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Nonischemic Donor Heart Preservation: New Milestone in Heart Transplantation History

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Heart transplantation is considered the gold standard for the treatment of advanced end-stage heart failure. However, standard donors after brain death are decreasing, whereas patients on the heart transplant waitlist are constantly rising. The introduction of the *ex vivo* machine perfusion device has been a turning point; in fact, these systems are able to significantly reduce ischemic times and have a potential effect on ischemia-related damage reduction. From a clinical standpoint, these machines show emerging results in terms of heart donor pool expansion, making marginal donors and donor grafts after circulatory death suitable for donation. This article aims to review mechanisms and preclinical and clinical outcomes of currently available *ex vivo* perfusion systems, and to explore the future fields of application of these technologies.
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Key Words: heart failure, heart transplantation, *ex vivo* machine perfusion

Since the first heart transplantation was performed by Dr. Barnard in 1967,[1] significant improvements have been achieved in terms of donor graft selection, surgical technique, and immunosuppressive therapy.[2] However, despite these advancements, donor heart preservation has not significantly changed over time: conventionally, the donor heart is arrested by means of cold heart-preservation solution and, after cardiectomy, it is stored in an icebox (static cold storage [SCS]).[3] This strategy is simple, cheap, and reproducible. Standard criteria donor hearts can be preserved for up to 6 hours, granting a low percentage of posttransplant graft dysfunction.[4] Unfortunately, SCS can negatively impact myocardial metabolism because it introduces time-dependent ischemic injury, which primes reperfusion injury upon implantation (ischemia/reperfusion injury [IRI]). This is epidemiologically relevant: in fact, end-stage heart failure is increasing over time, as well as the number and the age of patients listed for heart transplantation. Nevertheless, available donor grafts are limited and patients remain on list for years with an increased risk of death before transplantation.[5] Ischemia time is usually related to the geographical distance between donor and recipient center, which is not always solved by available transport services. During the last 10 years, *ex vivo* perfusion techniques have emerged as a promising substitute to SCS, showing robust results in terms of lower primary graft dysfunction (PGD) when compared with SCS.[6] Taking advantage of these nonischemic preservation strategies, both marginal donors and donor grafts after circulatory death (DCD) hearts can become suitable for donation.[7]

The aim of this review was to describe the physiopathologic mechanisms of ischemia-related injury and IRI, the current preclinical and clinical *ex vivo* perfusion strategies used in heart transplantation practice, and the future perspectives in the field.

Cardiac Ischemic Metabolism and Ischemia/Reperfusion Injury

IRI is a multifactorial inflammatory condition that involves different mechanisms[8] (**Figure 1**). Hypoxia is the initiating insult, which switches heart cells from aerobic to anaerobic metabolism. Adenosine 5'-triphosphate (ATP) decrease (secondary to anaerobic) dysregulates Na/K pump functioning, with intracellular sodium and calcium increase. Calcium overload produces reactive oxygen species (ROS) generation, which results in the activation of pathways that lead to cell death associated with IRI. IRI-induced cellular death includes different pathways (apoptosis, necrosis, ferroptosis). During cellular death, heart cells release proinflammatory molecules which activate "'sterile'" inflammation, the complement, and the immune system ("'sterile'" means that no microbes are involved in the inflammatory process). During ischemia, endothelial cells also secrete substances that promote vasoconstriction to attenuate tissue edema. When reperfusion begins, ischemia-primed endothelial cells favor leukocyte adhesion, transmigration, and activation into tissues. Once activated, leukocytes release cytokines and proteases that amplify tissue damage. In addition, during the reperfusion phase, respiratory activity and ATP synthesis are restored and intracellular pH rapidly normalizes. The increased availability of ATP upon reperfusion and the increased calcium intracellular uptake exceeds the sarcoplasmic reticulum capacity of calcium absorption. This calcium excess produces ROS, proteases activation (*e.g.*, calpain), uncontrolled myofibrillar hypercontraction, and mitochondrial swelling with consequent membrane damage and cell death.[9] Indeed, the vasoconstriction is exacerbated by decreased endothelial nitric oxide synthase expression during the ischemic time, and increased sensitivity to vasoconstrictive molecules, with impairment at the microcirculatory level. The endothelial dysfunction caused by IRI might be involved in chronic allograft vasculopathy and myocardial cell death,[9] with adverse effects on allograft durability.

