Interactive CardioVascular and Thoracic Surgery 20 (2015) 510–519
doi:10.1093/icvts/ivu432 Advance Access publication 23 December 2014



Cite this article as: Minasian SM, Galagudza MM, Dmitriev YV, Karpov AA, Vlasov TD. Preservation of the donor heart: from basic science to clinical studies. Interact CardioVasc Thorac Surg 2015;20:510–19.

# Preservation of the donor heart: from basic science to clinical studies

**Sarkis M. Minasian[a,b], Michael M. Galagudza[a,b,*], Yuri V. Dmitriev[a], Andrey A. Karpov[a,b] and Timur D. Vlasov[a,b]**

[a] Institute of Experimental Medicine, Federal Almazov Medical Research Centre, St Petersburg, Russian Federation
[b] Department of Pathophysiology, First Pavlov State Medical University of St Petersburg, St Petersburg, Russian Federation

* Corresponding author. Institute of Experimental Medicine, Federal Almazov Medical Research Centre, Akkuratova Str. 2, 197341 St Petersburg, Russian Federation. Tel: +7-812-4997035; fax: +7-812-4997069; e-mail: galagoudza@mail.ru (M.M. Galagudza).

Received 3 September 2014; received in revised form 7 November 2014; accepted 3 December 2014

## Abstract

The methods of donor heart preservation are aimed at minimizing graft dysfunction caused by ischaemia–reperfusion injury (IRI) which inevitably occurs during the *ex vivo* transport interval. At present, the standard technique of heart preservation is cardiac arrest followed by static cold storage in a crystalloid heart preservation solution (HPS). This technique ensures an acceptable level of heart protection against IRI for <6 h. In clinical trials, comparable levels of myocardial protection against IRI were provided by various HPSs. The growing shortage of donor hearts is one of the major factors stimulating the development of new techniques of heart preservation. Here, we summarize new HPS formulations and provide a focus for optimization of the composition of existing HPSs. Such methods of donor heart preservation as machine perfusion, preservation at sub-zero temperature and oxygen persufflation are also discussed. Furthermore, we review experimental data showing that pre- and post-conditioning of the cardiac graft can improve its function when used in combination with cold storage. The evidence on the feasibility of cardiac donation after circulatory death, as well as the techniques of heart reconditioning after a period of warm ischaemia, is presented. The implementation of new techniques of donor heart preservation may contribute to the use of hearts from extended criteria donors, thereby expanding the total donor pool.

**Keywords:** Donor heart preservation • Cold storage • Preservation solutions • Cardioprotection • Myocardial ischaemia–reperfusion injury

## INTRODUCTION

Donor heart preservation is aimed at minimizing graft dysfunction caused by ischaemia–reperfusion injury (IRI) which inevitably occurs during the *ex vivo* transport interval. At present, the standard technique of heart preservation is cardiac arrest followed by static cold storage (SCS) in a crystalloid preservation solution. Although research has made significant strides in improving the technology of donor heart preservation, IRI still occurs in a certain proportion of cardiac grafts owing to ineffective preservation and/or prolonged transportation. The quality of the cardiac graft is directly proportional to the cold ischaemic time, which should not exceed 6 h [1]. Consequently, there are two basic strategies to further improve the results of donor heart preservation: shortening of cold ischaemic time and refining preservation techniques, primarily by developing novel formulations of heart preservation solutions (HPSs). The problem of donor heart preservation was brought to light after the first human heart transplantation performed by Barnard in 1967. At that time, open heart surgery with cardiopulmonary bypass and cardioplegia was already introduced into clinical practice, and several cardioplegic solutions (CPSs) were successfully adopted for preservation of cardiac grafts. It should be noted, however, that the extent of myocardial IRI in heart transplantation may be greater than that observed in open heart surgery because of the lack of intermittent infusions of CPS and generally longer cold ischaemic times. Therefore, special HPSs with improved anti-ischaemic properties have been developed for donor heart preservation.

In this review, we summarize new HPS formulations and provide a focus for optimization of the composition of existing HPSs. Different methods of donor heart preservation are also discussed. For identification of relevant literature, electronic searches were performed of Ovid MEDLINE and PubMed from inception to August 2014. The search strategy used a combination of 'donor heart preservation' or 'heart preservation solution', or 'machine perfusion' or 'donation after circulatory death' as keywords. Manual searches of reference lists were used to identify any studies not found in the initial search.

## Standard heart preservation solutions

According to the systematic review of Demmy *et al*., as many as 167 HPSs were used for heart preservation in the USA in the middle of the 1990s [2]. At present, the histidine–tryptophan–ketoglutarate (HTK) solution, University of Wisconsin (UW) solution and Celsior solution are most commonly used for heart preservation.

HTK (or Custodiol) was introduced by Bretschneider in the 1970s as a cardioplegic solution and has subsequently been

applied for organ preservation, including the heart, liver and pancreas. Its usage has been increasingly popular in cardiac surgery because a single administration of cold HTK in the coronary vascular bed provides reliable protection of the heart from IRI for at least 2 h [3]. The chemical composition of HTK, as well as several other HPSs, is presented in Table 1. The Euro-Collins solution is an intracellular HPS with high concentrations of glucose (194 mmol/l) and $K^+$ (115 mmol/l) and the presence of bicarbonate and phosphate buffer systems. The Stanford solution is similar to Euro-Collins with the exception of an even higher glucose level (250 mmol/l) and a lower $K^+$ concentration (27 mmol/l). The UW solution developed by the group of Belzer typically has low $Na^+$ concentration, although an extracellular modification of this HPS that includes 125 mmol/l $Na^+$ is also known [4, 5]. Along with intracellular-type HPSs, there are several widely accepted extracellular solutions with high $Na^+$ and low $K^+$ concentrations. The most common extracellular HPS is Celsior. Celsior is enriched with the reactive oxygen species scavengers lactobionate and glutathione, which ensures prevention of oxidative stress during reperfusion. In contrast to other HPSs, Celsior contains 20 mmol/l glutamate, which has anti-ischaemic activity and exerts cardioprotective effects. In addition, Celsior, HTK and the Stanford solution contain the polyatomic alcohol mannitol, which can prevent oedema and quench reactive oxygen species.

**Table 1:** Chemical composition of the most commonly used heart preservation solutions

| | HTK | STF | UW | UW-1 | Celsior |
|---|---|---|---|---|---|
| Cations | | | | | |
| $Na^+$ | 15.00 | 20.00 | 30.00 | 125.00 | 100.00 |
| $K^+$ | 10.00 | 27.00 | 125.00 | 30.00 | 20.00 |
| $Mg^{2+}$ | 4.00 | – | 5.00 | 5.00 | 13.00 |
| $Ca^{2+}$ | 0.015 | – | – | – | 0.25 |
| Anions | | | | | |
| $Cl^-$ | 50.00 | 27.00 | – | – | 41.50 |
| $HPO_4^{2-}$ | – | – | – | – | – |
| $H_2PO_4^-$ | – | – | 25.00 | 25.00 | – |
| $HCO_3^-$ | – | 20.00 | – | – | – |
| $SO_4^{2-}$ | – | – | 5.00 | 5.00 | – |
| Substrates and metabolites | | | | | |
| Glucose | – | 250.00 | – | – | – |
| Glutamate | – | – | – | – | 20.00 |
| Ketoglutarate | 1.00 | – | – | – | – |
| Tryptophan | 2.00 | – | – | – | – |
| Adenosine | – | – | 5.00 | 5.00 | – |
| Metabolically inactive osmotic agents | | | | | |
| Mannitol | 30.00 | 60.00 | – | – | 60.00 |
| D-Raffinose | – | – | 35.40 | 35.40 | – |
| HES (g/l) | – | – | 50.00 | 50.00 | – |
| Antioxidants | | | | | |
| Lactobionate | – | – | 100.00 | 100.00 | 80.00 |
| Allopurinol | – | – | 1.00 | 1.00 | – |
| Glutathione | – | – | 3.00 | 3.00 | 3.00 |
| Organic buffers | | | | | |
| Histidine | 180.00 | – | – | – | 30.00 |
| Histidine-HCl | 18.00 | – | – | – | – |
| Others | | | | | |
| Osmolarity | 310 | 409 | 320 | 320 | 360 |

All concentrations except otherwise mentioned are in mmol/l.
HES: hydroxyethyl starch; HTK: histidine–tryptophan–ketoglutarate solution; STF: Stanford solution; UW: University of Wisconsin solution; UW-1: modified University of Wisconsin solution.

