



**University of
Sunderland**

Mownah, O.A., Khurram, M.A., Kanwar, A., Stamp, S., Rees, D., Brassil, J., Majo, J., Dark, J.H., Carter, Noel and Talbot, D (2014) Development of an ex vivo technique to achieve reanimation of hearts sourced from a porcine donation after circulatory death model. *Journal of Surgical Research*, 189 (2). pp. 326-334. ISSN 0022-4804

Downloaded from: <http://sure.sunderland.ac.uk/id/eprint/4946/>

Usage guidelines

Please refer to the usage guidelines at <http://sure.sunderland.ac.uk/policies.html> or alternatively

contact_sure@sunderland.ac.uk

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Development of an *ex vivo* technique to achieve reanimation of hearts sourced from a porcine donation after circulatory death model

Omar A. Mownah, MRCS,^{a,b,*} Muhammad A. Khurram, MRCS,^{a,b}
 Christopher Ray, MRCS,^{a,b} Aditya Kanwar, MRCS,^{a,b} Susan Stamp, HNC,^c
 Douglas Rees, PhD,^d John Brassil, MS,^e Joaquim Majo, LMS,^a
 John H. Dark, FRCS,^{a,c} Noel M. Carter, PhD,^b
 and David Talbot, PhD, FRCS^{a,b}

^a Institute of Transplantation, Freeman Hospital, Newcastle Upon Tyne, UK

^b Department of Pharmacy, Health and Well-being, University of Sunderland, Sunderland, UK

^c Institute of Cellular Medicine, Newcastle University, Newcastle Upon Tyne, UK

^d AQIX, Ltd, London BioScience Innovation Centre, London, UK

^e Functional Circulation LLC, Northbrook, Illinois

ARTICLE INFO

Article history:

Received 1 September 2013

Received in revised form

2 January 2014

Accepted 22 February 2014

Available online 28 February 2014

Keywords:

Donation after circulatory death

Heart transplantation

Cardiac reanimation

Persufflation

ABSTRACT

Background: This study reports on the development of a novel method for achieving *ex vivo* reanimation of hearts from a porcine donation after circulatory death (DCD) model without the use of donor pretreatment.

Methods: Porcine hearts ($n = 23$) were procured 10–29 min after confirmation of asystole. All hearts underwent initial flush with AQIX RS-I solution (London, UK). A 2-h preservation period followed: group 1 hearts ($n1-n11$) were preserved using static cold storage, group 2 hearts ($n12-n17$) were preserved using oxygenated, hypothermic machine perfusion (MP), and group 3 hearts ($n18-n23$) were subjected to retrograde oxygen persufflation. Reperfusion was performed on a Langendorff modification of a Model 33 Functional Circulation circuit. In hearts $n16-n23$, a dialysis circuit was incorporated into the circuit to facilitate removal of metabolites. The experimental protocol was allowed to follow an evolutionary course, with the aim of achieving greater success with reanimation.

Results: In group 1 (static cold storage), 7 of the 11 hearts (63.6%) achieved reanimation on the *ex vivo* circuit. Two of the six hearts (33.3%) in group 2 (MP) were successfully reanimated. All the six hearts (100%) in group 3 (persufflation) were successfully reanimated. The period of sustained reanimation increased when dialysis was incorporated into the circuit with a maximum of 300 min.

Conclusions: Porcine DCD hearts after 29 min of warm ischemia can be reanimated using the method described. A mechanism of reoxygenation (oxygenated MP or coronary sinus oxygen persufflation) during preservation appears mandatory for hearts from DCDs. Persufflation was associated with a higher probability of successful reanimation. Dialysis in the warm phase was useful in removing metabolites that could interfere with reanimation.

* Corresponding author. Institute of Transplantation, Freeman Hospital, Freeman Road, Newcastle Upon Tyne, UK. Tel.: +44 0 191 223 1351; fax: +44 0 191 223 1191.

E-mail addresses: omar.mownah@gmail.com (O.A. Mownah), david.talbot@nuth.nhs.uk (D. Talbot).

0022-4804/\$ – see front matter © 2014 Elsevier Inc. All rights reserved.

<http://dx.doi.org/10.1016/j.jss.2014.02.041>

The results demonstrate the potential of DCDs to counter the decline affecting heart transplantation.

© 2014 Elsevier Inc. All rights reserved.

1. Background

For patients with end-stage heart failure, heart transplantation remains the optimum treatment [1]. However, the increasing problem of shortage of suitable hearts from donors after brainstem death (DBD) has led to a decline in the number of heart transplants being performed (Fig. 1). In the United Kingdom, <150 procedures are occurring per annum compared with roughly 300 per annum during the 1980s and 1990s [2–4].

In kidney, liver, and lung transplantation, the decline in the number of DBDs has led to the expansion of donation after circulatory death (DCD) programs. Improvements in procurement and storage of DCD kidneys and lungs mean that outcomes are now comparable with DBD organs [5–7].

Cardiac transplantation has yet to extend into routine use of DCDs. Factors preventing the use of DCD hearts concern the significantly higher metabolic demand of the heart compared with other organs because of greater susceptibility to the deleterious effects of warm ischemia. It should be noted that the first human heart transplantation in 1967 involved a period of normothermic ischemia [8]. However, in the 1970s, after the formal definition of brainstem death, the cardiac transplant community established DBDs as the sole source of hearts.