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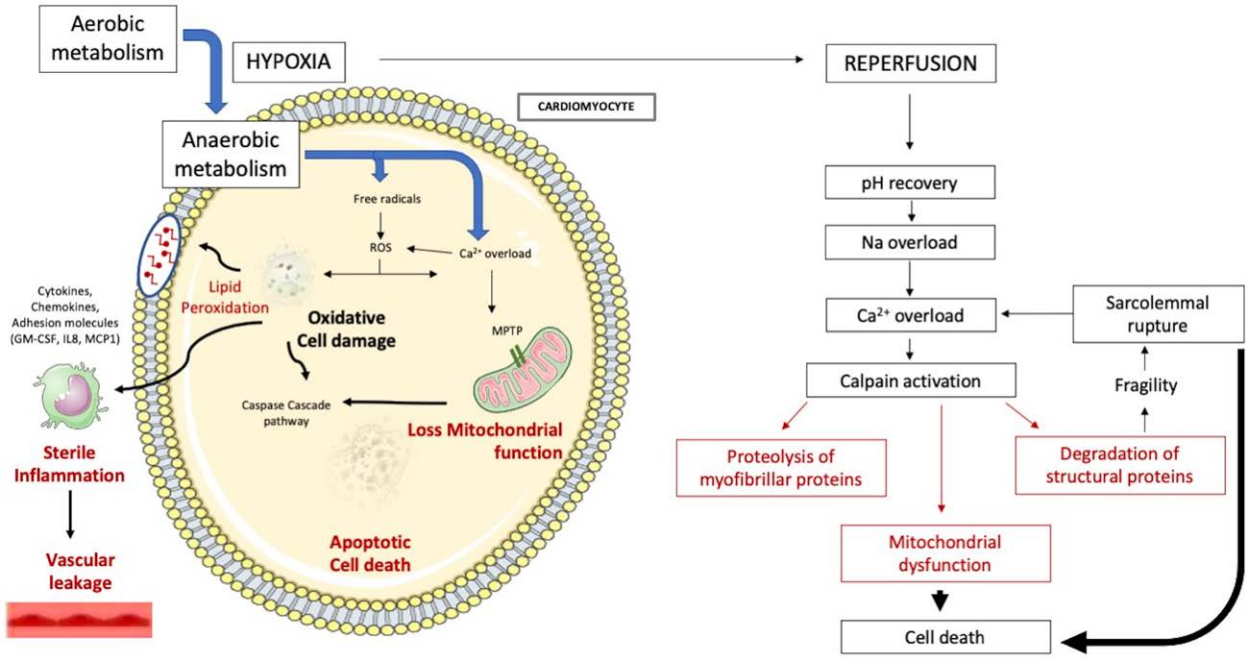

Figure 1. Picture showing cellular events involved in ischemia and ischemia/reperfusion injury (IRI): cellular hypoxia produces metabolic stress, vascular permeability, and cellular apoptosis. IRI generates additional cellular damage caused by an excess of intracellular calcium. GM-CSF, granulocyte-macrophage colony-stimulating factor; IL8, interleukin 8; MPTP, mitochondrial permeability transition pore; MCP1, monocyte chemotactic protein-1; ROS, reactive oxygen species.

Ischemic time is proportionally related to graft damage,[10] and it is a strong predictor of posttransplant PGD and survival.[11] Moreover, ischemic time might increase the deleterious effects of other risk factors (*e.g.*, donor age, left ventricular hypertrophy) to cause PGD and affect posttransplant survival.

Ex Vivo Heart Perfusion

Ex vivo machine perfusion of the heart has emerged as a way to diminish IRI by simultaneously providing oxygen and nutrients to the myocardium, and removing metabolic waste products from myocardial cells. With this strategy, donor graft ischemic time is reduced and, consequently, the related biochemical and cellular damage is lowered. Even if the concept of machine perfusion was first theorized during the 19th century by Ludwig and Cyon,[12] Langendorff was the first to pioneer a successful system (**Figure 2**). He was able to reanimate a dead explanted mammalian heart through coronary artery perfusion down the cannulated ascending aorta.[13]

Ex vivo perfusion devices under preclinical or clinical investigation, or approved and used in the clinical field, can be categorized into two groups: 1) hypothermic *ex vivo* nonbeating perfusion and 2) normothermic *ex vivo* beating perfusion.

Hypothermic *Ex Vivo* Nonbeating Machine Perfusion (HMP)

Hypothermia significantly slows cellular metabolism, reducing the energetic costs of ion-balancing ATPases, preserving transmembrane electrochemical gradients, and suspending the activation of apoptotic biochemical pathways. Nevertheless, hypothermia can also have deleterious effects on cells, including the maladaptive redistribution of membrane lipids and subsequent loss of membrane integrity. Consequently, hypothermic cooling is a delicate balancing act between the beneficial and detrimental effects of cooling, with a temperature at which damaging effects are minimized and protective effects maximized around 4°C. However, hypothermia is not sufficient to reduce IRI. In fact, SCS minimizes cardiac oxygen and energy requirements, but forces myocardial cells to adopt anaerobic metabolism with consequent acidosis and energy stress.[14] Nonbeating machine perfusion (HMPs) overcome this biochemical problem by providing oxygenated nutrient-rich perfusate while maintaining a low myocardial metabolism. Current evidence suggests that oxygen-rich perfusates protect cardiomyocyte integrity, preserve cellular ATP stores, and better maintain membrane conditions.[15] Furthermore, the continuous perfusion prevents build-up of toxic metabolites (*i.e.*, lactate and adenosine) which may contribute to poor ventricular function, or serve as a substrate for the generation of ROS upon reperfusion of the organ.[16] Unfortunately, hypothermic perfusion shows risks related to its narrow physiologic window of action such as organ edema, vascular bed injury, or insufficient perfusion. High perfusion pressure and low osmotic pressure of perfusate are risk factors for edema formation. For this reason, perfusate with high osmolality or high concentration of oncotic agents has demonstrated better results in edema prevention.[17,18]

The available HMPs preserve donor hearts in a perfused nonbeating state. These devices consist of an aortic cannula, a reservoir, a pump, and an oxygenator in a well-insulated container.