## Comparison of different heart preservation solutions in experimental studies

The majority of the available data on comparative effectiveness of HPSs were obtained in animal studies, both *in vitro* and *in vivo*. The most commonly used animal models include the isolated working rat heart [6] as well as models of SCS with subsequent ortho- or heterotopic transplantation [7, 8]. Experimental studies offer the advantage of using functional, biochemical, molecular and histological criteria as end-points of myocardial protection with HPSs. For example the recovery of heart function after a period of SCS is usually monitored in the isolated working heart model using such parameters as cardiac output, coronary flow and heart rate [9]. Left ventricular (LV) function could be precisely assessed using echocardiography and/or cardiac catheterization in the experiments with implantation of the donor heart to a recipient animal [10]. Among the biochemical markers of myocardial injury, the levels of cardiac troponins, creatine kinase (CK) and lactate dehydrogenase are usually determined in blood plasma [11] or in the reperfusate [12]. Important information about the status of myocardial energy metabolism could be obtained from measurements of the tissue adenosine triphosphate (ATP) level [9]. Another reliable parameter of transplant injury that is technically easy to obtain is the extent of myocardial oedema [13].

Some studies have utilized the activation of reperfusion injury salvage kinases, such as protein kinase B, extracellular signal-regulated kinase and glycogen synthase kinase-3β as a surrogate end-point of myocardial protection at the molecular level [14]. The infiltration of the myocardium with polymorphonuclear leucocytes and the integrity of the myocardial tissue are the main histological criteria used to assess the effectiveness of heart preservation [10]. In the majority of the studies on long-term donor heart storage, valuable information was obtained with electron microscopy showing ultrastructural signs of myocardial injury, such as sarcolemmal integrity and/or mitochondrial swelling [9, 13]. Endothelial function could be determined in the isolated heart after a period of SCS by means of measuring coronary flow reserve [15]. It must be emphasized that all the above criteria are more or less indirect and, therefore, they may not fully characterize the post-ischaemic function of the heart.

Several experimental studies compared the effects of different HPSs on post-ischaemic recovery of donor heart function. The comparative study of nine HPSs in the isolated rat heart model of SCS demonstrated the advantage of extracellular-type solutions [Celsior, the Lyon preservation solution, the St Thomas' Hospital cardioplegic solution No. 2 (STH2)] when compared with intracellular-type HPSs [5]. In fact, the intracellular Euro-Collins solution provided the same extent of myocardial protection as normal saline. Further, Celsior was found more effective than UW in canine and porcine orthotopic heart transplant models [8, 16]. Similar results were obtained in the isolated rat hearts preserved with one of four HPSs: Celsior, Krebs–Henseleit, HTK and STH2 [17]. The solutions were administered for 5 min either at +10°C or at +20°C, followed by 2-h global ischaemia at +20°C. Interestingly, the lower HPS temperature was associated with poorer recovery of LV function, potentially because of the injurious effects of hypothermia itself, while the best result was observed in the Celsior group in both temperature regimens.

A comparison of HTK and Celsior, representing classical intra- and extracellular-type solutions, respectively, in the Langendorff-perfused dog heart failed to demonstrate differences between the

two HPSs at 8 h of ischaemia, although HTK provided somewhat better recovery of LV function when the duration of ischaemia was increased to 12 h [18]. There is also some evidence that intracellular-type HPSs may be superior to extracellular-type HPSs. For example the use of HTK in the rat model of 6-h SCS and heterotopic heart transplantation was associated with lower levels of troponin and CK, less infiltration of the transplant with leucocytes and lower cardiac expression of proinflammatory cytokines than the use of Celsior [19]. Another study compared Celsior and UW in the isolated blood-reperfused rabbit heart subjected to 24 h of SCS. UW-treated hearts demonstrated improved recovery of LV function, a lower CK level in the recirculating blood and better preservation of endothelial function [15]. It follows, therefore, that the experimental studies produced controversial results with respect to the effectiveness of different HPSs in preserving heart function during cold storage. This fact might suggest that none of the currently available HPSs has a clear advantage over the others.

## Comparison of different heart preservation solutions in clinical settings

The number of heart transplantations has not changed significantly in the last decade owing to the shortage of donor organs and careful selection of recipients. The relatively low number of heart transplantations, that is, ∼3500 transplants per year worldwide, precludes testing of the effectiveness of HPSs in large-scale clinical trials. Therefore, our knowledge on the comparative effectiveness of HPSs is limited. One of the first randomized trials in the field demonstrated better myocardial protection with UW than with the Stanford solution [20]. A retrospective study of the two HPSs most commonly used in the USA, UW and Celsior, showed that the use of UW was associated with better survival over the periods of 30 days and 1 year [21]. Moreover, heart preservation with Celsior was associated with the use of higher doses of vasodilators and catecholamines postoperatively compared with UW, which is indicative of the presence of myocardial IRI manifesting as LV stunning [22].

A recent prospective randomized trial showed that there were no differences in mortality and incidence of biventricular graft failure between three groups of patients who received donor hearts arrested by and preserved in HTK, Celsior and STH2 [23]. To ensure more rapid cardiac arrest and minimize chamber distension, Lee *et al*. attempted to combine the use of extra- and intracellular HPSs in the same donor [24]. The technique included intracoronary infusion of 1l of cold STH2 solution followed by low-pressure administration of HTK. Although this approach has been demonstrated to be safe, the rationale for using two different HPSs in the same patient is questionable. Firstly, both of the above HPSs ensure rapid cardiac arrest. Secondly, prevention of heart distension does not depend on the effectiveness of cardioplegia and should be performed by means of appropriate heart venting.

## Strategies to improve donor heart preservation outcomes

Perhaps the main way to increase the viability of a donor heart is to limit the ischaemic time. However, in the majority of cases, this is impossible because of technical and logistical reasons. In this regard, several new approaches aimed at improved preservation of cardiac grafts and maintenance of graft function over extended time intervals have been suggested and experimentally validated in the last decades. These approaches include refinement of chemical composition of the HPSs, optimization of the regimen of HPS administration, machine perfusion of donor hearts, heart preservation at sub-zero temperatures, oxygen persufflation (PSF) and pre- and post-conditioning of cardiac grafts.

***Supplementation of standard heart preservation solutions with anti-ischaemic agents.*** Various active chemical compounds with putative anti-ischaemic properties have been tested as additives to standard HPSs in the preclinical models of SCS [6, 7, 9–14, 25–42]. The results of these studies, as well as the suggested underlying mechanisms, are summarized in Table 2. Although all the studies have demonstrated certain benefits in terms of myocardial IRI alleviation, none of the modified HPSs listed in Table 2 is currently approved for clinical use in the USA.

