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## Review

# Persufflation (or gaseous oxygen perfusion) as a method of organ preservation <sup>☆</sup>

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## ABSTRACT

Improved preservation techniques have the potential to improve transplant outcomes by better maintaining donor organ quality and by making more organs available for allotransplantation. Persufflation, (PSF, gaseous oxygen perfusion) is potentially one such technique that has been studied for over a century in a variety of tissues, but has yet to gain wide acceptance for a number of reasons. A principal barrier is the perception that *ex vivo* PSF will cause *in vivo* embolization post-transplant. This review summarizes the extensive published work on heart, liver, kidney, small intestine and pancreas PSF, discusses the differences between anterograde and retrograde PSF, and between PSF and other conventional methods of organ preservation (static cold storage, hypothermic machine perfusion). Prospective implications of PSF within the broader field of organ transplantation, and in the specific application with pancreatic islet isolation and transplant are also discussed. Finally, key issues that need to be addressed before PSF becomes a more widely utilized preservation strategy are summarized and discussed.

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## Introduction

The advancement of allotransplantation over the past half century has stimulated the development of techniques for whole organ preservation, especially in the face of common logistical challenges inherent in the delivery of the therapy (such as the need

for transportation and coordination of operating times). In addition to preserving the function and viability of cadaveric organs accepted via standard criteria, improved organ preservation has the potential to increase the fraction of marginal organs used for transplant [60,90]. It is generally believed that improved preservation techniques should contribute to improved maintenance of organ quality, minimize ischemia–reperfusion injury and result in more successful transplant outcomes, which has led to substantial research effort to optimize organ preservation protocols.

A key area of research interest lies in the optimization of oxygen delivery during hypothermic preservation. It has been shown that conventional static cold storage (SCS) techniques may not provide sufficient oxygen to the core of a larger organ [33,90], and only oxygenate to a maximum depth of a millimeter from the surface [63]. Efforts to improve the oxygen solubility of cold preservation solutions by using perfluorocarbons have proven largely ineffective, because these methods still rely on oxygen delivery by passive diffusion from the surface [2]. Even hypothermic machine perfusion (HMP), which has been designed to deliver cold preservation solution into the organ via the native vasculature, may deliver inadequate oxygen to an organ during preservation, particularly when the perfusate is not saturated with oxygen at a higher than atmospheric pO<sub>2</sub> [41,92,99]. It is in this regard that persufflation (PSF), or gaseous oxygen perfusion, may provide additional

**Abbreviations:** ADP, adenine diphosphate; AMP, adenine monophosphate; A-PSF, anterograde persufflation; ATP, adenine triphosphate; CPB, cardiopulmonary bypass; DCD, donation after cardiac death; HMP, hypothermic machine perfusion; POD, post-operative day; PSF, persufflation; R-PSF, retrograde PSF; SCS, static cold storage; SOD, superoxide dismutase; TLM, two-layer method; UNOS, United Network for Organ Sharing; UW, University of Wisconsin; WIT, warm ischemia time.

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advantages as compared to either SCS or HMP (see Table 1 for more detailed comparison of the advantages and disadvantages between SCS, HMP and PSF). PSF is not a new concept but can be considered an emerging technique for current-day organ preservation and deserves considerable attention for a variety of compelling reasons, including the unique capability to deliver oxygen gas or gas mixture directly into an organ by using the native vasculature. When compared with SCS and HMP, PSF may represent the best opportunity to fully oxygenate an entire, human-sized organ. This review details the historical development of PSF with heart, kidney, liver, small intestine and pancreas and discusses the differences between the 2 main approaches for PSF (anterograde versus retrograde). We also discuss the future research landscape for PSF in relation to established methods of preservation and describe some of the important issues that need to be addressed before the technique becomes more widely accepted.

### Early history with persufflation

PSF was first discovered in 1902 by Rudolf Magnus, when he made an unexpected observation while perfusing an isolated cat heart with defibrinated blood [42]. The reservoir storing liquid perfusate emptied inadvertently and was not re-filled immediately. Since compressed oxygen gas was used to pressurize the reservoir, the gas was pulled into the perfusion circuit and into the heart. Magnus observed that the heart continued to contract rhythmically for 9 min during PSF. This initial observation prompted the initiation of a series of more extensive studies designed to elucidate the utility of PSF in preserving cardiac function. Magnus illustrated that it was possible to maintain a beating heart in bradycardia during 69 min of PSF and that reperfusion of blood through the coronaries restored a normal heart rate (80 bpm). Interestingly, Magnus persufflated the cat heart with gaseous hydrogen and showed that it still beat at 20 min of treatment. Furthermore, he tried coronary persufflation with gaseous carbon dioxide and was able to demonstrate, unlike either oxygen or hydrogen gas, that the heart stops after just minutes. Even though

Magnus' findings were intriguing at the time, it was not until the mid-1950s that the significance of his studies was appreciated.

With the advent of clinical transplant on the near horizon [24,45], a group at McGill University in Montreal discovered in 1954 that PSF could preserve spinal reflexes in frogs and active cardiac and skeletal muscle contractions in rabbits [5,6]. Their first paper highlighted the benefits of PSF versus liquid perfusion in a frog spinal reflex model [5]. The authors showed that peripheral nerve reflexes and muscle contractions were preserved for up to 6–8 h when the gaseous oxygen was delivered into the systemic circulation. This paper described the significant benefit of PSF over liquid perfusion, citing the lack of edema formation and improved oxygenation. The authors even replaced oxygen gas with nitrogen gas to illustrate how anoxia eliminated these reflex activities. In a related, follow-up study the same group showed that a rabbit heart and skeletal muscle (tibialis anterior muscle) could be preserved with minimal depreciation of function during about 3 h of PSF [6].

These early reports establishing the potential virtues of PSF for improved organ preservation set the scene for exploration in a variety of tissues and organs.

### Whole organ persufflation

#### Heart

Although the earliest studies of PSF were focused on the heart, research in heart PSF had subsided for about 3 decades (1960s–1990s) in favor of research in liver and kidney PSF. More recently, cardiac PSF has been rekindled and several studies have been published in which PSF was used prior to transplant, including the use of PSF to preserve hearts having suffered short periods of warm ischemia. Collectively, these studies have at least established that cardiac PSF is technically possible and that it can preserve heart tissue.