HMP Preclinical Studies

Preclinical studies have been performed since the early 1980s in multiple animal models (pig,[19,20] dog,[21–23] baboon[24]) where heart viability has been assessed *in vitro* on a normothermic reperfusion system, or by autotransplantation or

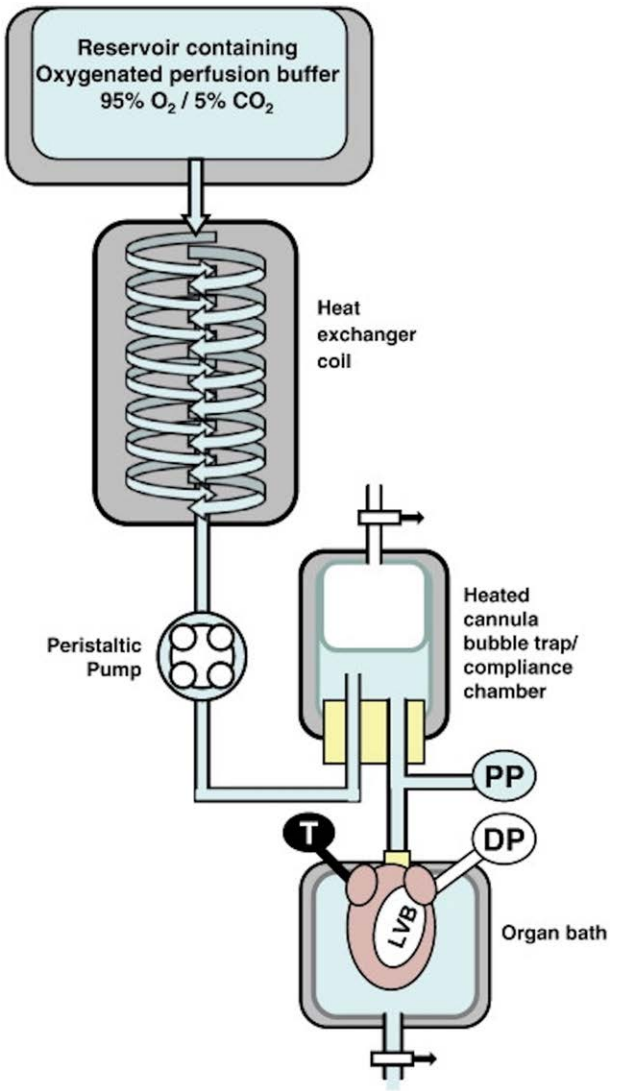

Figure 2. Scheme of the Langendorff system. DP, left ventricular developed pressure; LVB, left ventricular balloon, which measures DP; PP, perfusion pressure of the aortic cannula; T, thermocouple to monitor graft temperature.

orthotopic allotransplantation. Hearts were procured after terminal anesthesia of the animal except for three DCD,[25–27] and one donation after brainstem death (DBD) scenario.[18] All these studies have demonstrated the superiority of hypothermic oxygenated perfusion in terms of contractile function and aerobic metabolism (lower ROS production upon reperfusion) compared with SCS (with a perfusion time up to 48 hours). Indeed, at a microscopic level, cellular structures were better preserved in both endothelium and myocardium, with less cellular death. In both the preclinical DCD and DBD scenarios, hypothermic perfused hearts were able to support circulation after transplantation or showed better contractility and biochemical metabolism in *in vitro* settings. In all these cases, hypothermia was maintained between 4 and 10°C.

HMP Clinical Studies

Even if clinical experience with HMP started almost 40 years ago,[28] with the first experience of HMP in heterotopic transplantation, current clinical practice is still limited: to date, the Paragonix SherpaPak Cardiac Transport System is the only device approved by regulatory agencies (Food and Drug Administration [FDA] and European Community [CE]), but it is not machine perfusion. Only XVIVO NIHP system (XVIVO Perfusion AB, Göteborg, Sweden) is a true HMP, but it is still under clinical investigation (ongoing clinical trial), whereas the LifeCradle system (Organ Transport Systems, Inc, Frisco, TX) has been studied in human hearts only in *in vitro* settings.

Paragonix SherpaPak and SherpaPerfusion Cardiac Transport System

Paragonix Technologies (Cambridge, MA) has produced two different devices: SherpaPak Cardiac Transport System and SherpaPerfusion Cardiac Transport System.

The SherpaPak is not a perfusion machine, but offers uniform cooling through its proprietary CoolSafe technology that is capable of maintaining a consistent temperature of 4–8°C. The SherpaPak consists of multiple components: 1) outer transport shipper which contains within various nonice-based temperature-controlled packaging elements, 2) an inner and outer hard shell assembly that provides a rigid barrier enclosure in which the heart is immersed and suspended in a cold storage solution cleared for use in storing and transporting donors' hearts, 3) a data logger that monitors and displays the temperature of the cold storage solution in which the heart is stored during transport, and 4) four size heart connectors designed to accommodate various size aortic stem diameters by which donor hearts are attached. **Figure 3** summarizes the main components of the device. The system suspends the donor heart in a preservation solution for even cooling in a pressure-controlled, leak-proof, rigid canister that provides a consistent temperature range, prevents cold injury, and offers real-time monitoring and data reporting. Preliminary results from 10 sites on 569 patients (255 ice transports and 314 SherpaPak transports) of the ongoing retrospective/prospective large multicenter registry (Global Utilization And Registry Database for Improved heArt preservatioN [GUARDIAN] Registry)[6] have shown that early clinical outcomes, including PGD rates and intensive care unit length of stay are significantly improved in the SherpaPak group.