Additional improvements in the composition of HPSs may include increasing the buffer capacity and adding colloid components to the solutions. The currently available HPSs usually contain histidine or bicarbonate buffer, with phosphate and other buffer systems being less commonly used. The buffer capacity of an HPS is crucial for cardioprotection because the effective prevention of intra- and extracellular space acidification contributes to maintenance of glycolytic ATP production during ongoing ischaemia. Another important aspect of preservation solution composition is the presence of colloids. Such colloid components as high molecular weight dextran, gelatine and hydroxyethyl starch (HES) may effectively prevent intracellular oedema, ameliorate endothelial function and, at the same time, cause only minimal increase in total osmolarity of the HPS [43]. Supplementation of the HTK solution with a natural colloid, human albumin, resulted in improved endothelial integrity and myocardial function in the isolated guinea pig heart subjected to 4 h of SCS [44]. In this regard, it remains to be explored whether blood plasma-based HPSs may afford superior protection of the donor heart against IRI in marginal hearts.

***Novel formulations of heart preservation solutions.*** Over the last decade, several new formulations of HPSs have been developed and preclinically tested (Table 3). A new extracellular solution, Somah, includes energy substrates, metabolic modulators, antioxidants, L-arginine, and phosphate and bicarbonate buffers [45]. Four-hour preservation of porcine cardiac grafts harvested from donor animals with beating and non-beating hearts in Somah resulted in better viability of cardiac myocytes and endothelial cells, as well as a higher level of myocardial and endothelial protein expression when compared with that in controls [45]. Better cardioprotection was observed after 5-h preservation of a porcine heart in Somah at 21°C in comparison with Celsior and UW [46].

The CRMB solution was developed on the basis of Celsior by implementing several important differences: (i) the potassium concentration was decreased to the normal level in order to prevent potassium-mediated injury; (ii) histidine was excluded because of the putative histidine-mediated endothelial injury; (iii) adenosine, L-arginine and allopurinol were added [47]. Using the heterotopic heart transplantation model in rats, Desrois *et al*. showed that LV function, tissue high-energy phosphate level and nitrogen oxide production in the CRMB group were higher than in the Celsior group [47].

The new Krebs–Henseleit buffer-based (KHB) cardioplegic and preservation solution was developed by our group [48]. In

**Table 2:** Supplementation of heart preservation solutions with agents capable of reducing myocardial ischaemia–reperfusion injury

| Agent(s) | Mechanism(s) of myocardial protection | Model, duration of ischaemia and temperature | HPS | Main results | Ref. |
|---|---|---|---|---|---|
| Diadenosine tetraphosphate | Opening of mKATP channels | Isolated Langendorff-perfused rat heart, 8 h, +4°C | Euro-Collins | ↑ LV function, ↓ LDH and CK release | [25] |
| Cariporide and diazoxide or BMS-180448 | $Na^+/H^+$ exchanger inhibition and opening of potassium channels | Isolated working rat heart, 6 h, +4°C | Extracellular solution | ↑ LV function | [26] |
| Cyclosporin A | Inhibition of mitochondrial permeability transition pore opening | Isolated working rat heart, 12 h, +4°C | UW | ↑ LV function, ↑ tissue ATP level, ↓ tissue oedema, ↓ cytochrome c release | [27] |
| Cariporide | $Na^+/H^+$ exchanger inhibition | Orthotopic heart transplantation, pig, 4 h, +4°C | Extracellular solution | ↑ LV function, ↓ troponin I release | [28] |
| U74389G (16-desmethyl tirilazad) | Inhibition of lipid peroxidation | Orthotopic heart transplantation, pig, 6 h, +4°C | Aspartate-enriched extracellular solution | ↑ LV function, ↓ tissue oedema | [29] |
| Recombinant human hepatocyte growth factor | Inhibition of apoptosis | Isolated Langendorff-perfused rat heart; 4, 6 and 8 h, +4°C | Euro-Collins | ↑ LV function, ↓ apoptosis | [30] |
| Glyceryl trinitrate and cariporide | Increased NO and inhibition of $Na^+/H^+$ exchanger | Isolated working rat heart, 6 and 10 h, +4°C | Celsior | ↑ LV function | [6] |
| Taurine (2-aminoethanesulfonic acid) | Antioxidant activity, inhibition of apoptosis | Isolated Langendorff-perfused rat heart, 6 h, +4°C | STH2 | ↑ LV function, ↓ LDH and CK release, ↓ apoptosis | [31] |
| Nucleoside–nucleotide mixture | Favourable effect on energy metabolism | Isolated working rat heart, 12 h, +4°C | UW | ↑ LV function, ↑ tissue high-energy phosphates | [32] |
| Tetrahydrobiopterin | Increased activity of NO synthase | Isolated Langendorff-perfused rat heart, 8 h, +4°C | STH2 | ↑ LV function, ↑ tissue ATP level, ↑ nitric oxide availability | [33] |
| INO-1153 | Poly(ADP-ribose) polymerase 1 inhibition | Isolated working rat heart, 6 h, +4°C | Celsior | ↑ LV function | [34] |
| Hydrogen sulphide donor | Vasoprotection, opening of mKATP channels, inhibition of cytochrome c oxidase | Isolated Langendorff-perfused rat heart, 6 h, +4°C | Krebs–Henseleit solution | ↑ LV function, ↓ apoptosis | [35] |
| Bisindolylmaleimide derivative | Antioxidant effect, decreased $Ca^{2+}$ overload | Isolated working rat heart, 12 h, +4°C | UW | ↑ LV function, ↑ tissue ATP level, preserved myocardial structure | [9] |
| Carbon monoxide-releasing molecule (CORM-3) | Multiple cardioprotective effects of carbon monoxide | Isolated Langendorff-perfused rat heart, 4 and 6 h, +4°C | STH2 | ↑ LV function, ↓ LDH and CK release | [36] |
| Iloprost | Vasodilation, anti-inflammatory properties | Isolated Langendorff-perfused rat heart, 6 h, +4°C | STH2 | ↑ LV function, ↓ oxidative stress | [37] |
| Rho-kinase inhibitor | Inhibition of proinflammatory pathways, improved endothelial function, inhibition of apoptosis | Isolated working blood-perfused rabbit heart, 24 h, +4°C | UW | ↑ LV function, ↑ coronary blood flow, ↓ endothelial dysfunction | [38] |
| Small interfering RNA | Reduced expression of TNFα, complement 3 and Fas | Heterotopic transplantation, mouse, 48 h, +4°C | UW | ↑ LV function, preserved myocardial structure, ↓ PMN infiltration | [10] |
| Epoxomicin | 26S proteasome inhibitor | Heterotopic transplantation, rat, 12 and 24 h, +4°C | UW | ↓ troponin I release, preserved myocardial structure, ↓ tissue oedema | [13] |
| Pinacidil | Opening of mKATP channels | Isolated Langendorff-perfused rat heart, 8 h, +4°C | HTK | ↑ LV function, ↓ ultrastructural injury, ↓ troponin I release | [39] |
| Neuregulin-1, glyceryl trinitrate, cariporide | Increased NO, inhibition of $Na^+/H^+$ exchanger, inhibition of apoptosis | Isolated working rat heart, 6 and 10 h, +4°C | Celsior | ↑ LV function, ↑ phosphorylation of RISK | [14] |
| Levocarnitine | Metabolic protection | Isolated working rat heart, 4 and 6 h, +4°C | STH2 | ↑ LV function, ↑ tissue ATP level, ↓ tissue oedema | [40] |
| Clusterin | Recombinant chaperone protein | Heterotopic transplantation, mouse, 24 h, +4°C | UW | ↓ LDH release, ↓ PMN infiltration | [7] |