The advent of cardiopulmonary bypass (CPB) in the mid-1950s provided impetus for exploring the use of PSF with the heart. In 1959, Sabiston et al. at Johns Hopkins explored the use of PSF in

**Table 1**  
Potential advantages and disadvantages of hypothermic organ preservation techniques.

Preservation technique	Advantages	Disadvantages
Static cold storage (SCS)	Easiest and cheapest to implement Simplest logistical considerations	Significant nutrient delivery and oxygen diffusion limitations Extends core organ warm ischemia time and characterized by slow, inhomogeneous cooling Cannot extend allowable cold ischemia time or resuscitate ischemically-damaged organs
Hypothermic machine perfusion (HMP)	Can efficiently deliver nutrients and oxygen into the core of the organ under appropriate conditions Can continuously clear waste products during preservation period Can extend preservation period up to 48–72 h in kidneys and may in other organs May be able to monitor viability more easily	May increase risk of damage to vascular structures Can cause edema within organ ('perfusion nephropathy') Common perfusates have limited oxygen solubility, especially as compared with blood Perfusion pressures may damage endothelium (possibly affecting vascular function and/or inducing thrombosis) Useable substrates may be washed out Risk of transmitting reactive antibodies or pathogens may exist (if cryoprecipitated plasma is used) More challenging logistical considerations Fairly complicated and expensive technique to implement
Persufflation (PSF)	Can deliver more oxygen per gram tissue than SCS or HMP Gaseous perfusate has lower viscosity, may reach more regions of preserved organs and does not cause edema Can extend preservation time and may be able to resuscitate marginal organs May be able to monitor viability more easily May be simpler to implement than HMP	May increase risk of damage to vascular structures Depending on gaseous oxygen concentration, may induce hyperoxic damage in tissue Risk of damaging tissue by desiccation if gas is not properly humidified during long-term preservation PSF resembles iatrogenic gas embolization and challenges clinical dogma Cannot deliver nutrients like liquid perfusion and may be less efficient at removing waste products

conjunction with CPB [76]. In the first set of experiments, hearts from medium-sized dogs were cannulated at the coronary ostia, flushed with pre-oxygenated normal saline, and then persufflated with humidified gaseous carbogen (a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>). This approach, generally termed anterograde PSF (A-PSF), contrasts with retrograde PSF (R-PSF) which would be tried in the heart [8,87] and subsequently in the kidney [28,32]. Hearts maintained at 37 °C continued to beat for an average duration of 5.1 h (2.5–8 h, range). Cardiac contractility remained strong for the first 2–3 h and then gradually weakened. In some cases, the electrical activity of the heart continued for periods up to 4 h following cessation of a visible heartbeat. The second set of experiments examined *in situ* A-PSF of the heart. Once the heart had been isolated from the systemic circulation, A-PSF was performed for 25–30 min. A normal hemodynamic response was restored in 9 of 12 animals and some animals maintained a heartbeat for 48 h following the reestablishment of native coronary circulation. This study established the use of PSF in cardiac surgery by showing that oxygen gas can be used by the heart and that coronary blood flow may be reestablished after PSF. In 1960, a follow-up study introduced the concept of R-PSF [87]. At the time, retrograde perfusion of oxygenated blood via the coronary sinus was used to maintain a heartbeat and protect the heart from anoxia for short periods of time during open aortic valve procedures [22,43]. Talbert et al. used this knowledge, along with their previous work on A-PSF, to investigate whether R-PSF could be performed successfully in the heart. In their experiments, the coronary sinus was cannulated in 7 canine hearts, flushed with normal saline and started on R-PSF. These organs maintained a beat for an average duration of 3.5 h (2–4 h, range). In 3 separate hearts, the anterior cardiac veins were additionally cannulated and persufflated. These organs maintained a visible beat for an average duration of 5.5 h and up to 7 h. They noted that cardiac activity remained strong over the first 2 h of experimental conditions and then gradually became weaker until ventricular fibrillation or complete asystole had occurred. Talbert compared the R-PSF with A-PSF and determined that the heartbeat was visibly weaker and sustained for a shorter period of time using the retrograde approach. Nevertheless, they concluded that oxygen gas can be delivered retrogradely and that the method exhibits some efficacy.

In 1966, the Talbert et al. concept of R-PSF was applied during CPB by Camishion et al. [8]. They noted that continuous blood perfusion during open aortic valve procedures was cumbersome due to the obstruction of the surgical field caused by cannulation of the coronary vessels from within the aorta. Consequently, they tried to determine whether animals could survive CPB by using R-PSF of the coronary sinus as the main preservation technique. This was investigated by repeating previous work by Talbert et al. on *in situ* R-PSF using a similar canine model [87]. They reported that each of 10 canine hearts maintained a sinus rhythm for at least 31 min while being retrogradely persufflated. Following the loss of a sinus rhythm, 8 animals maintained a nodal or ventricular rhythm for up to 7 h and 2 animals developed and sustained ventricular fibrillation for up to 6 h. When hearts were persufflated with nitrogen gas, sinus rhythm was maintained for 5 min or less and a visible beat was lost in all 10 animals within an average duration of 11 min and no longer than 25 min. When persufflation was switched to oxygen gas, a 30-fold increase in the tissue oxygen partial pressure of the hearts was observed almost immediately. In 2 animals, asystole was converted to a persistent ventricular rhythm. In a second experimental study, the coronary sinus in 20 porcine and 10 canine hearts were cannulated and animals were placed on CPB using R-PSF for 1 h. During CPB, 25 of 30 animals maintained sinus rhythm for the entire hour. Of the remaining 5 animals, all developed ventricular fibrillation after an average of 30 min and 1 spontaneously reverted to a nodal rhythm after

