function is critical for post-transplant myocardial function and that endothelial mediated graft coronary disease is a key determinant of long-term graft survival [40,41]. IVUS studies have noted evidence of arterial remodeling and graft coronary disease as early as 1 year [42] post-transplant in those patients with perioperative endothelial

dysfunction, highlighting the importance of maintaining the functional integrity of the endothelium [43]. Ex vivo CS is associated with the release of endothelium derived interleukin-1 and other pro-inflammatory cytokines, intracellular endothelial edema and apoptotic changes [44], indicative of endothelial cell damage. MP may enhance microvascular protection, lessen the chance for perioperative endothelial dysfunction, and improve long-term graft survival through a reduction in chronic graft vascular disease when compared to hearts preserved with CS.

4. Use of MP to expand the donor pool

Although kidneys procured from NHBD have demonstrated good results after transplantation, the heart has greater potential for irreversible damage from the combination of hypoxia, ischemia and cardiac distension during the period of cardiac arrest that is legally mandated to procuring organs from these donors [45]. Nevertheless, an expansion of the cardiac donor pool with these grafts is worthy of serious investigation if their safety can be assured. Grafts from NHBD may actually have advantages over those obtained from the standard cadaveric donor exposed to an antecedent period of brain death. The Cushing response, a sympathetic and cytokine storm that follows brain death, is thought to play a role in the development of an activated or pro-inflammatory graft phenotype which may directly injure myocardial tissue [46]. These changes may increase the antigenicity of the organ, increasing the risk of acute and chronic graft rejection and adversely influencing long-term outcome [47]. Assuming the injury from warm ischemia is reversible, the NHBD organ may have an advantage when compared to the organ affected by the consequences of brain death. This advantage is perhaps best illustrated by the superior outcomes of the living but unrelated renal transplant donor compared to kidneys procured from standard cadaveric donors [48].

There are serious concerns about ischemic injury incurred to the heart during the period of warm hypoxia and arrest that is obligated by non-heart beating donation. Myocardial ATP levels decrease to 70% of their baseline level after only 10 min asphyxia [49] and canine hearts exposed to 60 min of global warm ischemia without further resuscitation show no evidence of systolic function, i.e. the stone heart (Fig. 4) [50]. In the face of this degree of energy depletion, the additional insult of anaerobic metabolism during a CS interval has nearly zero likelihood of success as a strategy for utilizing these organs. Clearly, a better approach is to utilize the ex vivo transport interval as an advantage to the organ using MP instead of a disadvantage using CS. Our studies showed that MP was able to reanimate grafts after 60 min of global warm ischemia, with a majority of hearts demonstrating full functional recovery [50]. However, recovery was highly variable with a few grafts unable to recovery from this severe ischemic injury (Fig. 4). Inconsistent recovery of hearts procured from the NHBD is a problem that has also been described with other MP techniques [45,51–54].

In addition to restoring myocardial energetics in the injured heart, the viability of these grafts can be evaluated prior to transplantation using MP. However, there is no optimal diagnostic strategy for discriminating reversible from lethal ischemic injury and predicting (before reperfusion) which organs are most likely to recover function (after transplantation) [55]. Clinical markers of myocardial damage such as troponin I and CK MB are dependent on reperfusion injury as much as the initial ischemic insult and as a result are of limited value when assessed during hypothermic MP with crystalloid perfusate prior to whole blood reperfusion [56,57]. Suehiro et al. [53] assessed viability of hearts exposed to 60 min of global warm ischemia using an ex vivo perfusion apparatus in which hearts were perfused with autologous whole blood for a short interval after cardiectomy followed by CS for the duration of the ex vivo period. Hearts that were able to eject against 80 mmHg afterload showed good function after orthotopic heart transplant, whereas those with poor ex vivo performance were unable to be weaned from inotropes. An alternative strategy is the dynamic monitoring of the

rate and magnitude of the organ's response to resuscitation during MP. Of greatest interest are viability markers that can be measured within a time period suitable for influencing decisions about transplantation. An improvement in LV myocardial pH, assessed in real time during the MP interval, has been predictive of good functional recovery [58] (Fig. 5). Biochemical markers of viability including ATP, caspase-3, and malonyldialdehyde levels can be measured in myocardial biopsies using commercially available assay kits. The level of these markers as well as the level of the vasoconstrictor endothelin-1 measured in coronary sinus effusate provides insight into the potential for recovery of the heart from the NHBD.