The SherpaPerfusion Cardiac Transport System consists of a single-use, disposable device for hypothermic oxygenated perfusion preservation and transport of the graft. This device is still not used in clinical practice, but its experience is limited to swine models. In the two preclinical settings described, a cannula was inserted in the aortic root and the heart was connected to the Sherpa organ carrier, which had been primed with Celsior solution without blood. The donor heart was completely immersed in Celsior solution (ZA La Croix Grand Borne 69930 Saint-Laurent-de-Chamousset, France), which was placed into the Sherpa shell, which provided a second, rigid barrier to protect the donor heart. This assembly was then inserted into the Sherpa shipper, which created a homogenous cooling environment. To operate the device in research mode, the SherpaPerfusion system was instrumented with multiple pressure and temperature sensors. Organ perfusion was achieved by the cyclical pressurization of two chambers: a lid and an organ container that are connected *via* a port through which solution enters the aortic root. Perfusion pressure was ~3 mmHg, coronary flow was ~44 mmHg, partial pressure of oxygen (pO_2) in the aortic root

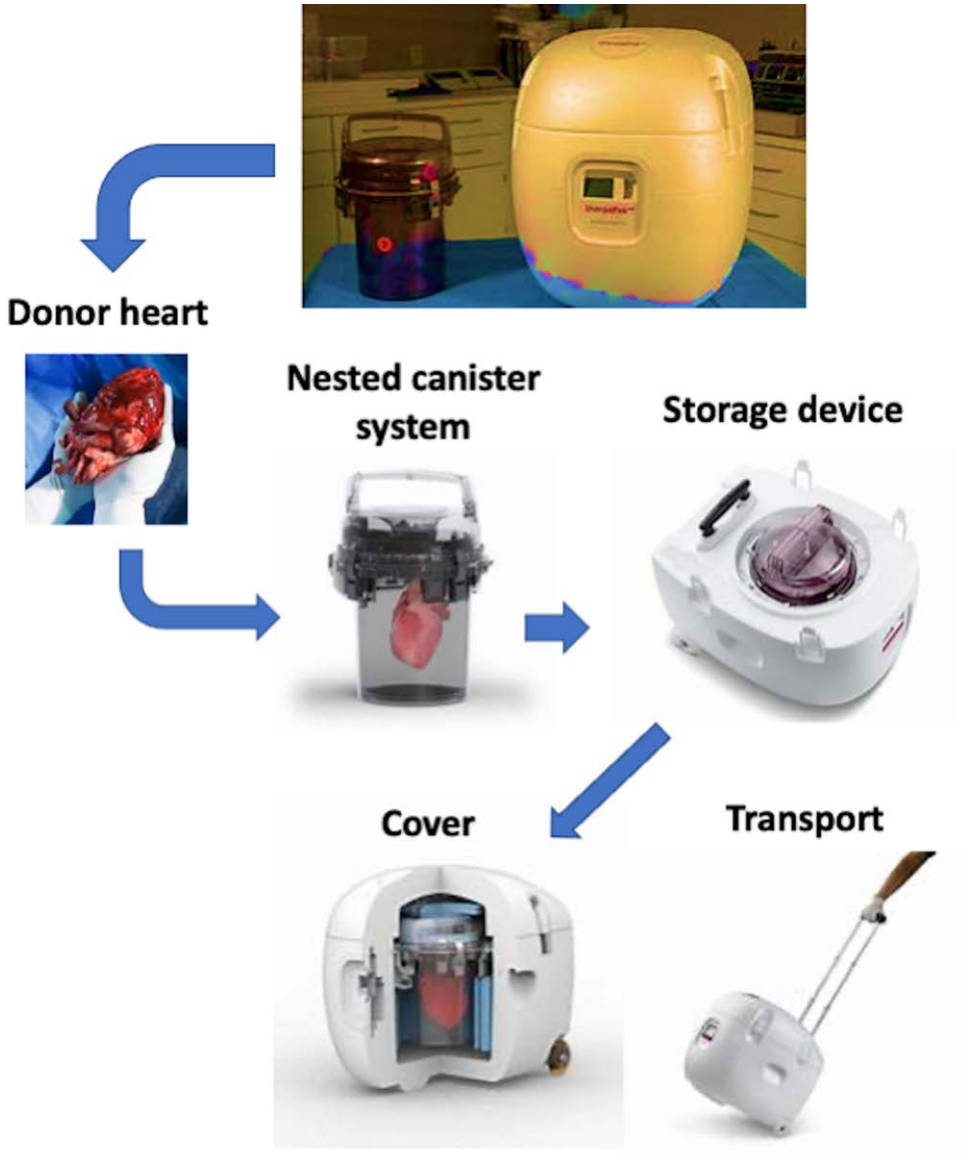

Figure 3. Picture showing how the Paragonix SherpaPak device works: the donor's heart is fully suspended and immersed in a preservation solution into a nested canister system; once in place, the storage box is covered, and the device is ready to be transported.