20 min of sustained ventricular fibrillation. Following removal of CPB, 22 of 30 animals remained in sinus rhythm. Four animals with ventricular fibrillation were converted electrically to sinus rhythm, 3 animals developed fibrillation after reperfusion, all of which could be converted electrically to sinus rhythm. The remaining animal exhibiting nodal rhythm converted to normal sinus rhythm spontaneously following cessation of CPB. After re-establishment of native coronary blood flow, mean aortic blood pressure was maintained between 60–120 mm Hg and central venous pressures remained 4.4–14.7 mm Hg in all animals. Of the 30 experimental animals in the second group, only 1 exhibited signs of heart failure postoperatively. This animal developed severe pulmonary edema following transfusion of 2500 mL of blood for ongoing hemorrhage. From the vantage point of a contemporary understanding of shock and transfusion medicine, it is conceivable that this animal may have developed a variant of acute respiratory distress syndrome or transfusion-related acute lung injury, which may have been misinterpreted as pulmonary edema from congestive heart failure – though the true pathology will never be known. Nevertheless, these studies illustrated that a heart could be preserved by PSF during CPB and that these organs recovered their function following reperfusion. No evidence of air embolization in the brain or viscera of any experimental animal. The authors commented on the fundamental difference between gas embolization and PSF; gas emboli are small bubbles that may occlude smaller vessels, whereas PSF is characterized by the free flow of a gas within the vascular system. This distinction is still not fully appreciated by the clinical community and services to highlight that this will need to continue to be proven experimentally. Camishion et al. also raised the possibility of using this preservation technique for the maintenance of donor hearts in cardiac transplant, even before the first successful heart transplant was performed in South Africa a few years later [3].

Also in 1966, Gabel et al. examined the physiology of the persufflated heart by using juvenile feline hearts that were anterogradely persufflated with gaseous carbogen mixture (95% O<sub>2</sub>, 5% CO<sub>2</sub>) via cannulae secured in the proximal aorta and compared with controls perfused with substrate-free Krebs solution [21]. They found that the heart rate in persufflated hearts declined rapidly over the first hour and then declined more slowly over the next 9 h, whereas the heart rate in the liquid-perfused heart exhibited a steady decline over the entire experimental period. Contractility measurements under A-PSF showed an initial rise in contractile force during the first 20 min and subsequent fall after 4 h. The persufflated hearts reached 50% of the initial contractile force after 7 h, whereas liquid-perfused hearts declined to 50% of initial contractile force in only 80 min. Metabolic studies revealed that glycogen, carbohydrate, lactate, and pyruvate levels decreased rapidly in the persufflated heart, but when these hearts were treated with pharmacologic agents they responded as expected. In addition, they found that rhythm changes in the A-PSF model reproducibly occurred above a certain threshold PSF pressure. Gabel et al. concluded that persufflated hearts exhibited stronger contractile forces, performed more work and were slower to fail than the hearts perfused with liquid. They theorized that oxygen gas allowed an increase in cardiac work, even though oxygen supply is not traditionally considered a major determinant of work capacity. It may be that cardiac oxygen extraction is altered when oxygen is delivered by PSF or that simply more oxygen is delivered using PSF. Another hypothesis emerging from these findings was that, in the case of PSF, active metabolites equilibrate solely between the extracellular fluid and the intracellular space, as opposed to being flushed away by a liquid perfusate.

Lochner et al. subsequently studied the metabolism and function of anterogradely persufflated guinea pig and rat hearts [40]. Hearts were persufflated with carbogen gas mixture (95% O<sub>2</sub>, 5%

CO<sub>2</sub>) at 37 °C for 1 h. With persufflated hearts, the peak systolic pressures and the first derivative of the left ventricular systolic pressure decreased, while exhibiting very little change in the heart rate. This seemed to indicate that the persufflated heart could continue to generate hemodynamic work. In a few of the hearts, A-PSF time was extended to 2 h, resulting in no additional decreases in heart rate, left ventricular systolic pressure and its first temporal derivative, or isovolumetric work. Isovolumetric work and the first derivative of left ventricular systolic pressure following PSF were calculated to be 16.4–18.4% of values characteristic for liquid-perfused hearts, respectively. Measurements of tissue creatine phosphate and ATP were similar between the PSF and liquid perfusion groups. This led the authors to suggest that the decrease in cardiac work was likely not due to a lack of available cellular energy. They also discovered that work capacity could be enhanced by increasing the PSF pressure or the diastolic filling pressure.

Following these early studies, there was a gap of several decades during which no work was published on the gaseous perfusion of hearts. It was not until the late 1990s that interest in cardiac PSF rekindled, largely as a result of the successful application of PSF in other organs, in particular the kidney and liver. What had been previously referred to as 'gaseous oxygen perfusion' was eventually termed 'persufflation' by Denecke [10]. After pursuing extensive work with kidneys and livers, Fischer's group in Cologne explored cold preservation via cardiac PSF in 1998 [35]. Porcine hearts were flushed and stored at 0–1 °C using 3 different methods: (1) SCS using modified Euro-flush solution with glutathione (based on Euro-Collins solution); (2) SCS with University of Wisconsin (UW) solution; and (3) A-PSF via the ascending aorta in combination with SCS using Histidine-Tryptophan-Ketoglutarate solution modified by adding hyaluronidase. The overall mean preservation time was 14.5 h. Hearts were orthotopically transplanted into recipient pigs of comparable body weight using standard CPB, reperfused on CPB using whole blood for an average of 154 min before being weaned off CPB to allow the hearts to take over normal circulation. Following transplantation, hemodynamic parameters were measured to estimate cardiac function and serum creatine kinase values were obtained as an indicator of myocardial damage. Prior to sacrificing the porcine recipient, left ventricular biopsies were taken to estimate myocardial water content and ATP levels. Persufflated hearts exhibited stroke work similar to preoperative values, whereas comparable measurements could not be obtained in static cold-stored organs due to severe arrhythmia and ventricular dyskinesia. Measurements of cardiac output, left ventricular systolic pressure and its first temporal derivative revealed that the persufflated hearts fared significantly better than hearts stored in modified Euro-flush solution alone and had better cardiac output than hearts stored in UW solution alone. Equivalent creatine kinase levels between each group indicated that the degree of cellular damage using A-PSF may have been similar to conventional SCS. However, persufflated hearts exhibited significantly higher ATP levels than UW/Euro-flush solution-stored hearts. Collectively, these data seem to indicate that A-PSF may permit superior recovery of post-transplant heart function compared with SCS using either UW or modified Euro-flush solution. Importantly, myocardial water content measurements indicated that there was significantly less myocardial edema with A-PSF than static UW solution alone. Decreased myocardial edema is a distinct benefit of PSF, as it is known that tissue edema can significantly impair cardiac function [37]. A follow-up study examined the effects of A-PSF on myocardial tissue quality and post-transplant cardiac function by comparing against SCS in histidine-tryptophan-ketoglutarate solution with and without hyaluronidase [36]. The cohort of hearts preserved by coronary A-PSF showed significantly higher left ventricular systolic pressure and its first temporal derivative, and cardiac output compared to hearts preserved by SCS with histi-

dine-tryptophan-ketoglutarate solution, but not when modified with hyaluronidase. Persufflated hearts maintained normal circulatory function for longer when compared to either SCS methodology. Additionally, tissue ATP levels were significantly higher in transplanted hearts following A-PSF than after SCS only. Post-transplant myocardial water content was not elevated in persufflated hearts over controls.