Perhaps the most rapid means to obtain a global assessment of myocardial viability is through the use of ex vivo cardiac imaging during MP. Data obtained by diffusion tensor MRI is able to directly assess viability at the cellular level. Fractional anisotropy (FA) is a parameter derived from this modality that describes the differences between isotropic (assigned a value of 0) and linear (assigned a value of 1) diffusion within the tissues. A high FA value, indicating that water movement is largely confined within intact cell membranes, has been shown to correlate with better neurologic prognosis when analyzed in brain tissue after stroke [59]. When assessed in the heart at the end of the preservation interval, FA showed a strong correlation with conventional biopsy based biochemical markers of viability discussed above. More importantly FA showed strong correlation with parameters of functional recovery following restoration of whole blood flow (r = 0.6, 0.96, and 0.96 for developed pressure, rate of pressure generation, and rate of relaxation, Fig. 6) [50]. The fact that cardiac MRI can provide a global dynamic assessment of myocardial viability at the cellular level using a rapid scan suggests a potentially important role for this technology in the assessment of these high-risk grafts.

The feasibility of using hearts from NHBDs depends on a reliable method to differentiate grafts that have reversible versus irreversible damage that can be conveniently completed during the ex vivo transport interval. NHBDs are classified based on the Maastricht criteria, with type I and II representing out of hospital and in hospital cardiac arrest, type III representing the patient who expires on the basis of cardiac death after withdrawal of care in hospital, and type IV representing cardiac death in a brain-dead donor. The majority of abdominal organs in the United States are obtained from controlled (Maastricht category III (controlled) donors) [60]. Uncontrolled NHBDs (Maastricht type I, II) may potentially yield a larger number of organs, but are more difficult and complex to procure due to a larger interval between cardiac arrest and organ retrieval. However, select centers have successfully developed protocols to preserve abdominal and thoracic organs from uncontrolled donors in situ using extra corporeal membranous oxygenation [61,62]. As it is unclear how the human heart will respond to the variable periods of warm hypoxia seen in these donors it is important to validate animal MP protocols using human hearts from extended criteria donor and controlled NHBD rejected for clinical use.

Our group has recently reported the preservation of five human hearts from extended criteria donors, including two from controlled NHBDs [30], one of which was successfully resuscitated by MP with full recovery of function. The patient in this successful resuscitation was a 48-year-old female who had experienced out of hospital cardiac arrest with successful resuscitation but experienced severe anoxic brain injury and required mechanical ventilation. After consent for organ retrieval, care was withdrawn in the operating room with a warm hypoxia time (the time from withdrawal of ventilatory support till the infusion of cold cardioplegia) of 23 min. The heart was preserved with our standard protocol and resuscitated on a Langendorff whole blood reperfusion with excellent functional recovery. The second controlled NHBD was a 17-year-old male with end stage Duchenne muscular dystrophy and a warm hypoxia time of 17 min. Despite ex vivo MP, the heart showed poor functional recovery on whole blood reperfusion. Importantly, functional recovery was predicted during the ex vivo period using the viability assessments described above. This experience provides clinical validation of our

animal data and supports a future role for MP in the procurement of these organs for heart transplant.