A reexamination of the potential of DCD heart transplantation is becoming current because of a number of animal models which have reported that hearts transplanted into pigs and dogs can work sufficiently to provide circulatory support in the early stages after implantation [9,10], with Gundry *et al.* [11] demonstrating success with a primate model. As well as the initial experiences with heart transplantation described by Barnard *et al.* [8], there has been recent experience of human heart transplantation using hearts from DCDs in a pediatric setting.

A notable element of the protocols involved in DCD heart transplantation studies thus far, in both the animal models and the clinical environment, is the use of donor pretreatment. All cases found in the literature included donor anticoagulation before the determination of circulatory arrest. Additional pretreatments have included cardioprotective

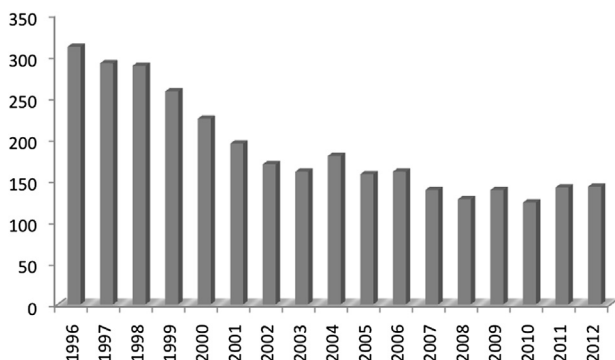


Fig. 1 – Heart transplantation decline. The decline in the number of heart transplants performed in the United Kingdom over the last 15 y [3,4].

therapies such as dysrhythmia prevention during the agonal period [10]. In several nations including the United Kingdom, such donor pretreatments for DCDs are currently prohibited [12]. An experimental model of DCD heart reanimation in a model with no pretreatment has yet to be described.

In this study, we report on our investigations into DCD heart reanimation using a porcine DCD model. We have devised a protocol devoid of any donor pretreatments, in accordance with current United Kingdom law, and using an *ex vivo* circuit to perform organ perfusion and reanimation under normothermic conditions. These experiments were designed to follow an evolutionary course with adjustments made to the experimental protocol based on the ongoing findings. The overall aim was to have a retrieval and preservation method conducive to facilitating reanimation. The design of the *ex vivo* circuit was similarly improved with sequential experiments to permit satisfactory cardiac perfusion aiming for prolonged periods of reanimation.

2. Methods

2.1. Porcine DCD model

All animal work was performed under conditions of the Home Office Project License (PPL 6004164). Twenty-three cross-Yorkshire Landrace pigs with a mean weight of 29.7 ± 5.9 kg were used in the study. Before the experiments, the animals were housed and treated humanely in accordance with the United Kingdom Home Office Animals (Scientific Procedures) Act 1986. Animals were brought to the facility 7 d before euthanasia for acclimatization.

A summary of the experimental protocol can be viewed in Figure 2. Before simulation of a DCD scenario, each animal was premedicated with an intramuscular injection of ketamine (300 mg), medetomidine (1.2 mg), and diazepam (10 mg). Circulatory death was caused by one of the two methods: excess administration of phenobarbitone or exsanguination. For n1–n11, n14, n15, n18, n22, and n23, an ear vein was cannulated, and an overwhelming dose of intravenous phenobarbitone (4.5 g) was delivered. Circulatory arrest was confirmed by auscultation of heart sounds. General anesthesia was administered to animals n12, n13, n16, n17, and n19–n21, with exsanguination performed after thoracotomy by opening of the inferior vena cava. Confirmation of circulatory arrest was performed on visual inspection of the heart.

2.2. Surgical procedure and initial flush

Hearts were explanted after 10–29 min after determination of circulatory arrest. The blood (500 mL) was collected from the inferior vena cava using a suction device. The suction tubing and container were pretreated with low-molecular-weight heparin (25,000 IU) and streptokinase (375,000 IU). The blood was then passed through a leukocyte filter (LeukoGuard RS;