Up to this point, most of the research into cardiac PSF had involved experimental operations with hearts experiencing no "down-time" or conventional warm ischemia as experienced with donation after cardiac death (DCD). The opportunity to resuscitate DCD hearts inspired the group in Cologne to study PSF following warm ischemic damage. Thus, Yotsumoto et al. studied the effects of several hypothermic preservation techniques on post-transplant cardiac function following a mean warm ischemia time (WIT) of 16.7 min in a porcine autotransplant model [102]. Three hours of A-PSF was compared with SCS, with an additional set of controls not damaged by warm ischemia and also stored in histidine-tryptophan-ketoglutarate solution modified with hyaluronidase. As with previous studies examining the effects of preservation on heart function, a number of physiologic parameters were recorded and samples were taken to assess the metabolic recovery of the cardiac tissue. It was reported that control and persufflated hearts were completely weaned from CPB within 2 h of transplantation, whereas the static cold-stored hearts exhibited significantly diminished functional recovery. Near the end of the 3-h reperfusion period – cardiac output, left ventricular contractility, and the relaxation velocity were significantly higher in the A-PSF group as compared to SCS. It appeared that persufflated DCD hearts had functional outcomes similar to hearts procured from heart-beating donors using conventional storage methods. Importantly, Troponin T levels were significantly higher under SCS than for undamaged controls and hearts preserved by A-PSF at 1 h after reperfusion. These data indicate that A-PSF may limit myocardial injury incurred during WIT. The authors noted that the transplant field is reluctant to adopt PSF as a legitimate cardiac preservation technique largely due to concerns about resulting endothelial damage. More recent studies have shown that the coronary arteries of porcine hearts following 3 h of oxygen A-PSF had normal functioning endothelium post-transplant [14,18,34]. Additionally, hearts transplanted following 14 h of A-PSF exhibited no topographic signs of endothelial damage, as assessed by scanning electron microscopy [34]. Fischer has recently reviewed work done by his group, and has described the detailed experimental approach in which A-PSF is recommended as the preferred method [16]. Collectively, these works have shown that cardiac PSF has considerable potential as an emerging preservation technique, and Table 2 summarizes the published work on heart PSF presented in this review.

### Kidney

The initial studies with kidney PSF occurred in the 1960s, shortly after the early development of heart PSF. It was in the kidney that PSF has been most extensively evaluated, likely because the vascular anatomy and associated transplant models are considered to be most straightforward (amongst the major transplantable organs). Following an initial study by Talbert et al. at Johns Hopkins in 1961 [88], most work on kidney PSF was performed by Fischer, Isselhard and others in Cologne, Germany. Early research efforts were comprehensive in developing the technical aspects of PSF (including optimization of flow pressures, oxygen partial pressures, temperature, and type of approach – whether antero- or retrograde – used during kidney PSF) by evaluating their effects on the bioenergetic status and function post-reperfusion. The groundwork produced by the researchers in Cologne

**Table 2**

Summary of published work on heart PSF.

Year	Author [Ref.]	Model(s)	Approach	WIT (min)	Duration of PSF (h)	Gas used	Temp (°C)	Primary endpoint
1902	Magnus [42]	Cat	A-PSF	–	≤1.15	O <sub>2</sub> , H <sub>2</sub> , CO <sub>2</sub>	24–28	Cardiac activity during PSF
1958	Burns [6]	Rabbit	A-PSF	–	>3	Carbogen <sup>a</sup> , N <sub>2</sub>	37	Cardiac activity during PSF
1959	Sabiston [76]	Dog	A-PSF	–	<8	Carbogen <sup>a</sup> , N <sub>2</sub>	37	Cardiac activity during PSF and after reperfusion
1960	Talbert [87]	Dog	R-PSF	–	2–7	Carbogen <sup>a</sup>	37	Cardiac activity during PSF and reperfusion
1966	Camishion [8]	Dog, Pig	R-PSF	–	<7	O <sub>2</sub> , N <sub>2</sub>	38	Cardiac activity during PSF
1966	Gabel [21]	Cat	A-PSF	–	10	Carbogen <sup>a</sup>	40	Cardiac activity and metabolic profile during PSF
1968	Lochner [40]	Guinea pig, Rat	A-PSF	–	<1.5	Carbogen <sup>a</sup>	4–37	Cardiac activity, WOOCR and metabolic profile during PSF
1998	Kuhn-Regnier [35]	Pig <sup>Tx</sup>	A-PSF	–	14.5	O <sub>2</sub>	0–1	Cardiac function and metabolic profile post-allotransplant
2000	Kuhn-Regnier [36]	Pig <sup>Tx</sup>	A-PSF	–	14.5	O <sub>2</sub>	0–1	Cardiac function and metabolic profile post-allotransplant
2003	Yotsumoto [102]	Pig (DCD) <sup>Tx</sup>	A-PSF	16.7	2.3	O <sub>2</sub>	0–1	Cardiac function and metabolic profile post-allotransplant
2001	Fischer J [14]	Pig (DCD)	A-PSF	16	3.3	O <sub>2</sub>	0–1	Coronary endothelial function
2004	Kuhn-Regnier [34]	Pig <sup>Tx</sup>	A-PSF	–	14	O <sub>2</sub>	0–1	Endothelial and myocardial cell function post-allotransplant
2004	Fischer [18]	Pig (DCD)	A-PSF	16	3.3	O <sub>2</sub>	0–1	Coronary endothelial function

<sup>a</sup> 95% O<sub>2</sub>, 5% CO<sub>2</sub>; DCD, donation after cardiac death; Tx, specifies transplant model; WOOCR, whole organ oxygen consumption rate.