5. Conclusions

The use of MP for the graft preservation has been shown to be a potentially effective alternative to conventional static CS. In the ideal donor heart, preservation with MP resulted in improved hemodynamics, improved myocardial acidosis, energy storage, apoptosis, oxidative/ischemia-reperfusion injury and endothelial function. These benefits are dependant upon homogeneous tissue perfusion, achieved through the use of a low potassium perfusate and optimal temperature management. In addition to the above benefits, MP also allows for the ex vivo resuscitation and evaluation of hearts severely damaged by ischemia, with viability predicted on the basis of MRI imaging, real time pH assessment and conventional biopsy based markers of viability. Taken as a whole, these data suggest that the dual benefits of MP are to resuscitate and preserve the heart ex vivo and screen for the irreversible injured heart. While studies utilizing MP in the ideal heart are clearly needed to establish the safety MP in clinical transplantation, MP may lead to reduced early graft dysfunction and might provide a novel avenue for use of NHBD hearts in clinical cardiac transplantation.

Abbreviations

MP
: machine perfusion

CS
: cold storage

NHBD
: non-heart beating donor

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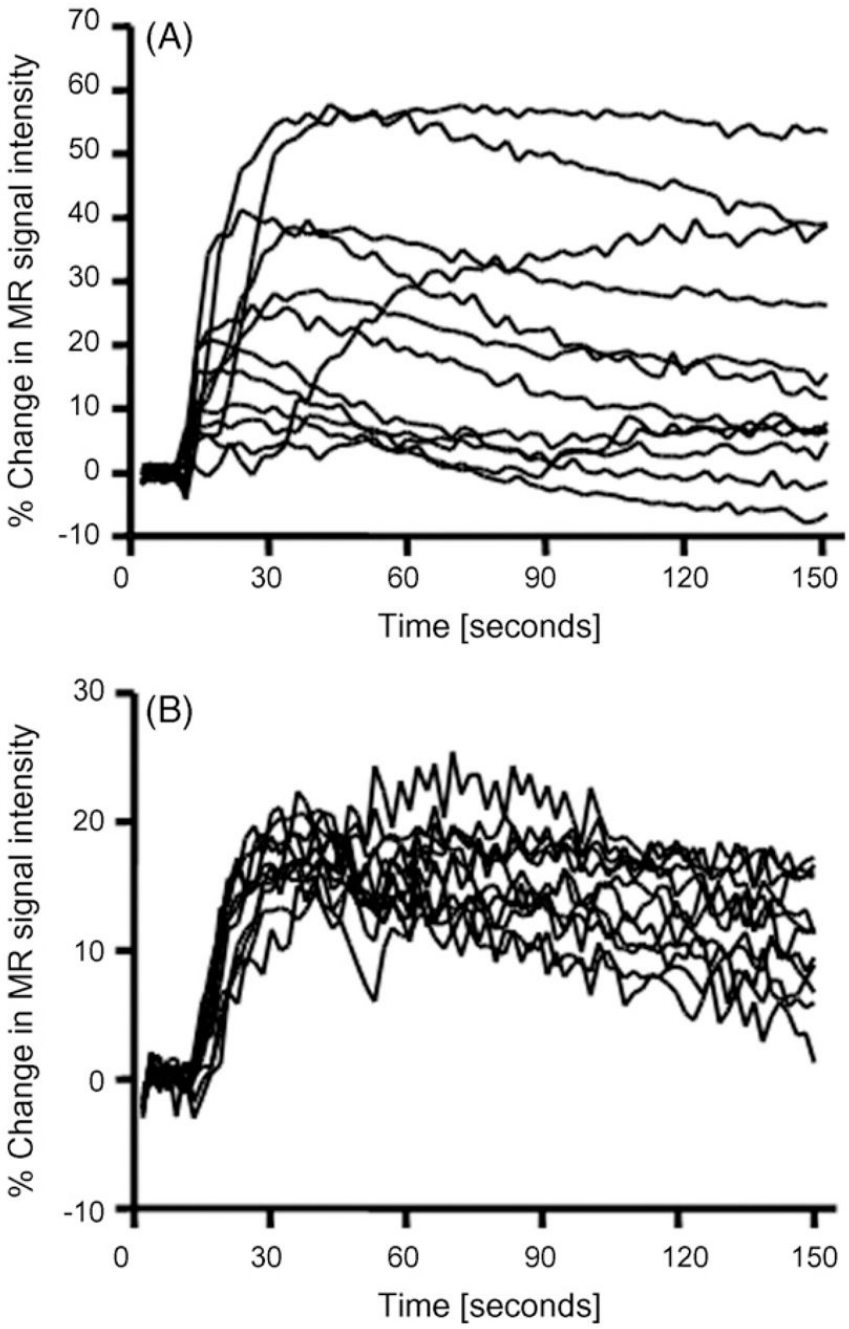