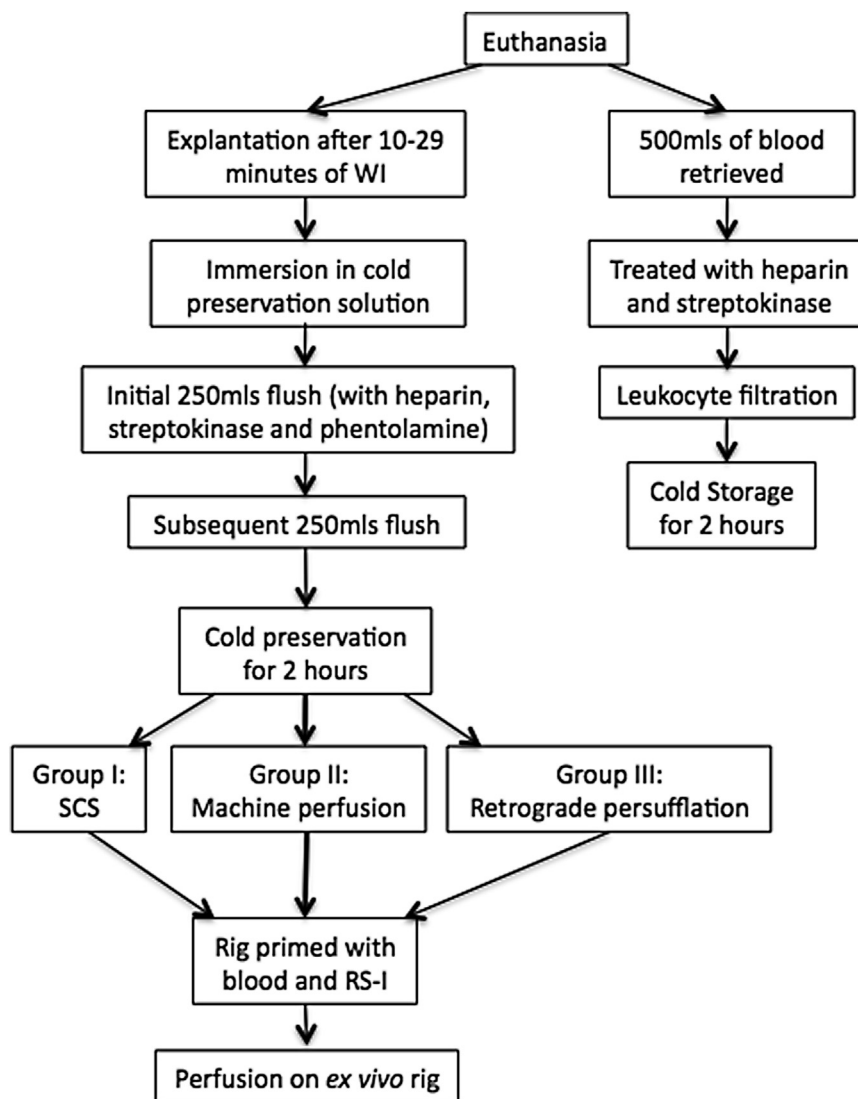


Fig. 2 – The experimental protocol. Flow chart showing the experimental protocol. WI = warm ischaemia.

Pall Medical, Port Washington, NY) and placed in storage at 4°C–8°C until perfusion on the *ex vivo* circuit. After the 10–29 min of warm ischemia, the heart was placed in a bowl of cold AQIX RS-I solution. RS-I is a novel non-phosphate-buffered preservation and normothermic perfusion solution with a formulation based on constituents present in human interstitial fluid (Table 1).

Immediately after hypothermic immersion in RS-I, an initial flush was delivered to the heart using a Tibbs arterial cannula directly into both coronary artery ostia. Group 1 (n1–n11) underwent initial flush with 250 mL of RS-I at room temperature. This initial flush also contained low-molecular-weight heparin (12,500 U), streptokinase (375,000 U), and phentolamine (0.25 mg). The initial flush for groups 2 and 3 (n12–n23) was preoxygenated by persufflating the RS-I with oxygen at a flow rate of 2 L/min for 15 min. Furthermore, the initial flush for groups 2 and 3 was supplemented with the addition of *N*-acetylcysteine (100 mg). For n18–n23, the following drugs were added to the initial flush: insulin (60 IU), dexamethasone (16 mg), and co-amoxiclav (120 mg). A

subsequent flush of 250 mL was delivered in the same manner immediately afterward. This second flush was either cold RS-I (n1–n6) or cold University of Wisconsin (UW) solution (n7–n11). Hearts from n12–n23 did not receive a second flush.

2.3. Cardiac preservation groups 1–3

After the flush, group 1 hearts were preserved as follows: n1–n8 were preserved for 2 h using static cold storage (SCS) in RS-I solution with n9–n11 undergoing SCS for the same period in UW solution. Group 2 hearts (n12–n17) underwent oxygenated, hypothermic machine perfusion (MP) with UW solution for 2 h. This was performed using a LifePort (Organ Recovery Systems, Zaventem, Belgium) with a pressure into the aortic root set at 30 mm Hg. Oxygen was persufflated into the circulating UW solution at a rate of 0.5 L/min. Group 3 hearts (n18–n23) underwent retrograde oxygen persufflation via the coronary sinus, at a pressure of 10–12 mm Hg, for a period of 2 h. At the onset of persufflation, a 21-gauge needle was used to make 12 holes in the myocardium to permit the

Table 1 – The components of AQIX RS-I solution.

Components	Concentration
NaCl	110 mmol/L
KCl	5 mmol/L
CaCl ₂	1.25 mmol/L
MgCl ₂	0.45 mmol/L
NaHCO ₃ (buffer)	25 mmol/L
BES	5 mmol/L
D-glucose	10 mmol/L
Glycerol	0.11 mmol/L
L-glutamate	0.30 mmol/L
L-glutamine	0.40 mmol/L
L-aspartate	0.02 mmol/L
L-carnitine	0.05 mmol/L
Choline chloride	0.01 mmol/L
TPP (cocarboxylase)	40 nmol/L
Human (recombinant) insulin	28 mIU
pH	7.13 – 7.41 ± 0.5 at 10°C–37°C
Osmolarity	286 mOsmol/L

BES = N,N-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid; TPP = thiamine diphosphoric acid ester tetrahydrate.

escape of excess oxygen. A manometer was fashioned to monitor the persufflation pressure, and the heart was immersed in cold RS-I during this time (Fig. 3).

2.4. Heart preparation for perfusion

A cannula was secured in the aorta with a silk tie, to lie 5 mm distal to the coronary ostia. The openings of the pulmonary veins entering the left atrium were closed with silk ties, except for one vein, which was cannulated. The inferior and superior vena cavae were closed with silk ties, and the proximal pulmonary artery vented to allow cardiac venous blood to drain back into the circuit.