*Continued*

**Table 2:** (*Continued*)

| Agent(s) | Mechanism(s) of myocardial protection | Model, duration of ischaemia and temperature | HPS | Main results | Ref. |
|---|---|---|---|---|---|
| Hydrogen (1.27 ± 0.05 μg/l) | Antioxidant and anti-inflammatory effect | Heterotopic transplantation, rat, 6 and 8 h, +4°C | Celsior | ↓ troponin and CK release, ↑ tissue ATP level, preserved myocardial structure, ↓ PMN infiltration | [11] |
| Erythropoietin (5 U/ml) | Activation of RISK pathway, inhibition of apoptosis | Isolated working rat heart, 6 and 10 h, +4°C | Celsior | ↑ LV function, ↓ LDH release | [12] |
| Doxycycline | Inhibition of matrix metalloproteinase-2 | Isolated working rat heart, 1 h, +4°C | Krebs buffer | ↑ LV function, ↓ arrhythmia, ↓ apoptosis, ↑ expression of Akt | [41] |
| Levosimendan | $Ca^{2+}$ sensitization, positive inotropy, inhibition of programmed cell death | Isolated Langendorff-perfused rat heart, 9 h, +4°C | Celsior | ↑ LV function, ↓ apoptosis | [42] |

CK: creatine kinase; HPS: heart preservation solution; LDH: lactate dehydrogenase; LV: left ventricle; mKATP: mitochondrial ATP-sensitive potassium channels; NO: nitric oxide; PMN: polymorphonuclear leucocytes; RISK: reperfusion injury salvage kinase; STH2: St Thomas' Hospital cardioplegic solution No. 2; TNFα: tumour necrosis factor α; UW: University of Wisconsin solution.

comparison with STH2, this solution afforded better myocardial protection in the isolated rat heart not only in the mildly hypothermic state, but also at normothermia. The robust protective effect of the KHB solution might be attributed to the presence of glucose at a concentration of 11 mmol/l, concerted effect of phosphate and bicarbonate buffer systems, decreased $Ca^{2+}$ concentration and mild hyperosmolarity. One more example of a new formulation of extracellular HPS is Dsol, which was developed on the basis of UW with high-sodium and low-potassium content [49]. The major differences of this HPS from its prototype are the lack of HES and replacement of raffinose with such cheaper impermeants as sucrose and mannitol. In addition, 30% of water in the solvent is replaced by deuterium oxide, which has been shown to exert anti-ischaemic effects [49]. It was demonstrated in the rat model of heterotopic heart transplantation that 24- or 36-h graft preservation in Dsol resulted in increased graft survival and lower extent of graft injury.

The novel solution Custodiol-N is a typical intracellular-type HPS which differs from HTK by a lower concentration of histidine, addition of certain amino acids and iron chelators, and replacement of mannitol with sucrose [50]. The superiority of Custodiol-N over HTK was demonstrated in the rat model of heterotopic heart transplantation [50]. Thus, several new HPSs of both extra- and intracellular types have been shown to be more effective than standard solutions. Despite these encouraging results, further rigorous preclinical testing of new HPSs will be required before clinical trials can be contemplated.

***Hyperpolarizing heart preservation solutions.*** It is known that standard high-potassium CPSs and HPSs cause cell depolarization resulting in inactivation of voltage-dependent sodium and calcium channels, which in turn prevents action potential generation. There are some indications that intracellular HPS HTK containing elevated $K^+$ concentrations may cause hyperpolarization rather than depolarization of the cell because of its low $Na^+$ content [51]. This concept requires further experimental validation because the mechanism of cell hyperpolarization in the absence of increased $K^+$ efflux from the cell and very low resting membrane permeability to $Na^+$ remains unclear.

To induce cardiomyocyte hyperpolarization, different chemical agents have been used as additives to CPSs, including adenosine, lidocaine, acetylcholine, an ultra-short-acting β-blocker esmolol and even the blocker of fast $Na^+$ channels tetrodotoxin [52]. Despite the fact that hyperpolarizing CPSs produce more physiological cardiac arrest, which is not associated with severe ionic imbalance, they are virtually not used in the clinics. There are several reports on the use of hyperpolarizing HPSs in experimental settings. The addition of the $K^+$ channel opener pinacidil to an intracellular histidine- and lactobionate-containing HPS resulted in improved LV function recovery upon reperfusion in comparison with the unmodified HPS and UW solution [53]. A substantial protective effect was also found after preservation of a rat cardiac graft in the polarizing normokalemic solution Adenocaine containing adenosine and lidocaine [54]. In a special series of experiments, Adenocaine with insulin, melatonin and double concentrations of adenosine and lidocaine (400 and 1000 μmol/l, respectively) was compared with standard HPSs [55]. This version of Adenocaine ensured the best LV recovery, whereas standard Adenocaine, Celsior and Custodiol provided inferior protection. It is clear from these data that hyperpolarizing solutions may hold promise for clinical use.

***Donor heart perfusion.*** In the very beginning of the heart transplantation era, donor heart function was maintained by *ex vivo* perfusion with blood or a crystalloid perfusion fluid. However, the introduction of the SCS technique in combination with cardiac arrest has supplanted the use of perfusion systems, mainly because of their high cost and technical complexity. Over the last 10 years, the interest to the technique of machine perfusion has been renewed (for review, see [56]). Machine perfusion of the heart can be performed in two different ways: (i) continuous perfusion of the arrested heart by oxygenated cold CPS; and (ii) perfusion of the beating heart with blood at normothermia. The second approach is especially useful for reconditioning of the hearts obtained after circulatory death (see the next section), but it is much more technically demanding in terms of supporting heart function during transportation and ideally should be complemented by monitoring of heart viability. Several techniques

**Table 3:** Chemical composition of new heart preservation solutions

| | Somah | CRMB | Dsol | HTK-N | KHB |
|---|---|---|---|---|---|
| Cations | | | | | |
| $Na^+$ | 130.19 | 120.00 | 125.00 | 16.00 | 143.00 |
| $K^+$ | 7.44 | 4.00 | 25.00 | 10.00 | 25.00 |
| $Mg^{2+}$ | 1.00 | 13.00 | 5.00 | 8.00 | 16.00 |
| $Ca^{2+}$ | 1.30 | 0.25 | – | 0.02 | 0.30 |
| Anions | | | | | |
| $Cl^-$ | 135.60 | 28.50 | – | 30.00 | 141.80 |
| $HPO_4^{2-}$ | 0.19 | – | – | – | 1.20 |
| $H_2PO_4^-$ | 0.44 | 2.00 | 25.00 | – | – |
| $HCO_3^-$ | 5.00 | – | – | – | 25.00 |
| $SO_4^{2-}$ | 0.50 | – | 5.00 | – | 16.00 |
| Substrates and metabolites | | | | | |
| Glucose | 11.00 | – | – | – | 11.00 |
| Glutamate | – | 20.00 | – | – | – |
| Ketoglutarate | – | – | – | 2.00 | – |
| Tryptophan | – | – | – | 2.00 | – |
| Adenosine | 2.00 | 0.50 | 5.00 | – | – |
| L-Arginine | 5.00 | 2.00 | – | – | – |
| L-Citrulline malate | 1.00 | – | – | – | – |
| Creatine orotate | 0.50 | – | – | – | – |
| Creatine × $H_2O$ | 2.00 | – | – | – | – |
| L-Carnosine | 10.00 | – | – | – | – |
| L-Carnitine | 10.00 | – | – | – | – |
| Dichloroacetate | 0.50 | – | – | – | – |
| Insulin (g/l) | 1.00 | – | – | – | – |
| Glycine | – | – | – | 10.00 | – |
| Aspartate | – | – | – | 5.00 | – |
| Alanine | – | – | – | 5.00 | – |
| Deferoxamine | – | – | – | 0.025 | – |
| LK-614 | – | – | – | 0.0075 | – |
| Metabolically inactive osmotic agents | | | | | |
| Mannitol | – | 30.00 | 10.00 | – | – |
| D-Raffinose | – | 30.00 | – | – | – |
| Sucrose | – | – | 20.00 | 33.00 | – |
| Antioxidants | | | | | |
| Lactobionate | – | 100.00 | 100.00 | – | – |
| Allopurinol | – | 1.00 | 1.00 | – | – |
| Glutathione | 1.50 | 3.00 | 3.00 | – | – |
| Ascorbic acid | 1.00 | – | – | – | – |
| Organic buffers | | | | | |
| L-Histidine | – | – | – | 124.00 | – |
| *N*-α-Acetyl-L-histidine | – | – | – | 57.00 | – |