Fig. 1.

Representative traces of signal intensity vs time (SIVT) curves during machine perfusion (MP). SIVT were generated using gadolinium-DPTA MRI standard first-pass imaging methodology for each of four areas of interest (posterior, anterior, lateral, and septal walls) in each slice for a total of 12 SIVT curves per heart. Variable penetration of gadolinium-DPTA in hearts preserved with high potassium perfusate (panel A) indicates inhomogeneous perfusion. Hearts perfused with a low potassium perfusate (panel B) showed homogenous perfusion in hearts. Reproduced with permission, Circ J [10].

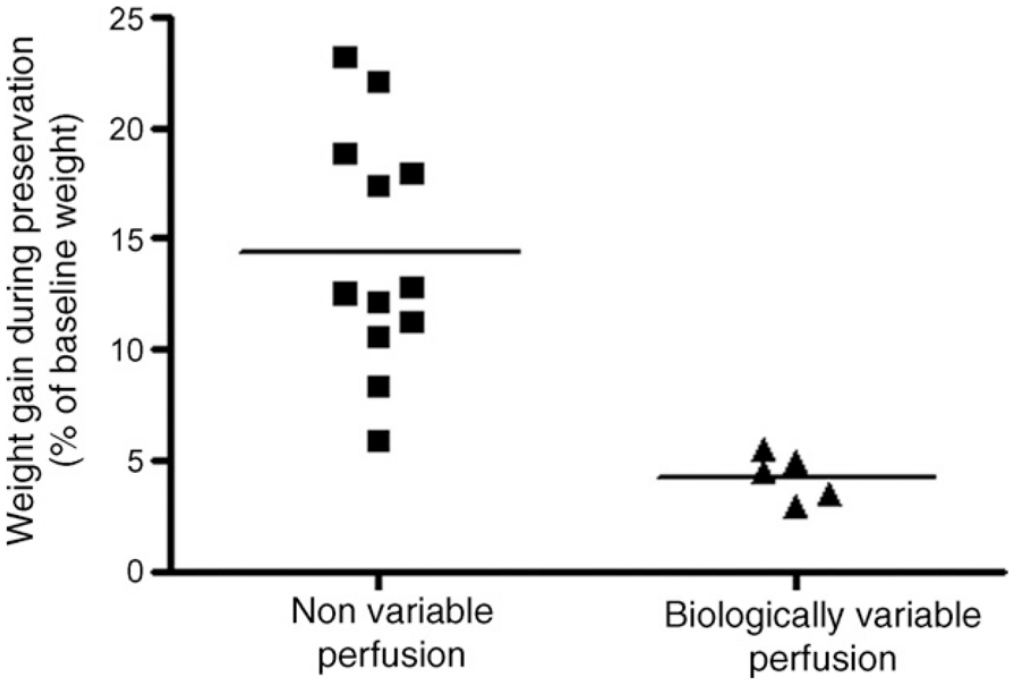

Fig. 2.

Weight gain during ex vivo MP (measured as a % of baseline weight) for hearts perfused at a constant, non-variable perfusion pressure and hearts perfused with biologically variable perfusion pressure. By mimicking physiologic perfusion pressures, biologically variable perfusion significantly reduces weight gain during ex vivo preservation.