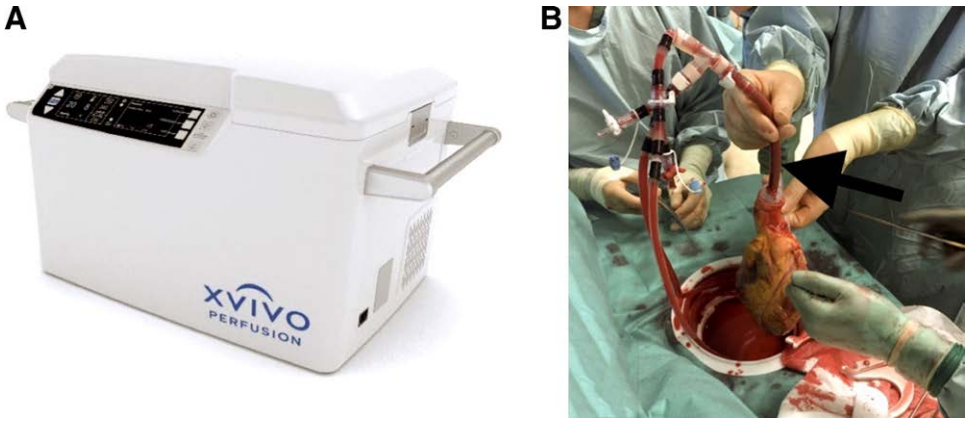

Figure 4. Hypothermic machine perfusion system - XVIVO device. **A**: XVIVO external perfusion machine; **B**: picture showing how the heart appears once extracted from the machine for implantation (black arrow showing the aortic cannula which is responsible for aortic perfusion during transportation).

solution was ~300 mmHg. When analyzed in an *in vitro* reperfusion machine, hearts harvested with the help of SherpaPerfusion showed less microscopic endothelial dysfunction and less myocardial cell injury. However, no differences were found in terms of function, and SherpaPerfusion showed greater heart weight (edema). Further larger studies in human cases compared with currently available alternative devices are required to check which machine can achieve the best outcomes in terms of graft dysfunction, heart rejection, and survival.

XVIVO NIHP Technology

The XVIVO NIHP system is a portable device approved for ground and airborne transportation (**Figure 4A**). The heart is continuously perfused with a cold (8°C) oxygenated cardioplegic nutrition–hormone solution containing erythrocytes from the blood bank.

The equipment consists of a reservoir, a pressure-controlled roller pump, an oxygenator, an arterial-leukocyte filter, a heater–cooler unit, oxygen and carbon dioxide containers, a gas mixer, sensors, and a programmable control system. The reservoir is filled with 2.5 L of the perfusion solution plus ~500 ml compatible irradiated and leukocyte-reduced blood cells, providing a hematocrit of ~15%. Perfusion is provided through the aortic cannula to the coronary vessels. Technically, the donor heart is arrested with the heart-preservation solution without erythrocytes (1,200 ml). Then the donor's heart is harvested using the same procedure as that used for the SCS group. Thereafter the distal ascending aorta is cannulated from the device with a special double-lumen cannula for easy deairing and a soft 3/8 inch silicon tube is placed into the left ventricle through the atrium to maintain the ventricle in a decompressed state. The venae cavae and pulmonary artery are left open for a free outlet of perfusate from the coronary sinus. The double-lumen cannula supplying the aorta with the preservation medium is fixed in a vertical position and the heart is submerged into the heart-preservation solution, which is actively regulated to maintain a pH of ~7.4 and a temperature of 8°C. The device's software is adjusted to maintain a mean blood pressure of 20 mmHg in the aortic root, providing an intermittent coronary flow between 150 and 250 ml/min. After explantation of the recipient's heart, the continuous perfusion is switched to intermittent perfusion. During the implantation of the heart, the aortic cannula is kept in the aortic root, thereby facilitating stability of the heart (**Figure 4B**). Intermittent perfusions with 200–300 ml of the preservation solution were administrated through the cannula every 15 minutes during the implantation procedure to avoid ischemia. The cannula was withdrawn before the aortic anastomosis was performed. Blood samples were retrieved from the coronary sinus in the right atrium.

Current clinical evidences are based on a nonrandomized phase II study in which 25 patients were assigned to SCS and 6 to XVIVO NIHP[29]: preliminary results have confirmed the feasibility and safety of XVIVO NIHP device for clinical use in heart transplantation. A randomized clinical multicenter trial involving different European centers is enrolling and ongoing and has the aim to evaluate early and 1 year mortality and graft dysfunction between randomized hearts procured by means of the XVIVO device *versus* those transported using SCS. This technology has been used for organ preservation in the first-in-human heart xenotransplantation.[30]

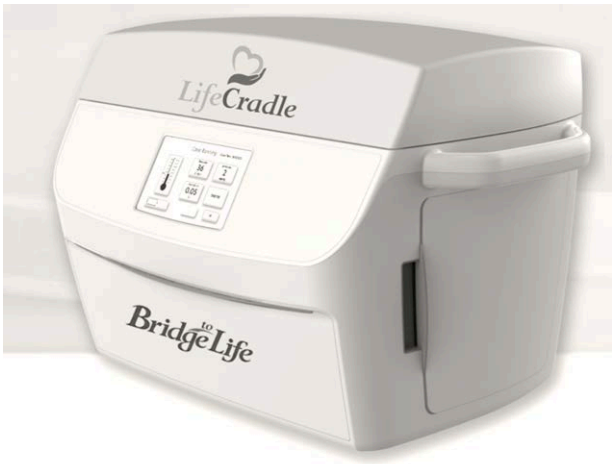

Figure 5. Figure showing the LifeCradle Heart Perfusion System.