All concentrations except otherwise mentioned are in mmol/l.



have been suggested for viability monitoring, including real-time assessment of myocardial pH, determination of fractional anisotropy by diffusion tensor magnetic resonance imaging and monitoring nicotinamide adenine dinucleotide and flavin adenine dinucleotide autofluorescence [56, 57].

At present, two randomized clinical trials evaluating new heart perfusion systems are underway. In these trials, the Transmedics organ care system (Transmedics, Inc., USA) and the LifeCradle® system (Organ Transport Systems, Inc., USA) are used to support heart function during transportation. In parallel to clinical studies, the donor heart perfusion methods are compared with the SCS technique in animal experiments. For example the effects of 4-h SCS of a pig heart in Celsior on LV function, CK level and myocardial water content were compared with the effects of 4-h heart perfusion with oxygenated cold Celsior (10 ml/100 g/min) in the orthotopic transplant model [58]. Perfusion resulted in better LV function recovery and lesser myocardial injury, and it was not associated with increased oedema. Another study compared two techniques of porcine heart preservation: (i) cardiac arrest with cold HTK followed by 30-min cold storage and normothermic perfusion with leucocyte-depleted blood; and (ii) immediate heart perfusion with leucocyte-depleted blood without using cardioplegia and hypothermia [59]. After 12 h of perfusion, the structural integrity of the myocardium was better in the second group. Retrograde pig heart perfusion with leucocyte-depleted blood for 8 h at normothermia resulted in greater cardiac output, lower incidence of arrhythmia and better preservation of myocardial ultrastructure compared with the hearts arrested in HTK at +4°C [60]. Very recently, a novel device for pulsatile oxygenated hypothermic perfusion of the donor heart (Paragonix Sherpa Perfusion™ Cardiac Transport System) was tested in the pig hearts subjected to 4-h *ex vivo* perfusion versus SCS in Celsior solution [61]. It was found that hypothermic machine perfusion resulted in lesser endothelial dysfunction and better preservation of myocardial ultrastructure.

Despite the growing body of evidence on the feasibility of using cardiac graft *ex vivo* perfusion to preserve myocardial function, the optimal perfusion regimen and composition of the perfusion fluid remain to be determined. A detailed description of heart perfusion systems, as well as the analysis of outcomes is beyond the scope of this review; the interested reader is referred to a comprehensive review [62].

***Donor heart preservation at sub-zero temperature.*** Although myocardial oxygen consumption decreases dramatically during SCS of the donor heart at +4°C, several attempts to minimize cardiac energy demand by graft cooling to sub-zero temperatures have been reported. To prevent freezing-mediated irreversible cell injury, preservation solutions have been supplemented with antifreeze proteins (AFPs) possessing cryoprotective properties. For instance the rat hearts stored in the UW solution containing AFP I or AFP III at −1.3°C for 21 h displayed better LV function and a lower rate of apoptosis after heterotopic transplantation in comparison with controls [63]. An interesting method of sub-zero heart preservation has been recently described by Kato *et al.* [64], who placed UW-solution-submerged rat hearts into a variable magnetic field at −3.0°C. The hearts were stored for 24 h, followed by 120-min reperfusion. The sub-zero preservation resulted in an improved post-ischaemic LV function, increased myocardial ATP level and decreased tissue oedema.

***Perfusion (persufflation) of donor heart with gaseous oxygen.*** Oxygen PSF emerged in the early 1960s as an experimental strategy of *ex vivo* donor heart preservation involving infusion of humidified gaseous oxygen into the coronary vascular bed [65]. The rationale for using PSF in heart preservation was to avoid possible myocardial hypoxia during SCS and ensure adequate oxygen delivery to the heart during transportation. It is noteworthy that significant acidosis and depletion of ATP stores were observed in hearts preserved with SCS as early as after 4 h [66]. It was shown that antegrade PSF of pig hearts for 14 h resulted in better functional recovery of the LV function after orthotopic transplantation than after SCS [67]. PSF was also successfully used for reconditioning and preservation of non-beating pig hearts subjected to 16.7 min of warm ischaemia [68]. In controls, the hearts were harvested in the beating state with subsequent SCS. Of note, 3 h after orthotopic transplantation, both the LV function and histological myocardial structure were similar in the PSF and control groups. Despite the existence of promising experimental data, PSF is still considered to be an 'exotic' technique of donor heart preservation, which might be attributed, firstly, to the subjective reluctance of most clinicians to introduce gas into the vasculature and, secondly, serious concerns about the development of PSF-mediated endothelial injury. However, large animal experiments have convincingly demonstrated that PSF for 3 h is not associated with coronary endothelial dysfunction or alterations in the endothelial ultrastructure [69]. Thus, oxygen PSF might be considered an interesting option for donor heart preservation, which deserves further experimental and clinical investigation.

***Pre- and post-conditioning of cardiac graft.*** The increased myocardial tolerance to IRI after a single or several brief episodes of ischaemia–reperfusion is referred to as local ischaemic preconditioning (PreCon) [70]. A large body of experimental evidence suggests that PreCon represents one of the most effective endogenous cardioprotective interventions developed thus far [71]. The predictability of ischaemia onset and direct access to the heart are major prerequisites for successful clinical use of PreCon, and both of these conditions are fulfilled during heart procurement for transplantation. The first experimental evidence on the protective effect of PreCon in the settings of prolonged hypothermic heart storage came from the study demonstrating improved post-ischaemic LV functional recovery after one 5-min cycle of normothermic ischaemia–reperfusion prior to 10 h of global ischaemia at 4°C [72]. Using the orthotopic heart transplantation model in sheep, Landymore *et al.* showed that a single episode of PreCon significantly reduced myocardial stunning and increased the tissue ATP level [73]. Subsequent studies have demonstrated that the protective effect of PreCon on cardiac grafts could be further enhanced by administration of the $Na^+/H^+$ exchanger inhibitor cariporide during the PreCon episode [74]. Myocardial ischaemic post-conditioning (PostCon) is viewed as a way of cardiac protection against reperfusion injury, which is achieved by the induction of several brief episodes of ischaemia during the initial stage of reperfusion after prolonged ischaemic insult. A PostCon protocol consisting of three 30-s episodes of global ischaemia performed after 4 h of hypothermic rat heart storage resulted in improved postischaemic LV function, suppressed reperfusion arrhythmias and reduced superoxide anion production [75].

Despite technical feasibility and encouraging experimental results, donor heart PreCon and PostCon have not been tested clinically. It seems that these methods may be of value in protecting human cardiac grafts against IRI when used in combination with the standard techniques of heart preservation.