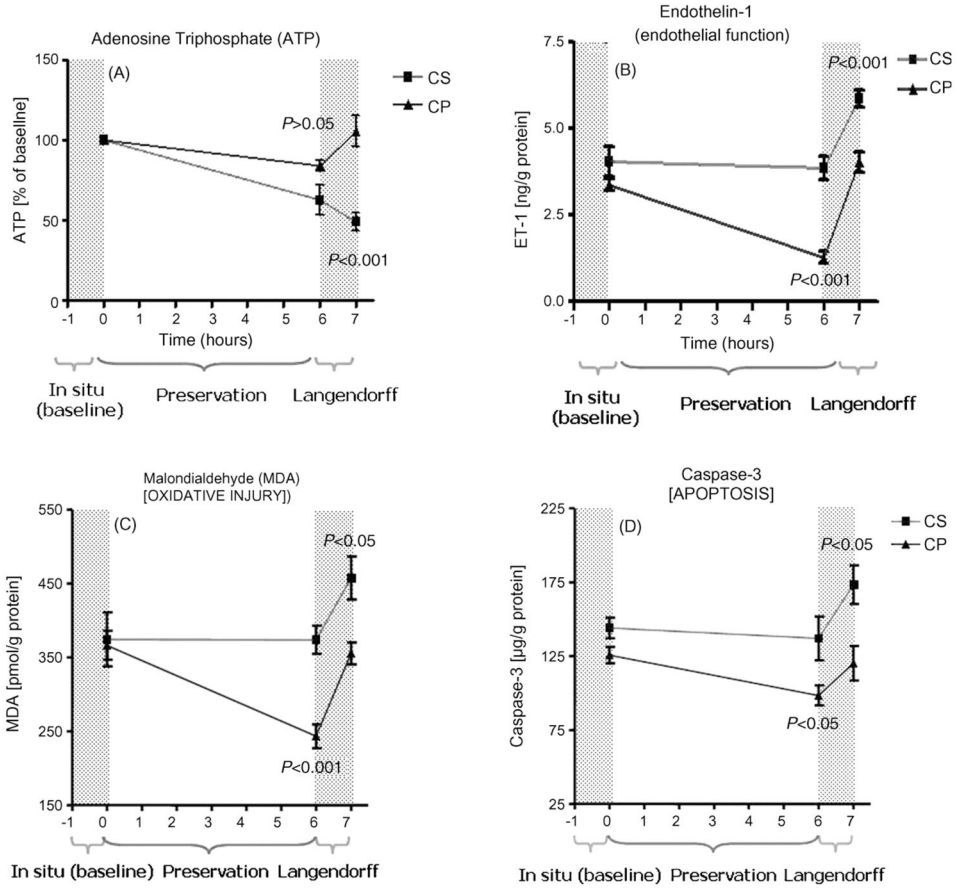

Fig. 3.

Three biopsies were obtain for all hearts preserved with either ex vivo machine perfusion (MP) or cold storage (CS); the first biopsy was obtained in situ prior to preservation, the second after ex vivo preservation and the third after 1 h of reperfusion with whole blood on a Langendorff apparatus. Hearts preserved with MP showed improved ATP levels after preservation with a return to baseline ATP levels after 1 h of whole blood reperfusion (panel A). Similarly, hearts preserved with MP showed significantly lower levels of endothelin-1 (marker of endothelial injury, panel B), malondialdehyde (MDA, marker of oxidative damage, panel C) and caspase-3 (marker of apoptosis, panel D) compared with hearts preserved with CS.