LifeCradle Heart Perfusion System

The LifeCradle Heart Perfusion System (**Figure 5**) is a device that delivers temperature-regulated, oxygenated perfusate to the donor heart both in an anterograde or retrograde fashion. Perfusate temperature, flow, and pressure are monitored continuously. FDA and CE approval is still pending to date. Technically, anterograde fashion is characterized by perfusion with cardioplegia through the ascending aorta at a flow rate of 10 ml/100 g heart weight/min at 5 ± 2°C. Retrograde perfusion is carried out by means of a retrograde cardioplegia cannula (Medtronic, Inc, Minneapolis, MN) sewn into the coronary sinus with cardioplegia at a flow rate of 13–20 ml/100 g/min, also at 5 ± 2°C. This device was tested in canine models. A recent study performed in human donor hearts either not offered for transplantation or rejected for transplantation has shown that aerobic metabolism is better preserved with this machine.

Normothermic *Ex Vivo* Beating Machine Perfusion (NMP)

Normothermic ex vivo beating machine perfusions (NMPs) are more recent than HMPs, with the first preclinical studies published in the late 1990s: both DCD[31] and terminally anesthetized animal settings[32,33] have shown that these donor grafts can be safely stored for up to 4 hours after 30 minutes of warm ischemia time, and for up to 12 hours, respectively, at a temperature between 34 and 37°C.

Currently, the only clinically authorized NMP both in United States and Europe is the organ care system (OCS) developed by Transmedics (Andover, MA) (**Figure 6**). This machine allows the donor heart to be preserved beating and perfused in normothermia until its implantation in the recipient. Because of this normothermic maintenance, the cold ischemic time is shortened, distant procurement of organs is achieved and the possibility of sharing organs across greater distances is theoretically possible.

The OCS is a portable device with a wireless monitor, a perfusion module, and a "maintenance" solution, that is given *via* a standard intravenous infusion pump into the donor blood circulating in the system (Figure 6). When used, 1,200–1500 ml of donor blood is collected and used before cross-clamping

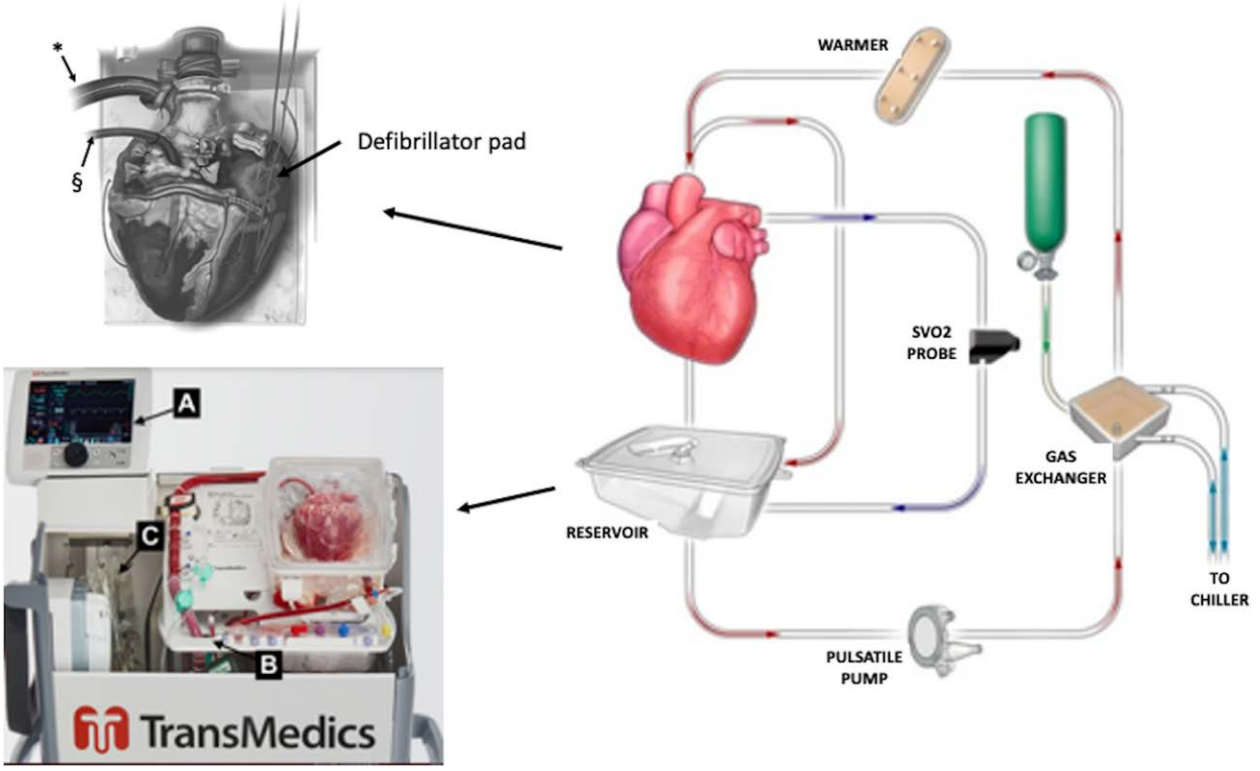

Figure 6. Picture showing the organ care system (OCS) circuit (right part of the figure) and components (left part of the figure). **A**: Wireless monitor; **B**: Perfusion model; **C**: Maintenance solution. The donor's heart is placed into the reservoir box; the graft is perfused by the aortic root with a specific aortic cannula, the left ventricle is unloaded through an atrial vent (§), and the coronary sinus blood is drained by means of a pulmonary artery cannula (*). A defibrillator pad is placed underneath the heart to avoid unexpected arrhythmias.