2.5. The ex vivo circuit and dialysis

Reperfusion was performed on a Langendorff modification of a Model 33 Functional Circulation (Functional Circulation, Northbrook, IL) circuit (Fig. 4). The top reservoir provided a gravity-driven column of oxygenated blood into the coronary

arteries. The second reservoir provided inflow into the left atrium to deliver preload to the heart when in working mode. Venous blood was driven by a roller pump through a hollow fiber oxygenator (Chalice Medical, Nottinghamshire, UK) before being returned to the top reservoir. Temperature control was provided by a circulating water bath (9100 Series Temperature Controller; PolyScience, Niles, IL), which provided heat exchange at the oxygenator and the reactor (the glass container which housed the heart).

For n16–n23, a dialysis filter was added to run in parallel to the circuit (Fig. 5). The arrangement used an FX8 Capillary Dialyser (Fresenius Medical Care, Bad Homburg, Germany) with two roller pumps. Venous blood from the reactor was diverted to the dialysis filter and returned to the circuit proximal to the oxygenator. The dialysate was made by mixing deionized water and phosphate to a commercially available hemodialysate concentrate (A/5; Fresenius Medical Care).

2.6. Perfusion

The circuit was primed with the leukocyte-depleted blood (500 mL) plus 300 mL of RS-I. Clamps were placed on the outflow tubes draining both reservoirs. The heart was connected to the circuit by attachment of the aortic cannula to the top reservoir outflow and the left atrial cannula to the second reservoir outflow. Perfusion was commenced together with cautious rewarming. For hearts n1 and n2, the top reservoir was unclamped completely to deliver high initial flows into the aortic root. For subsequent hearts, a variable clamp was used to control initial flow with a steady increase of flow over the first 30 min “Venous” and oxygenated blood were regularly sampled and tested in a blood gas analyser (RAPIDLab 1200; Siemens AG, Munich, Germany). This provided data including pO₂, pH, lactate, and potassium concentrations.

Activity of the heart was monitored. Fibrillatory activity, as well as time and duration of reanimation, was recorded. Interventions were carried out, such as electrical defibrillation and drugs (calcium, magnesium, and adrenaline) were administered to facilitate reanimation. With the heart in

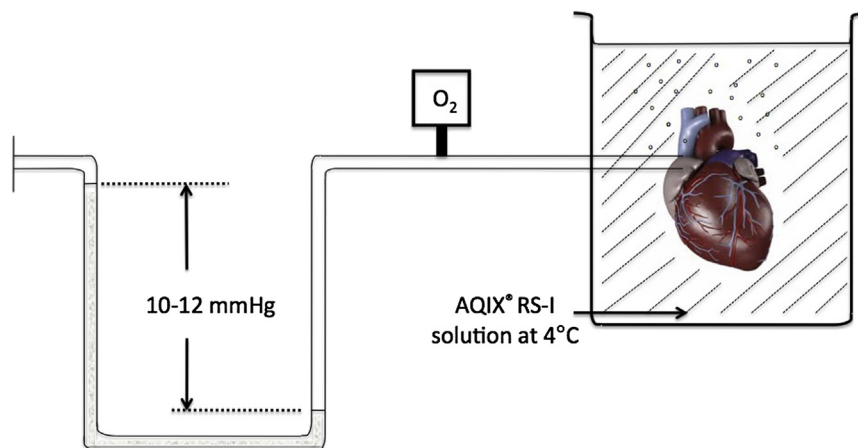


Fig. 3 – The persufflation procedure. The procedure used to deliver oxygen at a pressure of 10–12 mm Hg (measured by a simple manometer) into the coronary sinus. Small punctures in the myocardium allowed excess oxygen to escape. (Color version of figure is available online.)

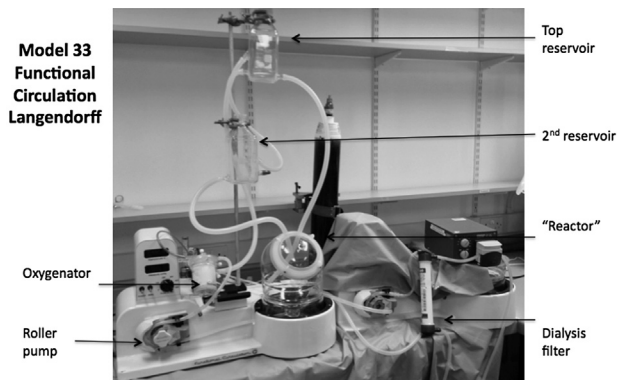


Fig. 4 – The *ex vivo* circuit. The *ex vivo* reperfusion circuit (Langendorff modification of a Model 33 Functional Circulation) together with the dialysis arrangement.

working mode, the clamp on the second reservoir was released allowing preload to enter the left atrium.

3. Results

Reanimation of the DCD hearts on the *ex vivo* circuit was achieved in 15 of the 23 hearts (65%). The first two hearts failed (as well as the *n*4 and *n*10). Four of the six hearts in group 2 (oxygenated MP) failed to reanimate, whereas all group 3 (persufflation) hearts reanimated. On the instances of failure to reanimate, the myocardium was noted to have developed gross edema soon after the start of perfusion. After these initial experiments, electrical defibrillation was used routinely to establish sinus rhythm (from *n*3 onward) with consistent success. Drugs were also introduced into the protocol with good effect. Adrenaline as well as calcium, magnesium, and antibiotics administered into the circuit were all found to

contribute to successful reanimation (refer to Table 2 for a detailed account of the experiments).