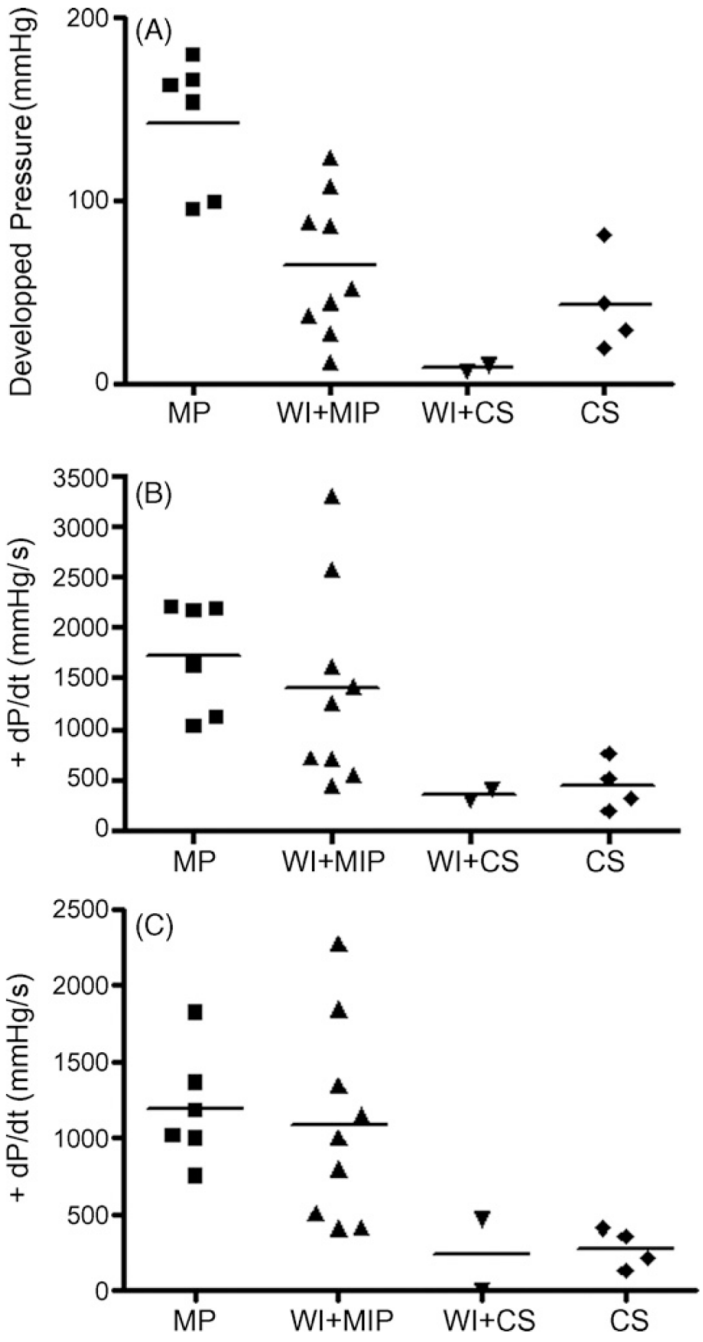

Fig. 4.

To simulate a NHBD in a large animal model, canine hearts were exposed to 60 min of global warm ischemia prior to cardiectomy then preserved with 6 h of continuous ex vivo perfusion (WI + MP group). Hearts exposed to warm ischemia followed by cold storage (WI + CS) and hearts preserved with 6 h of machine perfusion (MP) or cold storage (CS) without any prior warm ischemia served as control groups. Despite 60 min of warm ischemia, mean functional recovery of the WI + MP group, based on the developed pressure (panel A), rate of pressure generation (+d*P*/d*t*, panel B) and rate of relaxation (−d*P*/d*t*, panel C) was not significantly different from hearts preserved without prior warm ischemia (MP group). However functional recovery in the WI + MP group was highly variable indicating that not all hearts in this

homogeneous canine population are able to recovery from such severe ischemic injury. Hearts in the WI + CS group showed no functional recovery.