to prime the perfusion module. After retrieving the heart (previously arrested with conventional heart-preservation solution) and once at the back table, the aorta and pulmonary artery are cannulated before *ex vivo* reperfusion. The heart is placed within the system and warm oxygenated donor blood is pumped into the aorta allowing the coronary arteries to be perfused. Both vena cava are closed and the coronary sinus flow then passes through the tricuspid valve and is ejected by the right ventricle into the pulmonary artery that is cannulated and returns the blood into a reservoir. Grafts are usually perfused depending on the size, degree of ventricular hypertrophy, and coronary artery disease, with an aortic flow of 1 L/min, aortic pressure of around 70 mmHg, and a coronary blood flow of around 700 ml/min, at 34°C. Arterial and venous lactate samples are regularly taken to assess the adequacy of perfusion and ensure a favorable myocardial lactate uptake. Synchronization of the pulsatile perfusion pump to the electrocardiograph during preservation allows lower aortic root pressure and coronary flow with optimized myocardial perfusion during ventricular diastole with the aim of reducing the risk of myocardial edema during prolonged preservation cases. Grafts are perfused with warm, oxygenated donor blood supplemented with epinephrine infusion, and a proprietary maintenance solution containing adenosine, glucose, and several amino acids. Maintenance infusion requirements are titrated depending on aortic pressure which indicates coronary artery resistance to perfusion.

The PROCEED II trial was a prospective, randomized study conducted between June 2010 and September 2013 that compared 67 hearts preserved with OCS to 130 preserved with SCS.[34] No significant differences were observed regarding cardiac-related adverse events or 30 day patient survival, and researchers concluded that preservation with OCS and SCS yield similar short-term functional outcomes. A more recent study, the EXPAND II trial,[35] was a prospective, multicenter trial that evaluated the ability of OCS to preserve and evaluate extended criteria donor hearts before transplant. Of the 93 donor hearts that met inclusion criteria and were subsequently preserved with OCS, 75 were successfully transplanted with a mean perfusion time of 6.35 hours. A 30 day survival was 94.7% and researchers concluded that cardiac preservation with OCS results in high rates of utilization with excellent short-term functional outcomes. PGD rate in OCS grafts was 10.7%. Additionally, OCS has also been evaluated in human DCD with successful results. Because of the inevitable warm ischemia time between the withdrawal of life support and circulatory arrest, the already energy-depleted heart poorly tolerates additional cold ischemia. Preretrieval function is incompletely assessed so DCD heart quality has to be evaluated. The OCS provides a platform to partially reverse the warm ischemic injury, safely preserve hearts over long-distance transport, and allow subjective examination of ventricular contractility. Unfortunately, quantitative functional assessment is difficult to perform because the heart is unloaded, and it is technically demanding (epicardial echocardiography), with no sufficient experience available. To date, more than 50 DCD heart transplantations have been performed in Australia[36] and United Kingdom[37] by means of OCS

technology, with excellent short-term outcomes, comparable with those of DBD transplantation. A recent meta-analysis (comparing DCD and DBD) has shown that among 87 OCS-DCD patients, 30 day survival in the DCD cohort was 96.6% (95% confidence interval, 89.9–98.9%), with no difference compared with DBD. Similarly, long-term survival (4 years) in the DCD group was 85.3%, with no significant difference when compared with OCS-DBD cases.[38] Unfortunately, longer follow-ups are required. The OCS-DCD US trial was the first randomized trial comparing DCD heart transplant to DBD standard criteria heart transplant clinical outcomes[39]: to date, 180 patients (90 DCD *vs*. 90 DBD) were enrolled and transplanted. One year patient and graft survival was greater than 90%, with a higher rate compared with DBD. Unfortunately, incidence of moderate-to-severe ISHLT PGD was around 20% (DCD) *versus* 9.1% (DBD), raising the idea that warm ischemia (even if < 30 min) can negatively affect early graft function and that research should focus on methods for positively conditioning the DCD hearts.

Discussion

Both HMP and NMP have advantages and disadvantages (Table 1). Regarding HMP, XVIVO NIHP has the advantage of being simple to use and it grants a hypothermic environment for the heart. The hypothermic preservation provides increased safety and protection against external impacts on the system such as power failure. With normothermic preservation, an interruption in *ex vivo* perfusion can result in irreversible damage to the heart. Creating an artificial environment similar to the physiologic state in which a warm beating heart is supposed to work is both complicated and risky. Moreover, it involves additional surgical and technical support and appropriate transport, inevitably resulting in more expensive management compared with what is needed for SCS. Additionally, in the DCD Australian and British experience, 12.5–25% of carefully selected Maastricht category III donors did not proceed to circulatory arrest, and among the hearts perfused by the OCS, 10–30.3% were declined for implantation because of various reasons, including unsatisfactory function or metabolic profile. These unsuccessful retrieval attempts with the OCS further raised the cost of transplantation program. Unfortunately, HMP does not allow a functional assessment of donor graft, and for this reason, many heart transplant teams might not be confident in using these devices for extended criteria donor hearts.