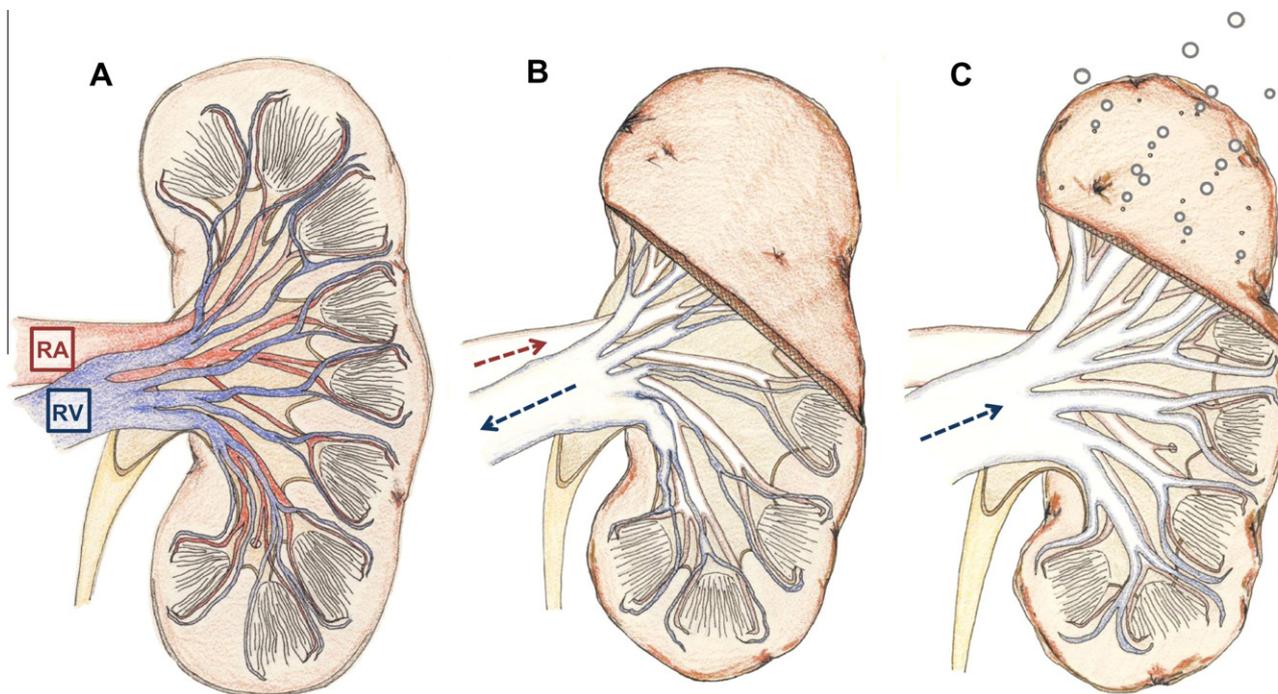
stimulated interest in the field and by the 1980s a number of other institutions had initiated studies to explore the value of kidney PSF.

The initial study by Talbert et al. involved *in situ* PSF of 7 canine kidneys [88] and showed that A-PSF with gaseous carbogen mixture (95% O<sub>2</sub> and 5% CO<sub>2</sub>) could be used to preserve kidney function for 4 h. A-PSF was performed by feeding a catheter through the left iliac artery and positioning it at the renal artery. Once the catheter was appropriately positioned and the proximal renal artery around the catheter was sealed, the left renal vein was clamped distally and the left gonadal vein was divided and used for drainage. Once the blood was flushed using normal saline, A-PSF was started at a pressure of 120–150 mm Hg (to expel the liquid perfusate) and gradually decreased to 80–100 mm Hg. The left kidney was persufflated for 2–4 h, flushed with normal saline until no visual evidence of gas appeared in the venous effluent and then renal blood flow was re-established. This was performed by pulling back on the renal arterial catheter, removing the renal venous clamp and ligating the proximal stump of the left gonadal vein. The animals were then monitored for up to a year. The study included 2 sets of controls. In the first set, 4 dogs had the left renal artery isolated and clamped for 2 h, while in the second set of controls the left renal artery was cannulated, flushed and the renal circulation was re-established after 2 h of warm ischemia. Compared to controls, persufflated kidneys functioned for some time after the treatment. Renal function was determined primarily through intravenous pyelography and also by assessing left kidney function following contralateral nephrectomy. Furthermore, histologically, most of the persufflated kidneys exhibited some signs of tubular atrophy and scarring, but these findings were considered minimal in comparison with ischemic controls. The authors concluded that PSF has the potential to prevent the harmful consequences of warm ischemia and that the afforded protective effects are likely a result of tissue being able to utilize and survive by consumption of gaseous oxygen. They noted that simply clearing the renal vasculature of blood (to prevent coagulation during the ischemic period) was not enough to preserve organ function. These findings are highly significant in that PSF appeared to keep kidneys alive during 2 h of WIT.

It would be 10 years before these encouraging observations reported by Talbert et al. were pursued further by others. In 1971, Denecke et al. developed an *in situ* canine renal ischemia

model [10], similar to the one developed by Talbert et al. [88]. This study involved a comparison between hypothermic A-PSF at 100 mm Hg and SCS. Kidneys undergoing either treatment were initially flushed clear of blood by perfusion with an unspecified crystalloid solution. Following 4 h of A-PSF or SCS, contralateral nephrectomy was performed and circulation to the remaining experimental kidney was re-established. It was reported that A-PSF was actually more harmful to the kidneys than SCS alone; 4 of 5 dogs had died within 7 days, while the remaining dog survived but exhibited marked uremia. Of the 3 dogs having their kidneys preserved by SCS, all survived. Additionally, it was determined that persufflated kidneys had difficulty maintaining normal blood flow following the treatment, with perfusion having decreased to roughly one-third of normal. Moreover, despite an increase in tissue levels of ATP during A-PSF, the ATP levels quickly diminished following reperfusion. The authors postulated that as a result of enhanced oxygenation, the renal vasculature had responded reflexively by increasing the resistance to flow, thereby decreasing global reperfusion of the kidney. As far as we are aware, further evidence has not been provided to substantiate this claim. In our opinion, the physiological response invoked to explain these observations does not seem tenable under hypothermic conditions. It is more likely that the decrease in perfusion may have been related to vascular damage caused by hyperoxia and elevated PSF pressures. On a historical note, this was the first time that the term ‘persufflation’ was substituted for gaseous oxygen perfusion.