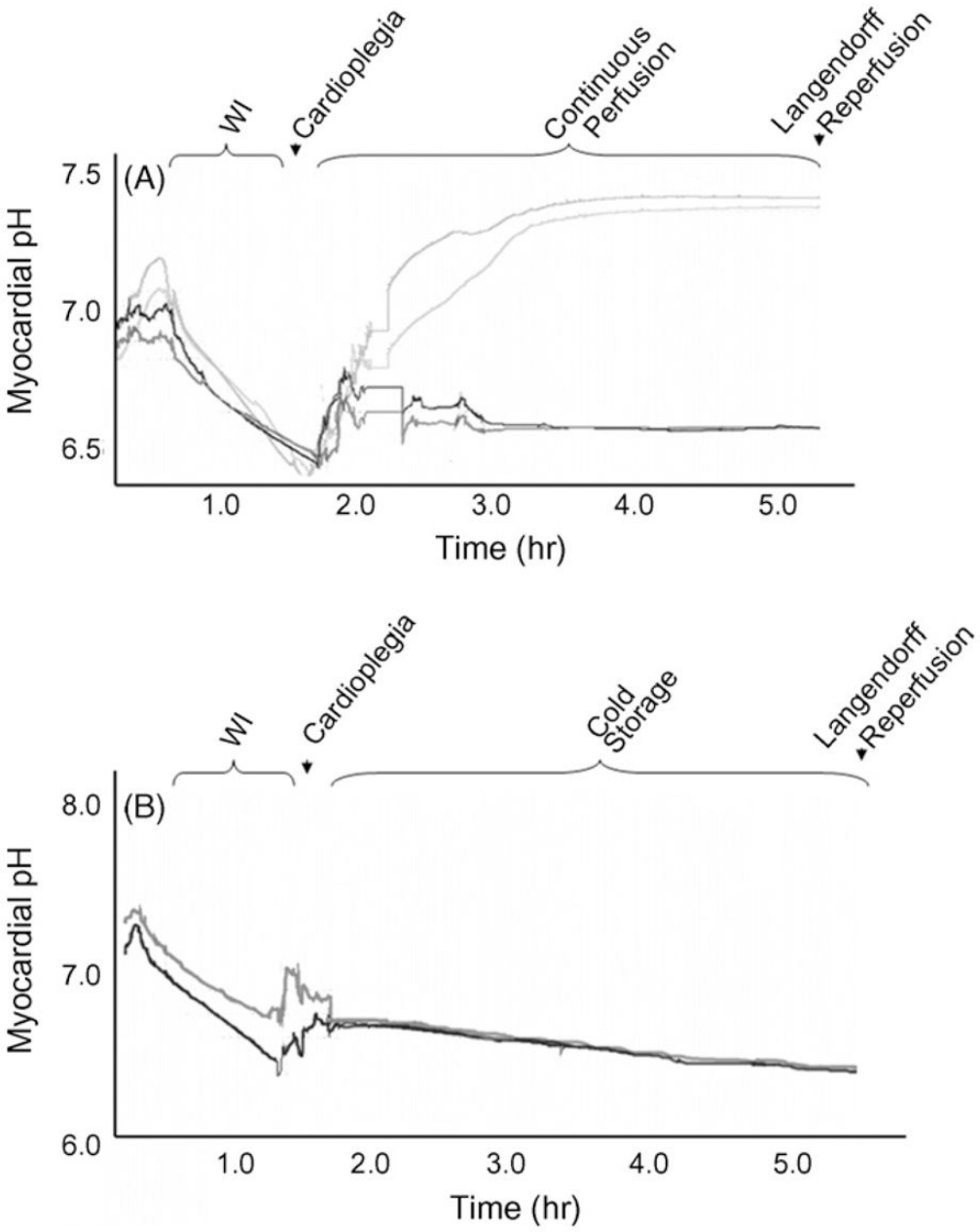

Fig. 5.