## Reconditioning of donor hearts obtained after circulatory death

At present, donation after circulatory death (DCD) has been successfully adopted for such organ transplants as the kidney and liver. The use of hearts procured after DCD might strongly contribute to the expansion of the donor pool. The possibility of heart resuscitation and transplantation after circulatory death has been explored in several experimental studies. In particular, pig hearts harvested after 30 min of warm ischaemia were reconditioned by perfusion with a leucocyte-depleted blood-based CPS containing adenosine and an inhibitor of the $Na^+/H^+$ exchanger followed by orthotopic transplantation [76]. The hearts were preserved in HTK for 3 h prior to implantation. The outcomes of transplantation were similar between non-heart-beating and heart-beating donors. In the rat model, the hearts were procured 30 min after euthanasia and preserved for 24 h in Somah at 4, 10, 21 or 37°C. Myocyte viability, mitochondrial function, tissue ATP level and expression of contractile proteins were best preserved in the hearts stored at 21°C [77]. Reconditioning of rat hearts obtained after 25 min of warm ischaemia by means of perfusion with diluted autologous blood with subsequent 4-h preservation in cold HTK resulted in post-ischaemic LV function comparable with that in non-reconditioned hearts and hearts that were not exposed to warm ischaemia [78]. Interesting preliminary data were obtained on reconditioning of a human heart exposed to warm ischaemia for 17 min [79]. The heart was first arrested by infusion of a custom-developed CPS followed by 2.5 h of perfusion with an organ perfusion solution at 10°C at a rate of 20 ml/min. After that, the heart was placed on an *ex vivo* rig and perfused with warm donor blood for 12 h. Although the diastolic pressure in the LV at

the end of perfusion averaged ~70 mmHg, there were no histological signs of myocardial necrosis.

At present, there is only one published clinical study describing the outcome of heart transplantation after DCD in three children [80]. In this study, the mean warm ischaemic time averaged 18.3 min. The outcomes of transplantation were not different from those obtained in paediatric patients receiving heart transplants from donors after brain death. Therefore, the use of cardiac grafts procured from DCD donors may increase the number of heart transplantations.

## CONCLUSION

The main factor limiting the number of heart transplantations in patients with end-stage heart failure is the critical shortage of donor hearts suitable for transplantation. This problem becomes especially pressing if one takes into consideration the fact that patients on the waiting list have very poor prognosis and high mortality. These challenges point out the urgent need for improving the quality of donor heart preservation. The implementation of new techniques of donor heart preservation may contribute to the use of hearts from extended criteria donors, thereby expanding the total donor pool.

## Funding

This work was supported by a grant from Russian Scientific Foundation [project 14-15-00473 (Myocardial protection against ischaemia–reperfusion injury using targeted delivery of cardioprotective substances)] and also by a grant of the President of the Russian Federation for support of leading scientific groups 1611.2014.7.

**Conflict of interest:** none declared.

## REFERENCES

[1] Hicks M, Hing A, Gao L, Ryan J, Macdonald PS. Organ preservation. Methods Mol Biol 2006;333:331–74.
[2] Demmy TL, Biddle JS, Bennett LE, Walls JT, Schmaltz RA, Curtis JJ. Organ preservation solutions in heart transplantation—patterns of usage and related survival. Transplantation 1997;63:262–9.
[3] Gebhard MM, Preusse CJ, Schnabel PA, Bretschneider HJ. Different effects of cardioplegic solution HTK during single or intermittent administration. Thorac Cardiovasc Surg 1984;32:271–6.
[4] Wahlberg JA, Southard JH, Belzer FO. Development of a cold storage solution for pancreas preservation. Cryobiology 1986;23:477–82.
[5] Michel P, Vial R, Rodriguez C, Ferrera R. A comparative study of the most widely used solutions for cardiac graft preservation during hypothermia. J Heart Lung Transplant 2002;21:1030–9.
[6] Gao L, Hicks M, MacDonald PS. Improved preservation of the rat heart with celsior solution supplemented with cariporide plus glyceryl trinitrate. Am J Transplant 2005;5:1820–26.
[7] Guan Q, Li S, Yip G, Gleave ME, Nguan CY, Du C. Decrease in donor heart injury by recombinant clusterin protein in cold preservation with University of Wisconsin solution. Surgery 2012;151:364–71.
[8] Mohara J, Morishita Y, Takahashi T, Oshima K, Yamagishi T, Takeyoshi I *et al*. A comparative study of Celsior and University of Wisconsin solutions based on 12-hr preservation followed by transplantation in canine models. J Heart Lung Transplant 1999;18:1202–10.
[9] Katare RG, Zhitian Z, Sodeoka M, Sasaguri S. A novel bisindolylmaleimide derivative enhances functional recovery of heart after long-term hypothermic heart preservation. Transplantation 2007;83:1588–94.
[10] Zheng X, Lian D, Wong A, Bygrave M, Ichim TE, Khoshniat M *et al*. Novel small interfering RNA-containing solution protecting donor organs in heart transplantation. Circulation 2009;120:1099–107.
[11] Noda K, Shigemura N, Tanaka Y, Kawamura T, Hyun Lim S, Kokubo K *et al*. A novel method of preserving cardiac grafts using a hydrogen-rich water bath. J Heart Lung Transplant 2013;32:241–50.
[12] Watson AJ, Gao L, Sun L, Tsun J, Jabbour A, Ru Qiu M *et al*. Enhanced preservation of the rat heart after prolonged hypothermic ischemia with erythropoietin-supplemented Celsior solution. J Heart Lung Transplant 2013;32:633–40.
[13] Baker TA, Geng Q, Romero J, Picken MM, Gamelli RL, Majetschak M. Prolongation of myocardial viability by proteasome inhibition during hypothermic organ preservation. Biochem Biophys Res Commun 2010; 401:548–53.
[14] Jabbour A, Gao L, Kwan J, Watson A, Sun L, Qiu MR *et al*. A recombinant human neuregulin-1 peptide improves preservation of the rodent heart after prolonged hypothermic storage. Transplantation 2011;91:961–7.
[15] Kajihara N, Morita S, Tanoue Y, Boku N, Eto M, Nishida T *et al*. The UW solution has greater potential for longer preservation periods than the Celsior solution: comparative study for ventricular and coronary endothelial function after 24-h heart preservation. Eur J Cardiothorac Surg 2006; 29:784–9.
[16] Warnecke G, Schulze B, Haverich A, Klima U. Celsior solution provides superior post-ischemic right ventricular function as compared with UW solution in a porcine heart transplantation model. J Heart Lung Transplant 2002;21:586–9.
[17] Lima ML, Fiorelli AI, Vassallo DV, Batista PR, Simoes FV, Fiorim J *et al*. Deleterious effect of hypothermia in myocardial protection against cold ischemia: a comparative study in isolated rat hearts. Transplant Proc 2012; 44:2326–32.
[18] Ackemann J, Gross W, Mory M, Schaefer M, Gebhard MM. Celsior versus custodiol: early postischemic recovery after cardioplegia and ischemia at 5 degrees C. Ann Thorac Surg 2002;74:522–9.
[19] Lee S, Huang CS, Kawamura T, Shigemura N, Billiar TR, Nakao A *et al*. Histidine-tryptophan-ketoglutarate or celsior: which is more suitable for cold preservation for cardiac grafts from older donors? Ann Thorac Surg 2011;91:755–63.
[20] Stein DG, Drinkwater DC Jr, Laks H, Permut LC, Sangwan S, Chait HI *et al*. Cardiac preservation in patients undergoing transplantation. A clinical trial comparing University of Wisconsin solution and Stanford solution. J Thorac Cardiovasc Surg 1991;102:657–65.
[21] George TJ, Arnaoutakis GJ, Baumgartner WA, Shah AS, Conte JV. Organ storage with University of Wisconsin solution is associated with improved outcomes after orthotopic heart transplantation. J Heart Lung Transplant 2011;30:1033–43.
[22] Wildhirt SM, Weis M, Schulze C, Conrad N, Rieder G, Enders G *et al*. Effects of Celsior and University of Wisconsin preservation solutions on hemodynamics and endothelial function after cardiac transplantation in humans: a single-center, prospective, randomized trial. Transpl Int 2000; 13(Suppl 1):S203–11.
[23] Cannata A, Botta L, Colombo T, Russo CF, Taglieri C, Bruschi G *et al*. Does the cardioplegic solution have an effect on early outcomes following heart transplantation? Eur J Cardiothorac Surg 2012;41:e48–52.
[24] Lee KC, Chang CY, Chuang YC, Sue SH, Yang HS, Weng CF *et al*. Combined St. Thomas and histidine-tryptophan-ketoglutarate solutions for myocardial preservation in heart transplantation patients. Transplant Proc 2012; 44:886–9.
[25] Ahmet I, Sawa Y, Nishimura M, Kitakaze M, Matsuda H. Cardioprotective effect of diadenosine tetraphosphate (AP4A) preservation in hypothermic storage and its relation with mitochondrial ATP-sensitive potassium channels. Transplantation 2000;69:16–20.
[26] Cropper JR, Hicks M, Ryan JB, Macdonald PS. Enhanced cardioprotection of the rat heart during hypothermic storage with combined Na+ - H+ exchange inhibition and ATP-dependent potassium channel activation. J Heart Lung Transplant 2003;22:1245–53.
[27] Rajesh KG, Sasaguri S, Ryoko S, Maeda H. Mitochondrial permeability transition-pore inhibition enhances functional recovery after long-time hypothermic heart preservation. Transplantation 2003;76:1314–20.
[28] Ryan JB, Hicks M, Cropper JR, Garlick SR, Kesteven SH, Wilson MK *et al*. Cariporide (HOE-642) improves cardiac allograft preservation in a porcine model of orthotopic heart transplantation. Transplantation 2003; 75:625–31.
[29] Ryan JB, Hicks M, Cropper JR, Nicholson A, Kesteven SH, Wilson MK *et al*. Lazaroid (U74389G)-supplemented cardioplegia: results of a double-blind, randomized, controlled trial in a porcine model of orthotopic heart transplantation. J Heart Lung Transplant 2003;22:347–56.