The dialyser was able to efficiently remove lactate from the system and maintain physiological pH and potassium concentration. The experiments, which incorporated dialysis (*n*16–*n*23), involved sustained reanimation from 3–5 h on the circuit. The heat exchange aspect of the circuit maintained the perfusate at 33°C–34°C. The oxygenator was able to provide pO_2 measurements of 80–100 kPa.

4. Discussion

In this study, we have described the steps taken to achieve a reproducible method for reanimating porcine hearts after exposure to 10–29 min of warm ischemia. The results of the experiments underline the potential for using hearts sourced from DCDs in clinical heart transplantation. This protocol underwent various amendments, detailed in the methods, to improve organ preservation and the *ex vivo* circuit. Several early hearts failed, but as the experiments progressed, a reproducible method was established leading to reanimation of a heart subjected to 29 min of warm ischemia with prolonged activity on the circuit lasting 5 h. This study has shown the potential of AQIX RS-I as a perfusate for DCD hearts. The benefits of RS-I have been reported in a study of rat hearts showing superior cardioprotection when using an RS-I based solution versus St Thomas' Hospital solution [13].

The amendments made to the experimental protocol during the course of the study were based on observations during the initial hearts with the aim of achieving successful reanimation for prolonged periods. The best results came from the last six hearts. These hearts underwent oxygen persufflation during the preservation process and dialysis while on the circuit. Using persufflation as a means of introducing oxygen into the organ during the cold phase has been shown to

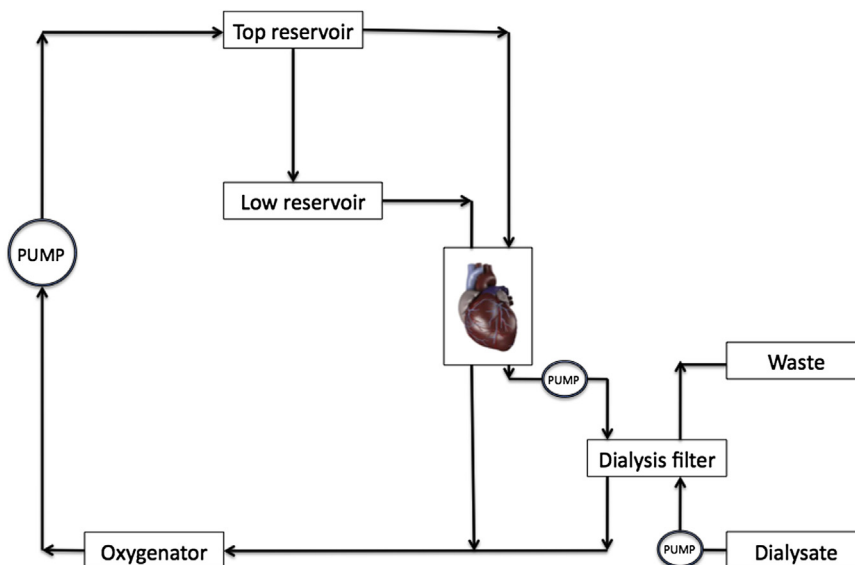


Fig. 5 – Schematic of the circuit. This schematic diagram illustrates the circuit with the heart in “working” mode with the parallel dialysis circuit. (Color version of figure is available online.)

Table 2 – Results table.

Heart number	Mode of death	Warm ischemic time (min)	Cold phase		Normothermic phase			
			Flush	Preservation	Drugs	Electrical defibrillation	Dialysis	Activity
Group 1								
1	Phenobarbitone	10	250 mL of RS-I	SCS in RS-I	None	None	None	Fibrillation only and nil ventricular contractions
2	Phenobarbitone	10	250 mL of RS-I	SCS in RS-I	None	None	None	Fibrillation only and nil ventricular contractions
3	Phenobarbitone	10	250 mL of RS-I	SCS in RS-I	Adrenaline	Yes	None	Reanimation
4	Phenobarbitone	10	250 mL of RS-I	SCS in RS-I	Adrenaline	Yes	None	Fibrillation only and nil ventricular contractions
5	Phenobarbitone	10	250 mL of RS-I	SCS in RS-I	Adrenaline	Yes	None	Reanimation
6	Phenobarbitone	10	250 mL of RS-I	SCS in RS-I	Adrenaline	Yes	None	Reanimation
7	Phenobarbitone	10	250 mL of RS-I	SCS in RS-I	Adrenaline and calcium	Yes	None	Reanimation
8	Phenobarbitone	10	250 mL of RS-I	SCS in RS-I	Adrenaline and calcium	Yes	None	Reanimation
9	Phenobarbitone	10	250 mL of RS-I	SCS in RS-I	Adrenaline and calcium	Yes	None	Reanimation
10	Phenobarbitone	10	250 mL of RS-I	SCS in UW solution	Adrenaline and calcium	Yes	None	Fibrillation only and nil ventricular contractions
11	Phenobarbitone	10	250 mL of RS-I	SCS in UW solution	Adrenaline and calcium	Yes	None	Reanimation
Group 2								
12	Exsanguination	12	250 mL of oxygenated RS-I	MP in oxygenated UW solution	Adrenaline and calcium	Yes	None	Fibrillation only and nil ventricular contractions
13	Exsanguination	12	250 mL of oxygenated RS-I	MP in oxygenated UW solution	Adrenaline and calcium	Yes	None	Nil reanimation
14	Phenobarbitone	16	250 mL of oxygenated RS-I	MP in oxygenated UW solution	Adrenaline and calcium	Yes	None	Nil reanimation
15	Phenobarbitone	18	250 mL of oxygenated RS-I	MP in oxygenated UW solution	Adrenaline, calcium, and magnesium	Yes	None	Reanimation
16	Exsanguination	14	250 mL of oxygenated RS-I	MP in oxygenated UW solution	Adrenaline, calcium, and magnesium	Yes	Yes	Nil reanimation
17	Exsanguination	12	250 mL of oxygenated RS-I	MP in oxygenated UW solution	Adrenaline, calcium, and magnesium	Yes	Yes	Reanimation
Group 3								
18	Phenobarbitone	22	250 mL of oxygenated RS-I	O ₂ persufflation (in RS-I)	Adrenaline, calcium, and magnesium	Yes	Yes	Reanimation
19	Exsanguination	18	250 mL of oxygenated RS-I	O ₂ persufflation (in RS-I)	Adrenaline, calcium, and magnesium	Yes	Yes	Reanimation—sustained for 2 h
20	Exsanguination	18	250 mL of oxygenated RS-I	O ₂ persufflation (in RS-I)	Adrenaline, calcium, and magnesium	Yes	Yes	Reanimation—intermittent for 2 h
21	Exsanguination	22	250 mL of oxygenated RS-I	O ₂ persufflation (in RS-I)	Adrenaline, calcium, magnesium, and co-amoxiclav	Yes	Yes	Reanimation—sustained for 5 h
22	Phenobarbitone	20	250 mL of oxygenated RS-I	O ₂ persufflation (in RS-I)	Adrenaline, calcium, magnesium, and co-amoxiclav	Yes	Yes	Reanimation—sustained for 3 h
23	Phenobarbitone	29	250 mL of oxygenated RS-I	O ₂ persufflation (in RS-I)	Adrenaline, calcium, magnesium, and co-amoxiclav	Yes	Yes	Reanimation—sustained for 5 h