Follow-up studies resulting from this original report are important for addressing the largely unexpected outcome that A-PSF had a distinctly detrimental effect as compared with R-PSF. Isselhard and his collaborators published a series of studies that examined the differences between A-PSF and R-PSF [28–32,77], which is PSF by delivering the gas in the direction opposite to physiologic flow (starting at the venous end). Historically, the technique of R-PSF also involved the introduction of small, pin-pricks into the surface of the organ – to facilitate gas efflux as illustrated in Fig. 1. In these studies, the effects of SCS, A-PSF and R-PSF on the bioenergetic profile of canine kidneys throughout preservation and after reperfusion were explored using their established *in situ* model. The degradation rate of high-energy phosphates at 37, 26 and 6 °C in canine kidneys was studied to better understand the effect of hypothermia on ATP, ADP and AMP levels.



**Fig. 1.** Cross-sectional illustration from the anterior view showing a native kidney (A) and kidneys being preserved by A-PSF (B) and R-PSF (C). Note the differences between A-PSF and R-PSF, in particular the relatively pronounced distension of the kidney during A-PSF and the capsular perforations only found with R-PSF – which allow for gas to escape during preservation and reduces overall resistance to gas flow.

Furthermore, they measured the levels of high-energy phosphates and lactate in kidneys undergoing A-PSF and R-PSF using pure gaseous oxygen (100% O<sub>2</sub>), 40% oxygen gas (mixed with 55% N<sub>2</sub> and 5% CO<sub>2</sub>), and room air (21% O<sub>2</sub>) to also study the effects of delivered oxygen concentration. A-PSF was performed at 60 or 100 mm Hg and R-PSF at 30 or 60 mm Hg. To study the impact of preservation protocol on metabolic status, renal cortical biopsies were taken at various time-points before and during preservation and after blood flow had been re-established. ATP depletion rate dropped by a factor of 2 and nearly 10 for kidneys preserved at 26 °C and 6 °C, respectively, as compared with measurements at 37 °C. These findings confirmed that hypothermia diminishes the pace of energy utilization during storage. In the same study, Isselhard et al. were able to illustrate that the operational pressures of both A-PSF and R-PSF needed optimization for the best outcomes. ATP levels during R-PSF at 26 °C and for 8 h were strongly dependent on the driving pressures, averaging 81% and 98% of control values at 30 and 60 mm Hg. They also reported that R-PSF was generally better at the lower pressure (30 mm Hg) than A-PSF at either 60 or 100 mm Hg, based on these metabolic assays. It also appeared that lowering the PSF pressure from 100 to 60 mm Hg during A-PSF had a stronger, negative effect on ATP metabolism than lowering the PSF pressure from 60 to 30 mm Hg during R-PSF. The authors specifically stated that pressures below 60 mm Hg were unable to sustain adequate gas flow during A-PSF and they also pointed out that the rate at which ATP was degraded increased inversely with gaseous oxygen concentration. Not surprisingly, lactate levels rose as the oxygen concentration decreased (from 100% to 40% and 21%) in both A-PSF (at 60 mm Hg) and R-PSF (at 30 mm Hg), but more dramatically during A-PSF. A remarkable accomplishment was the demonstration that ATP levels were maintained at 40% and 30% of control values under A-PSF (at 60 mm Hg, 66 h) and R-PSF (at 30 mm Hg, 72 h) at 6 °C, respectively. In contrast, during SCS – ATP levels were reduced to negligible levels within minutes. These investigators noted that despite an ability to maintain a healthy bioenergetic status in preserved kidneys by PSF, the energy dispar-

ity (between utilization and production) remains during hypothermic storage and is only slowed down.

Further studies by this group remained focused on the important differences between A-PSF and R-PSF, by assessing their effects on renal bioenergetic status and function after reperfusion [29,32]. *In situ* A-PSF (at 90–100 mm Hg) was performed on canine kidneys for 4 h at 6 °C. Following the preservation period, a contralateral nephrectomy was carried out and the treated kidneys were reperfused. These persufflated kidneys were compared with healthy control kidneys and kidneys preserved by SCS for 4 h and at 6 °C. In summary, it was shown that A-PSF was better at maintaining ATP levels than SCS alone. However, once blood flow was restored, kidneys preserved by A-PSF fared no better. Sixty minutes after reperfusion, static cold-stored kidneys restored their ATP to levels comparable to those achieved following A-PSF. It was observed that kidneys preserved by A-PSF exhibited healthy levels of ATP during the first 30 min of reperfusion, but that deterioration quickly ensued. The authors attributed this fall in ATP to the development of poor intrarenal blood flow following reperfusion and speculated that the cause was damage inflicted on glomerular vessels during A-PSF. *In vivo* renal function studies yielded findings that supported the belief that filtration had been most affected. Within 8 days, all dogs having a kidney preserved by A-PSF had died, while all dogs in the SCS control group survived. A progressive decline in renal function was documented through failing urine production, uremia and systematic elevation in serum creatinine. The glomerular filtration rate and renal plasma flow had dropped drastically by post-operative day (POD) 2 in all animals that died following A-PSF. Following this study, Isselhard pursued an identical study using R-PSF and, in contrast to A-PSF, R-PSF for 4 h at 30 mm Hg did not result in the same deterioration in kidney function. ATP levels remained similar after 60 min of reperfusion, were no different from healthy controls, and better than static cold-stored controls. Furthermore, serum urea and creatinine values were generally lower following R-PSF than with SCS, but remained above baseline at POD 10. Glomerular filtration rate and

renal plasma flow were normalized by POD 2 in persufflated kidneys, but static cold-stored kidneys did not fully normalize until POD 21, highlighting the accelerated recovery of renal function following R-PSF. As a direct result of these studies, the Cologne group primarily adopted R-PSF as the most promising of these approaches.