[30] Ryugo M, Sawa Y, Ono M, Fukushima N, Aleshin AN, Mizuno S *et al*. Myocardial protective effect of human recombinant hepatocyte growth factor for prolonged heart graft preservation in rats. Transplantation 2004; 78:1153–8.
[31] Oriyanhan W, Yamazaki K, Miwa S, Takaba K, Ikeda T, Komeda M. Taurine prevents myocardial ischemia/reperfusion-induced oxidative stress and apoptosis in prolonged hypothermic rat heart preservation. Heart Vessels 2005;20:278–85.
[32] Xing Y, Gopalrao Rajesh K, Suzuki R, Yamamoto M, Rao P, Sasaguri S. Supplementation of nucleoside-nucleotide mixture enhances functional recovery and energy metabolism following long-time hypothermic heart preservation. J Surg Res 2005;127:144–50.
[33] Yamashiro S, Kuniyoshi Y, Arakaki K, Uezu T, Miyagi K, Koja K. Cardioprotective effects of tetrahydrobiopterin in cold heart preservation after cardiac arrest. Ann Thorac Cardiovasc Surg 2006;12:95–104.
[34] Gao L, Kwan JC, Macdonald PS, Yang L, Preiss T, Hicks M. Improved post-storage cardiac function by poly (ADP-ribose) polymerase inhibition: role of phosphatidylinositol 3-kinase Akt pathway. Transplantation 2007;84: 380–6.
[35] Hu X, Li T, Bi S, Jin Z, Zhou G, Bai C *et al*. Possible role of hydrogen sulfide on the preservation of donor rat hearts. Transplant Proc 2007;39:3024–9.
[36] Musameh MD, Green CJ, Mann BE, Fuller BJ, Motterlini R. Improved myocardial function after cold storage with preservation solution supplemented with a carbon monoxide-releasing molecule (CORM-3). J Heart Lung Transplant 2007;26:1192–8.
[37] Dedeoglu BD, Aytac E, Suzer O, Balci H, Uzun H, Seymen P *et al*. Donor heart preservation with iloprost supplemented St. Thomas Hospital cardioplegic solution in isolated rat hearts. Prostaglandins Leukot Essent Fatty Acids 2008;78:415–21.
[38] Kobayashi M, Tanoue Y, Eto M, Baba H, Kimura S, Oda S *et al*. A Rho-kinase inhibitor improves cardiac function after 24-hour heart preservation. J Thorac Cardiovasc Surg 2008;136:1586–92.
[39] Yang L, Yu T. Prolonged donor heart preservation with pinacidil: the role of mitochondria and the mitochondrial adenosine triphosphate-sensitive potassium channel. J Thorac Cardiovasc Surg 2010;139:1057–63.
[40] Tao Z, Da-Guo Z, Dao-Kang X. Protective effects of St. Thomas no. 2 solution supplemented with levocarnitine on preservation of hypothermic heart ex vivo. J Clin Rehab Tissue Eng Res 2011;15:5809–12.
[41] Ozcinar E, Okatan EN, Tuncay E, Eryilmaz S, Turan B. Improvement of functional recovery of donor heart following cold static storage with doxycycline cardioplegia. Cardiovasc Toxicol 2014;14:64–73.
[42] Zhou HY, Zhang LN, Zheng MZ, Wang LL, Chen YY, Shen YL. Improved myocardial function with supplement of levosimendan to Celsior solution. J Cardiovasc Pharmacol 2014; 64:256–65.
[43] Zausig YA, Chappell D, Becker BF, Potschka D, Busse H, Nixdorf K *et al*. The impact of crystalloidal and colloidal infusion preparations on coronary vascular integrity, interstitial oedema and cardiac performance in isolated hearts. Crit Care 2013;17:R203.
[44] Jacob M, Paul O, Mehringer L, Chappell D, Rehm M, Welsch U *et al*. Albumin augmentation improves condition of guinea pig hearts after 4 hr of cold ischemia. Transplantation 2009;87:956–65.
[45] Thatte HS, Rousou L, Hussaini BE, Lu XG, Treanor PR, Khuri SF. Development and evaluation of a novel solution, Somah, for the procurement and preservation of beating and nonbeating donor hearts for transplantation. Circulation 2009;120:1704–13.
[46] Lowalekar SK, Cao H, Lu XG, Treanor PR, Thatte HS. Sub-normothermic preservation of donor hearts for transplantation using a novel solution, Somah: a comparative pre-clinical study. J Heart Lung Transplant 2014;33: 963–70.
[47] Desrois M, Caus T, Belles PM, Dalmasso C, Lan C, Cozzone PJ *et al*. Limitation of myocardial and endothelial injury of the rat heart graft after preservation with Centre de Resonance Magnetique Biologique et Medicale (CRMB) solution. Transpl Int 2008;21:276–83.
[48] Minasian SM, Galagudza MM, Dmitriev YV, Kurapeev DI, Vlasov TD. Myocardial protection against global ischemia with Krebs-Henseleit buffer-based cardioplegic solution. J Cardiothorac Surg 2013;8:60.
[49] Wakayama K, Fukai M, Yamashita K, Kimura T, Hirokata G, Shibasaki S *et al*. Successful transplantation of rat hearts subjected to extended cold preservation with a novel preservation solution. Transpl Int 2012;25:696–706.
[50] Loganathan S, Radovits T, Hirschberg K, Korkmaz S, Koch A, Karck M *et al*. Effects of Custodiol-N, a novel organ preservation solution, on ischemia/reperfusion injury. J Thorac Cardiovasc Surg 2010;139:1048–56.
[51] Edelman JJ, Seco M, Dunne B, Matzelle SJ, Murphy M, Joshi P *et al*. Custodiol for myocardial protection and preservation: a systematic review. Ann Cardiothorac Surg 2013;2:717–28.
[52] Chambers DJ, Hearse DJ. Developments in cardioprotection: "polarized" arrest as an alternative to "depolarized" arrest. Ann Thorac Surg 1999;68: 1960–6.
[53] Hoenicke EM, Sun X, Strange RG Jr, Damiano RJ Jr. Donor heart preservation with a novel hyperpolarizing solution: superior protection compared with University of Wisconsin solution. J Thorac Cardiovasc Surg 2000;120: 746–54.
[54] Snabaitis AK, Shattock MJ, Chambers DJ. Comparison of polarized and depolarized arrest in the isolated rat heart for long-term preservation. Circulation 1997;96:3148–56.
[55] Rudd DM, Dobson GP. Eight hours of cold static storage with adenosine and lidocaine (Adenocaine) heart preservation solutions: toward therapeutic suspended animation. J Thorac Cardiovasc Surg 2011;142:1552–61.
[56] Collins MJ, Moainie SL, Griffith BP, Poston RS. Preserving and evaluating hearts with ex vivo machine perfusion: an avenue to improve early graft performance and expand the donor pool. Eur J Cardiothorac Surg 2008; 34:318–25.
[57] Papayan G, Petrishchev N, Galagudza M. Autofluorescence spectroscopy for NADH and flavoproteins redox state monitoring in the isolated rat heart subjected to ischemia-reperfusion. Photodiagnosis Photodyn Ther 2014;11:400–8.
[58] Rosenbaum DH, Peltz M, DiMaio JM, Meyer DM, Wait MA, Merritt ME *et al*. Perfusion preservation versus static preservation for cardiac transplantation: effects on myocardial function and metabolism. J Heart Lung Transplant 2008;27:93–9.
[59] Aupperle H, Garbade J, Ullmann C, Schneider K, Krautz C, Dhein S *et al*. Comparing the ultrastructural effects of two different cardiac preparation- and perfusion-techniques in a porcine model of extracorporal long-term preservation. Eur J Cardiothorac Surg 2007;31:214–21.
[60] Yang Y, Lin H, Wen Z, Huang A, Huang G, Hu Y *et al*. Keeping donor hearts in completely beating status with normothermic blood perfusion for transplants. Ann Thorac Surg 2013;95:2028–34.
[61] Michel SG, La Muraglia GM 2nd, Madariaga ML, Titus JS, Selig MK, Farkash EA *et al*. Preservation of donor hearts using hypothermic oxygenated perfusion. Ann Transplant 2014;19:409–16.
[62] Balfoussia D, Yerrakalva D, Hamaoui K, Papalois V. Advances in machine perfusion graft viability assessment in kidney, liver, pancreas, lung, and heart transplant. Exp Clin Transplant 2012;10:87–100.
[63] Amir G, Rubinsky B, Basheer SY, Horowitz L, Jonathan L, Feinberg MS *et al*. Improved viability and reduced apoptosis in sub-zero 21-hour preservation of transplanted rat hearts using anti-freeze proteins. J Heart Lung Transplant 2005;24:1915–29.
[64] Kato H, Tomita S, Yamaguchi S, Ohtake H, Watanabe G. Subzero 24-hr nonfreezing rat heart preservation: a novel preservation method in a variable magnetic field. Transplantation 2012;94:473–7.
[65] Suszynski TM, Rizzari MD, Scott WE, Eckman PM, Fonger JD, John R *et al*. Persufflation (gaseous oxygen perfusion) as a method of heart preservation. J Cardiothorac Surg 2013;8:105.
[66] Tsutsumi H, Oshima K, Mohara J, Takeyoshi I, Aizaki M, Tokumine M *et al*. Cardiac transplantation following a 24-h preservation using a perfusion apparatus. J Surg Res 2001;96:260–7.
[67] Kuhn-Regnier F, Fischer JH, Jeschkeit S, Switkowski R, Bardakcioglu O, Sobottke R *et al*. Coronary oxygen persufflation combined with HTK cardioplegia prolongs the preservation time in heart transplantation. Eur J Cardiothorac Surg 2000;17:71–6.
[68] Yotsumoto G, Jeschkeit-Schubbert S, Funcke C, Kuhn-Rйgnier F, Fischer JH. Total recovery of heart grafts of non-heart-beating donors after 3 hours of hypothermic coronary oxygen persufflation preservation in an orthotopic pig transplantation model. Transplantation 2003;75:750–6.
[69] Fischer JH, Funcke C, Yotsumoto G, Jeschkeit-Schubbert S, Kuhn-Rйgnier F. Maintenance of physiological coronary endothelial function after 3.3 h of hypothermic oxygen persufflation preservation and orthotopic transplantation of non-heart-beating donor hearts. Eur J Cardiothorac Surg 2004;25:98–104.
[70] Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986;74:1124–36.
[71] Galagudza MM, Blokhin IO, Shmonin AA, Mischenko KA. Reduction of myocardial ischemia-reperfusion injury with pre- and postconditioning: molecular mechanisms and therapeutic targets. Cardiovasc Hematol Disord Drug Targets 2008;8:47–65.
[72] Karck M, Rahmanian P, Haverich A. Ischemic preconditioning enhances donor heart preservation. Transplantation 1996;62:17–22.
[73] Landymore RW, Bayes AJ, Murphy JT, Fris JH. Preconditioning prevents myocardial stunning after cardiac transplantation. Ann Thorac Surg 1998; 66:1953–7.