Results of each experiment with groups assigned according to the preservation technique used. Group 1 hearts underwent SCS, group 2 hearts underwent oxygenated MP, and group 3 hearts underwent retrograde coronary sinus persufflation. Dialysis was incorporated into the reperfusion protocol from n16 onward.

improve graft quality in kidney, liver, and pancreas preservation [14–16]. Fischer *et al.* [17] described the positive effects of persufflation on myocardial preservation, and following our observations, we would advocate its use in preserving DCD hearts. Furthermore, the technique as we have described is straightforward and inexpensive. An additional reason why the latter hearts may have shown superior reanimation was the inclusion of several drugs in the initial flush. N-acetylcysteine was used as a free-radical scavenger to counter the potential ischemia–reperfusion injury resulting from persufflating oxygen into the ischemic myocardium. Dexamethasone has an important role in the inhibition of proinflammatory mediators (e.g., tumor necrosis factor α , interleukin 6, and interleukin 8) produced during reperfusion [18]. Insulin promotes cellular potassium uptake, thereby stabilizing cardiomyocytes.

A problem encountered in the experiments was the increasing lactate concentrations in the circulating blood as the period on the circuit progressed. Lactate accumulation is associated with impairment of glycolytic reactions in the myocardium and reduced anaerobic adenosine triphosphate production [19]. This problem was addressed by including a modified dialysis circuit to run within the circuit. Lactate removal was found to enable prolonged periods of sustained reanimation on the circuit, with the longest period of sustained reanimation at 5 h. The dialysis feature also allowed pH buffering and maintenance of physiological potassium levels.

Eight of the 23 hearts failed to reanimate. These occurrences were broadly spoken at the beginning of the study and much was learned from these initial failures. These hearts were macroscopically found to be satisfactory at retrieval and after preservation. However, soon after warm perfusion had been commenced, they had rapidly developed gross edema with a fixed contracture of the myocardium. This phenomenon has previously been described in the literature as “stone heart” formation [20]. The basis for this complication lies in the failure of cardiomyocyte ion-exchange mechanisms. Ischemia causes rapid depletion in the adenosine triphosphate stores of the metabolically active cardiomyocytes with an accumulation intracellular Na^+ . This results in overwhelming Ca^{2+} influx via the $\text{Na}^+ - \text{Ca}^{2+}$ exchange mechanism causing activation of the myocardial contractile apparatus. The consequent contractile necrosis bands which form cause irreversible damage, with functional recovery no longer possible [21]. There is evidence that the use of $\text{Na}^+ - \text{H}^+$ exchange inhibitors (cariporide) in previous DCD heart transplantation models is associated with improved functional recovery [9,10]. A further technique demonstrating protection against stone heart formation is controlled initial myocardial reperfusion. When this has been coupled with steady rewarming, there has been an improvement in the functional recovery of DCD hearts [22]. The hearts in our study were found to reanimate consistently by using this technique of cautious initial reperfusion.

Crucially in our study, the donor animal received no pre-treatment. Several studies have reported on DCD animal experimentation [9–11] and the initial results of clinical pediatric DCD heart transplantation [23]. However, on all occasions, the donor was anticoagulated with intravenous heparin before circulatory arrest. Thus, our method, if extended to the clinical environment, would comply with the current laws in the United Kingdom concerning organ DCD [12].