In the mid-to-late 1970s, Fischer's group contributed some of the most fundamental work on kidney PSF. In 1978, Fischer et al. presented a study in which the functional recovery of kidneys was documented after 2 or 30 min of WIT and following 24 h of R-PSF, all of which preceded heterotopic autotransplantation into dogs [17]. For this, the authors developed a unique model where a contralateral nephrectomy was not used to isolate the functional output of each kidney – rather the preserved kidney was by transplanted into the collar region while the contralateral kidney was left in the retroperitoneum. It was demonstrated that 24 h of R-PSF, in the presence of up to 30 min of WIT, was capable of preserving post-transplant renal function. Key measurable parameters of kidney function were glomerular filtration rate and renal plasma flow during a 3-h period following transplantation. In kidneys subjected to only 2 min of WIT, the persufflated and autotransplanted kidney exhibited mean glomerular filtration rate and renal plasma flow that were 46% and 56% of the healthy contralateral kidney, respectively. Similarly, in kidneys undergoing 30 min of WIT – the preserved kidney had mean values of glomerular filtration rate and renal plasma flow that were 32% and 49% of the healthy control during the observation period. These results demonstrated that *ex vivo*, hypothermic R-PSF for 24 h can preserve renal function in the face of considerable WIT (30 min). At the same time, Isselhard suggested that the duration of cold preservation by R-PSF could possibly be extended even further, up to 48 h [27] – leading to a new line of investigation. In summary, they found that R-PSF maintained ATP levels for up to 120 h. A more significant finding was that 30 min of WIT did not have as profound an effect on the ability of R-PSF to resuscitate the organ. ATP levels were monitored for 72 h and were maintained at levels comparable to kidneys not damaged by warm ischemia. As cold ischemia time was prolonged, the capacity for aerobic metabolism measurably decreased but it was apparent that R-PSF may extend the life of kidneys during cold preservation.

At this point, the reported merits of PSF had not been tried clinically. However, in 1975, Flatmark et al. described a short study in which they reported their experiences with the accidental gas perfusion of human kidneys during HMP [19]. These kidneys were preserved in SCS (at 4 °C, for 4–7.5 h), transported from the site of procurement and then started on machine perfusion (at 8–10 °C, with the perfusate equilibrated to 66% N<sub>2</sub>, 33% O<sub>2</sub> and 1% CO<sub>2</sub>) once received at their institution in Oslo. At some time after the start of HMP (between 3 and 12.5 h), leaks were discovered near the oxygenator, which allowed air to be pulled into the flow circuit. In each of 4 kidneys the leak persisted and these organs were persufflated with air for about 60–120 min. Once the leak was identified, liquid perfusion was re-established and continued for the remainder of cold preservation, or 2–18.5 h. Each kidney was subsequently transplanted, all produced urine immediately and most achieved healthy renal function by 4 weeks post-operatively. One of the 4 patients unfortunately died, but the cause of death was not reported. In conclusion the authors stated that PSF (which they referred to as 'massive gas embolization') for up to 2 h did not adversely affect post-transplant renal function. This was the first time that PSF was performed on human organs, albeit inadvertently.

The significant contributions of the Cologne group provided inspiration for other scientists to pursue this field of investigation. In Australia, Ross and Escott explored 24-h PSF of canine kidneys following 30 min of WIT and the effects of PSF on heterotopic auto-

transplant outcomes [70,71]. These studies focused on how the composition of the gaseous perfusate affected post-transplant renal function and survival. Also, these investigators tried persufflating via the ureter for the first time. Three interesting observations were reported: First, that R-PSF was better at preserving renal function than A-PSF, as measured by serum creatinine values post-transplant. Second, that carbogen (95% O<sub>2</sub>, 5% CO<sub>2</sub>) may be better than pure oxygen gas (100% O<sub>2</sub>) in normalizing renal function post-transplant. Third, that ureteral PSF may work, but that it needs further exploration. One puzzling finding from these studies was the relatively high incidence of intravascular thrombosis in all groups studied. They had postulated that it may have been due to endothelial damage resulting from a gas-drying effect, yet the greatest incidence of thrombosis occurred in the group receiving humidified pure oxygen gas. Traumatic damage to the vasculature may have been an alternative explanation. An explanation not explicitly considered was the possibility of endothelial damage resulting from hyperoxia.

During this same era, Pegg and his group in Cambridge, England published a series of studies exploring the utility of PSF in kidney preservation. This group developed a fairly elaborate canine autotransplantation model that they used to examine the effects of varying lengths of WIT (30 and 60 min) in combination with R-PSF for 24 or 48 h duration [64,68,69]. They also explored the differences between pure oxygen, air, nitrogen and helium PSF on post-transplant renal function and survival (for up to 3 months). It was reported that no kidney transplanted after 60 min of WIT and 24 h of SCS was able to sustain recipient survival. On the contrary, R-PSF with pure oxygen gas was capable of sustaining long-term renal function and survival in most recipients. When air was substituted for oxygen, kidneys remained functioning, but the survival rates decreased. R-PSF with nitrogen and helium gases generated results similar to kidneys preserved by SCS. Kidneys that had undergone 30 min of WIT and 48 h of cold ischemia time performed significantly better post-transplant, particularly if they had been persufflated; most animals (80% of group) survived R-PSF, while only a single animal (20% of group) survived following transplantation of a control kidney. Another noteworthy finding was that they were not able to establish any differences in total adenine nucleotide content between persufflated and control kidneys, or between kidneys persufflated for 24 versus 48 h or with different gases. One explanation is that the authors do not report ATP, ADP or AMP levels, but rather total adenine nucleotide content, which is the sum of these three; it is possible that the individual ATP and ADP fluctuations are masked by their summation. It is also important to note that the R-PSF pressures used in their study were particularly low, which may have resulted in inadequate PSF of the entire organ. This may be corroborated by the better outcomes using pure oxygen gas rather than air. Since the total adenine nucleotide content measurements are derived from tissue processed following biopsy, it is possible that the sampled regions of the kidney were poorly persufflated or that these samples do not reflect real-time total adenine nucleotide content levels because the tissue processing involves several steps and takes some time. Nevertheless, these data are compelling because the authors raise very reasonable questions regarding the interpretation of ATP and ADP measurements and their use as accurate predictors of metabolic quality in organ preservation. It has been widely appreciated that high energy phosphates recycle rapidly and that a single measurement is only a snapshot into the tissue metabolic status that does not necessarily provide information regarding the ability of tissue to recover from an insult [86].