[74] Kevelaitis E, Oubenaissa A, Mouas C, Peynet J, Menaché P. Ischemic preconditioning with opening of mitochondrial adenosine triphosphate-sensitive potassium channels or Na/H exchange inhibition: which is the best protective strategy for heart transplants? J Thorac Cardiovasc Surg 2001;121:155–62.
[75] Lauzier B, Sicard P, Bouchot O, Delemasure S, Menetrier F, Moreau D *et al.* After four hours of cold ischemia and cardioplegic protocol, the heart can still be rescued with postconditioning. Transplantation 2007;84:1474–82.
[76] Martin J, Lutter G, Ihling C, Siepe M, Wagner S, Hilberath J *et al.* Myocardial viability twenty-four hours after orthotopic heart transplantation from non-heart-beating donors. J Thorac Cardiovasc Surg 2003;125:1217–28.
[77] Lowalekar SK, Lu XG, Thatte HS. Further evaluation of Somah: long-term preservation, temperature effect, and prevention of ischemia-reperfusion injury in rat hearts harvested after cardiocirculatory death. Transplant Proc 2013;45:3192–7.
[78] Tolboom H, Makhro A, Rosser BA, Wilhelm MJ, Bogdanova A, Falk V. Recovery of donor hearts after circulatory death with normothermic extracorporeal machine perfusion. Eur J Cardiothorac Surg 2015;47:173–9.
[79] Rosenfeldt F, Ou R, Woodard J, Esmore D, Marasco S. Twelve-hour reanimation of a human heart following donation after circulatory death. Heart Lung Circ 2014;23:88–90.
[80] Boucek MM, Mashburn C, Dunn SM, Frizell R, Edwards L, Pietra B *et al.* Pediatric heart transplantation after declaration of cardiocirculatory death. N Engl J Med 2008;359:709–14.