Ex vivo reanimation is proposed as the method central to DCD heart transplantation. Reanimation of a heart *in vivo* would likely be prevented by ethical concerns, that is, the donor has been declared dead after a period of circulatory arrest so that the donation can proceed, but now, the dead donor has a cardiac output. As well as circumventing this issue, the *ex vivo* technique allows the reanimated heart to (1) be transported if the recipient is at a distant site and (2) undergo functional testing. Conductance catheters could be introduced to assess contractility and echocardiography performed for assessment of ejection fraction. This form of viability testing would be essential before deciding on the suitability for transplantation [24]. Ex vivo lung perfusion has highlighted the importance of viability testing and has contributed to the high success rates achieved with DCD lung transplantation [25]. Furthermore, a period of normothermic *ex vivo* perfusion has been shown to improve the condition of DCD lungs [26].

The circuit designed in this study could also be extended for use in the assessment of marginal DBD hearts. There is currently a high rate of organ decline on the basis of poor function. Ex vivo perfusion would be the ideal method of assessing such organs, potentially allowing more transplants to successfully proceed. In addition, placing a DBD heart on the circuit would eliminate exposure of the graft to the deleterious effects of the “catecholamine storm” associated with brainstem death, which is known to cause myocardial dysfunction [27,28].

The cold phase reoxygenation step we used could be performed in practice during transport of a donated heart. The reoxygenation via MP was not successful with us, but this was probably because the flow rates were not high enough to close the aortic valve as we were using a renal MP system. So, reoxygenation with MP is still a possible alternative to persufflation [29].

The less transportable Langendorff circuit described here could be used at the base hospital. Our circuit perfused the coronary arteries under gravity. This method of gravity-driven flow provided adequate myocardial perfusion, whereas pressure-driven flow (using pumps) can be associated with myocardial damage. Gravity-driven reperfusion leads to filling in accordance with the coronary vascular resistance, avoiding ischemia with hypoperfusion, or hemorrhage with hyperperfusion. Furthermore, the height of the top reservoir providing the coronary perfusion can be altered to increase the perfusion pressure. In our system, the top reservoir provided a column of blood analogous to afterload when the heart was in working mode. Our modified Langendorff approach allows preload to be given via the second lower reservoir inflow into the left atrium during the working mode.

This study was treated as an evolutionary process to identify the ideal method and conditions to achieve reanimation of a porcine DCD heart. The primary end points were the presence and duration of reanimation. Quantitative data regarding the quality of the organs have not been studied at this early stage but will form the basis of future studies. There were difficulties in maintaining a physiological temperature of 36°C in our circuit, prompting several adjustments to be considered for our future experiments. The temperature averaged between 33°C and 34°C, which is short of the physiological conditions we aimed to simulate with our circuit.

Also of note is that transplantation of the reanimated hearts was not performed as part of this study. Incorporation of transplantation into our animal model is an essential step toward assessing the true potential for using DCD-sourced hearts in the clinical environment. Despite transplantation not occurring in this study, it remains a key part of this research and will form the basis of our future experiments.

Several studies have investigated the potential number of hearts that could be contributed to the donor pool by using DCDs. Noterdaeme et al. [30] reported a potential increase in donor hearts of 15%, which would correlate with a potential 40% decrease in the number of patients dying while on the waiting list. With an ever increasing number of patients requiring transplantation and based on the findings of this study, we would propose an increased focus on DCDs as an untapped resource to expand the donor pool.

5. Conclusions

Hearts sourced from DCDs using our porcine model can be successfully reanimated. Reanimation can be achieved following cold phase preservation using SCS, oxygenated MP, or retrograde coronary sinus persufflation. But DCD hearts were most likely to reanimate after the use of retrograde oxygen persufflation. The addition of dialysis to the *ex vivo* circuit was a straightforward method for maintaining the integrity of the blood in the circuit, thus enabling more success with reanimation. AQIX RS-I solution has shown promise as a potential preservation solution for hearts in transplantation.

Acknowledgment

This study was funded by a grant from the Technology Strategy Board. In addition, the authors acknowledge the support offered by AQIX Ltd (UK) in providing preservation solution and advice. Advice for circuit design was obtained from both AQIX Ltd (UK) and Functional Circulation LLC (USA) with regard to the reperfusion circuit.

Author contributions: O.A.M., M.A.K., C.R., and A.K. conducted the experiments. O.A.M. composed the article. D.T., N.M.C., S.S., J.M., D.R., J.B., and J.H.D. advised and supervised the study.

Disclosure

D.R. is the Founder, Director and Chief Scientific Officer of AQIX Ltd (UK). J.B. is the Chief Executive Officer of Functional Circulation LLC (USA). The remaining authors reported no proprietary or commercial interest in any product mentioned or concept discussed in the article.