Representative real time LV intramyocardial pH tracing during ex vivo preservation. Hearts that showed good functional recovery following restoration of whole blood flow had demonstrated improving anterior and posterior LV myocardial pH during ex vivo preservation (light tracings, panel A), in contrast hearts that showed poor functional recovery did not demonstrate ex vivo pH improvement and were acidotic at the time of blood reperfusion (dark tracings, panel A). Tissue pH may thus be an important variable to identify, during the ex vivo preservation period, hearts that are at risk for primary nonfunction. Hearts preserved with cold storage (panel B) showed a gradual decline in anterior and posterior tissue pH during the preservation period indicative of anaerobic metabolism.

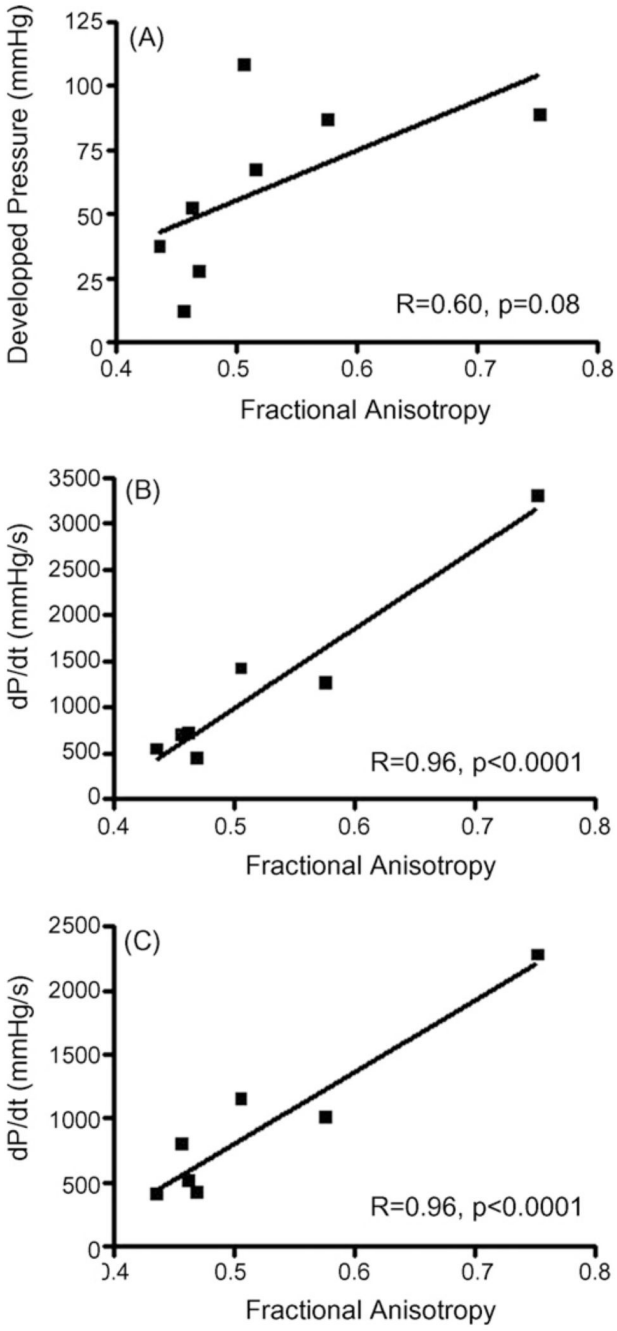

Fig. 6.

Correlation of fractional anisotropy with markers of functional recovery following restoration of whole blood flow. Fractional anisotropy, assessed ex vivo using diffusion tensor MRI, provides a direct measure of cellular viability. The strong correlations with developed pressure (panel A), rate of pressure generation (+d*P*/d*t*, panel B) and rate of relaxation (−d*P*/d*t*, panel C) suggest that this technology could be used to assess graft viability during the ex vivo period. Reproduced with permission, J Heart Lung Transplant [50].