On the other side, NMP like OCS maintains the heart in a normothermic working beating status, thus permitting a dynamic assessment of allograft function. This is crucial for extended criteria donor hearts with questionable pathology and contractility (in which a clinical case report and a preclinical study showed that OCS allows graft assessment by contrast echocardiogram[40] and coronary angiogram[41] during perfusion, respectively), as well as for DCD organs. OCS heart assessment in the beating scenario is primarily based on biochemical parameters analysis (*e.g.*, arterial lactates trends, artero-venous lactates difference, pH, Na, K, Ca, glucose levels) and their pharmacological correction (which is not possible with SCS). However, these conventional metabolic parameters are not sufficiently validated for heart assessment,[42] and better metabolic markers already used for other organs should be studied for heart *ex vivo* systems retrieval (*e.g.*, IL-1, flavin mononucleotide).[43,44] However, quantitative functional analysis (by means of echocardiograms) or anatomical coronary evaluation (by means of angiograms) is still not widely standardized and practiced[40,41] and should be a field of additional investigation.

A disadvantage of *ex vivo* perfusion systems (both hypothermic and normothermic) is myocardial edema. Multiple mechanisms might be involved in edema formation: hyperperfusion is caused by high aortic perfusion pressure, and can be managed by turning OCS machine settings to reduce aortic flow (aortic pressure around 70 mmHg, coronary flow around 700 ml/min). Indeed, blood exposure to extracorporeal circuits can trigger a proinflammatory response and cause edema. For this reason, OCS protocol includes the addition of methylprednisolone to the perfusate solution.[45] Oncotic pressure is another variable that can affect the development of myocardial edema. Unfortunately, even if OCS priming or Steen solution (used for XVIVO perfusion) is hyperoncotic, the optimal oncotic pressure and the ideal impermeant (albumin, mannitol, lactobionic acid, dextran) have not been established. Future research should develop systems or perfusate solutions able to limit endothelial cell exposure to oxidative stress and sterile inflammation.

Indeed, *ex vivo* perfusion may provide an opportunity to administer therapies to the donor heart before transplantation to improve the metabolic state of the heart after an insult such as recent cardiac arrest, or to modify the immunologic state of

Table 1. Pros and Cons of Currently CE- and FDA-Approved *Ex Vivo* Machine Perfusion Systems

Pros	Cons
SherpaPak Cardiac Transport System, Paragonix	
Stable hypothermia (4–10°C)	Costs (20,000€)
Technically simple	Inability to assess graft function
Single operator	No graft perfusion
Low risk of organ loss because of technical problems	
Organ care system, Transmedics	
Beating normothermic graft (34°C)	Edema
Oxygen and nutrition delivery	Complexity of use
Metabolic waste removal	Costs (200,000€ consolle; 35,000€ single-use kit)
Biochemical assessment (lactates)	Risks of losing usable grafts because of technical and mechanical errors
Functional assessment (contractility)	Need of trained team
Marginal donors	Special arrangement for transportation morpho-functional graft assessment (dimensions)
DCD	

CE, European Community; DCD, donor after circulatory death; FDA, Food and Drug Administration.

the heart and minimize rejection in the recipient. The delivery of novel therapies seems to be more effective in normothermic conditions because myocardial cells are at a near-normal rate of metabolism.[3] Because the mechanisms responsible for myocardial damage both in the ischemic and reperfusion phase are related to the production of proinflammatory cytokines, this "sterile" inflammation represents the emerging target for the delivery of therapies during *ex vivo* perfusion. Emerging fields of interest (in animal models) are represented by molecules that can block the apoptotic process (necrostatin-1, small interfering RNAs)[46,47] or ROS production (MCI-186),[48] with positive effect on ischemic heart recovery (especially in DCD scenarios). However, these molecules have never been tested in humans or in currently approved *ex vivo* machine perfusion systems. Preliminary preclinical results on delivery methods for heart transplantation gene therapy have demonstrated that gene therapy might be feasible in OCS hearts to biologically modify donor's hearts.[49]

Conclusions

Despite a constant increase in patients listed for heart transplantation, heart replacement therapy is nowadays primarily limited by the reduced number of available donors. Both HMP and NMP have been recognized as safe and feasible in clinical practice, and they might allow a 15–20% increase in transplant volumes by using hearts that were otherwise rejected. Besides, with longer extracorporeal time, allografts could be shared over a larger geographic area with more comprehensive and better donor–recipient matching. Transplantation will be done with less stress and time pressure, and more available resource and personnel support.

The future direction of *ex vivo* machine perfusion will be the implementation of novel laboratory and instrumental techniques for *ex vivo* heart assessment. Indeed, further investigations will be required to optimize perfusate composition, improve evaluation protocol, and explore novel therapies to realize the full benefits of *ex vivo* machine perfusion devices and therefore justify the additional cost and required resources.

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