REFERENCES

- [1] Stehlik J, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: 29th official adult heart transplant report—2012. *J Heart Lung Transplant* 2012;31:1052.
- [2] Macgowan GA, Parry G, Schueler S, Hasan A. The decline in heart transplantation in the UK. *BMJ* 2011;342:d2483.
- [3] NHS blood and transplant activity report 2012/13 [Internet]. [cited 2013 Aug 31]. Available from: http://www.organdonation.nhs.uk/statistics/transplant_activity_report/current_activity_reports/ukt/activity_report_2012_13.pdf.
- [4] Organ donation and transplantation—activity figures for the UK as at 12th April 2013 [Internet]. [cited 2013 Aug 31]. Available from: http://www.organdonation.nhs.uk/statistics/downloads/annual_stats.pdf.
- [5] Morris PJ. Kidneys donated after cardiac death are acceptable. *Lancet* 2010;376:1276.
- [6] Monbaliu D, Pirenne J, Talbot D. Liver transplantation using donation after cardiac death donors. *J Hepatol* 2012;56:474.
- [7] Mason DP, Brown CR, Murthy SC, et al. Growing single-center experience with lung transplantation using donation after cardiac death. *Ann Thorac Surg* 2012;94:406. discussion 411–412.
- [8] Barnard CN. Human cardiac transplantation. An evaluation of the first two operations performed at the Groote Schuur Hospital, Cape Town. *Am J Cardiol* 1968;22:584.
- [9] Martin J, Sarai K, Yoshitake M, et al. Successful orthotopic pig heart transplantation from non-heart-beating donors. *J Heart Lung Transplant* 1999;18:597.
- [10] Repse S, Pepe S, Anderson J, McLean C, Rosenfeldt FL. Cardiac reanimation for donor heart transplantation after cardiocirculatory death. *J Heart Lung Transplant* 2010;29:747.
- [11] Gundry SR, de Begona JA, Kawauchi M, Bailey LL. Successful transplantation of hearts harvested 30 minutes after death from exsanguination. *Ann Thorac Surg* 1992;53:772. discussion 774–775.
- [12] DCD consensus meeting report—DCD guidelines for British Transplant Society and Intensive Care Society [Internet]. [cited 2013 Aug 31]. Available from: <http://www.bts.org.uk/Documents/Guidelines/Active/DCD%20for%20BTS%20and%20ICS%20FINAL.pdf>.
- [13] Maruyama Y, Chambers DJ. Myocardial protection: efficacy of a novel magnesium-based cardioplegia (RS-C) compared to St Thomas' Hospital cardioplegic solution. *Interact Cardiovasc Thorac Surg* 2008;7:745.
- [14] Rolles K, Foreman J, Pegg DE. Preservation of ischemically injured canine kidneys by retrograde oxygen persufflation. *Transplantation* 1984;38:102.
- [15] Treckmann J, Minor T, Saad S, et al. Retrograde oxygen persufflation preservation of human livers: a pilot study. *Liver Transpl* 2008;14:358.
- [16] Scott WE 3rd, O'Brien TD, Ferrer-Fabrega J, et al. Persufflation improves pancreas preservation when compared with the two-layer method. *Transplant Proc* 2010;42:2016.
- [17] Fischer JH, Funcke C, Yotsumoto G, Jeschkeit-Schubbert S, Kuhn-Régnier 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.
- [18] Pearl JM, Drinkwater DC Jr, Laks H, Stein DG, Capouya ER, Bhuta S. Leukocyte-depleted reperfusion of transplanted human hearts prevents ultrastructural evidence of reperfusion injury. *J Surg Res* 1992;52:298.
- [19] Rovetto MJ, Lamberton WF, Neely JR. Mechanisms of glycolytic inhibition in ischemic rat hearts. *Circ Res* 1975;37:742.
- [20] Opie LH. Reperfusion injury and its pharmacologic modification. *Circulation* 1989;80:1049.
- [21] Piper HM, Balsler C, Ladilov YV, et al. The role of Na⁺/H⁺ exchange in ischemia-reperfusion. *Basic Res Cardiol* 1996;91:191.

[1] Stehlik J, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung

- [22] Osaki S, Ishino K, Kotani Y, et al. Resuscitation of non-beating donor hearts using continuous myocardial perfusion: the importance of controlled initial reperfusion. *Ann Thorac Surg* 2006;81:2167.
- [23] Boucek MM, Mashburn C, Dunn SM, et al. Pediatric heart transplantation after declaration of cardiocirculatory death. *N Engl J Med* 2008;359:709.
- [24] Suehiro K, Mohri M, Yamaguchi H, et al. Posttransplant function of a nonbeating heart is predictable by an ex vivo perfusion method. *Ann Thorac Surg* 2001;71:278.
- [25] Mason DP, Thuita L, Alster JM, et al. Should lung transplantation be performed using donation after cardiac death? The United States experience. *J Thorac Cardiovasc Surg* 2008;136:1061.
- [26] Yeung JC, Cypel M, Waddell TK, van Raemdonck D, Keshavjee S. Update on donor assessment, resuscitation, and acceptance criteria, including novel techniques—non-heart-beating donor lung retrieval and ex vivo donor lung perfusion. *Thorac Surg Clin* 2009;19:261.
- [27] Todd GL, Baroldi G, Pieper GM, Clayton FC, Eliot RS. Experimental catecholamine-induced myocardial necrosis. I. Morphology, quantification and regional distribution of acute contraction band lesions. *J Mol Cell Cardiol* 1985;17:317.
- [28] Stoica SC, Satchithananda DK, White PA, et al. Brain death leads to abnormal contractile properties of the human donor right ventricle. *J Thorac Cardiovasc Surg* 2006;132:116.
- [29] Fondevila C, Hessheimer AJ, Maathuis MH, et al. Hypothermic oxygenated machine perfusion in porcine donation after circulatory determination of death liver transplant. *Transplantation* 2012 15;94:22.
- [30] Noterdaeme T, Detry O, Hans M-F, et al. What is the potential increase in the heart graft pool by cardiac donation after circulatory death? *Transpl Int* 2013;26:61.