The Cambridge group also published a follow-up pilot study in which a number of DCD human kidneys were persufflated prior to transplantation and compared with static cold-stored kidneys post-transplant in a paired fashion (meaning that each donor

provided 1 kidney for R-PSF and another for SCS) [69]. The average WIT was 55 min, whereas the average cold ischemia time was 21.5 h. The persufflated organs performed better post-transplant, exhibiting a mean onset of function at  $8.4 \pm 2.6$  days versus  $13.9 \pm 1.4$  days in the paired controls. Furthermore, the reported mean serum creatinine levels at POD 15 were 457 and 826  $\mu\text{M}$  for the R-PSF and SCS groups, respectively. It was also explained that cyclosporine was largely omitted from the immunosuppressive regimen in order to limit the impact of calcineurin inhibitor-associated nephropathy on the study results. Even so, of the 6 recipients that received cyclosporine, 4 of them received a kidney that was preserved by R-PSF. This first clinical study exploring R-PSF in preserving DCD kidneys illustrated that: (1) R-PSF can be executed within the current clinical infrastructure, including time-frame; and (2) R-PSF also exhibits the ability to resuscitate organs that have suffered from significant warm ischemia. Collectively, these 2 observations suggest that R-PSF may be easily implemented and could make more DCD organs suitable for transplant.

In an attempt to further elucidate the mechanism of preservation by PSF, Pegg et al. carried out an interesting study in 1989 in which they compared 24 h of SCS to 24 h of R-PSF following 60 min of WIT in an *ex vivo* rabbit kidney model [64]. Their hypothesis was that gaseous oxygen PSF enables continued aerobic respiration and that hypothermic conditions only slow the rates governing energy turnover. They studied ATP levels following R-PSF in conjunction with pharmacologic treatment with ouabain or cyanide/iodoacetate. Concomitant administration of ouabain, a potent sodium pump inhibitor, led to elevated ATP levels over R-PSF controls, which exhibited significantly higher ATP levels if compared with kidneys preserved by SCS. Conversely, treatment with cyanide/iodoacetate resulted in a fall of ATP, below levels present in static cold-stored kidneys. These measurements confirmed that if oxygen is available, even as a gas at low temperatures, it will be consumed by viable tissue. If pumps that consume ATP, like the  $\text{Na}^+/\text{K}^+$ -ATPase, are actively blocked, ATP levels will transiently rise, suggesting that 'new' ATP has been produced. On the contrary, if the electron transport chain is inhibited, then the capacity to generate ATP drops and the available oxygen supply will not be consumed. These findings provided insight into the mechanistic principles governing oxygen consumption. Histology and electron microscopy revealed that renal tissue after R-PSF exhibited cellular damage over smaller foci and were largely surrounded by healthy tissue. Intriguingly, electron micrographs were capable of discriminating between the "orthodox" (energized) mitochondrial configuration present in persufflated samples and the "condensed" (de-energized) mitochondria of the cold-stored tissues [64]. As a whole, these findings support the belief that R-PSF can maintain renal tissue viability and prevent irreversible injury during prolonged cold storage.

During this time, Stowe et al. at the Cleveland Clinic pursued *ex vivo* R-PSF in conjunction with a canine autotransplantation model [84]. After resecting the left kidney and flushing immediately with chilled lactated Ringers solution, R-PSF was performed for 48 h (at 4–5 °C and 7–10 mm Hg). Two days following the start of cold preservation, a contralateral nephrectomy was performed and the preserved kidney was transplanted heterotopically. Only 25% of animals that were autotransplanted with kidneys preserved by SCS or R-PSF survived to POD 22. In addition to comparing R-PSF with SCS, 3 animals received an intramuscular dose (500 mg) of deferoxamine mesylate. All 3 animals receiving a kidney preserved by R-PSF and treated with intramuscular deferoxamine mesylate survived to POD 22. In general, persufflated kidneys exhibited better overall renal function than static cold-stored kidneys. Though the total number of transplants was low, it appeared that deferoxamine mesylate contributed to an elevated survival rate post-transplant. The authors postulated that this was possibly due to free radical-scavenging activity in deferoxamine mesylate.

Several years later, Kootstra's group at the University Hospital in Maastricht addressed the question of whether the presence of adenosine benefits the quality of warm ischemically-damaged (30 min of WIT) and preserved rat kidneys during both R-PSF and SCS [101]. Yin et al. used UW solution containing exogenous adenosine and UW solution with no adenosine. All kidneys, whether preserved by 24 h of R-PSF or SCS, were flushed with either of these UW solutions [101]. In brief, they determined that regeneration of ATP was not affected by the presence of adenosine in UW solution. The authors postulated that since the levels of hypoxanthine (degradation product of adenosine) were significantly higher in renal tissue preserved with exogenous adenosine than in tissue without, that most of the additionally available adenosine was degraded in the tissue and not used in the direct replenishment of ATP. However, hypoxanthine levels were significantly lower in persufflated kidneys than in static cold-stored kidneys, which were also flushed with adenosine-containing UW solution. Alternatively, it may be that adenosine found in the preservation solutions may not be able to cross the plasma membranes of viable cells. The authors also went further by transplanting 20 kidneys, 10 from each of the R-PSF and SCS groups. They reported that none of the rats transplanted with static cold-stored kidneys survived, whereas 3 of the rats transplanted with retrogradely persufflated kidneys survived an observation period of 2 weeks. Because the survival rates between the 2 groups were not significantly different, they concluded that singular measurements of ATP during the preservation period may not predict survival outcomes. These conclusions were similar to those generated at Cambridge [68]. However, a point of caution must be raised regarding the choice in animal model. In general, PSF (or HMP, for that matter) will impart its greatest benefit if an organ is large and cannot obtain adequate oxygen by passive diffusion from the surface alone. In other words, success in a rat model may not be comparable to the human clinical situation because of significant size disparity. This scaling problem has already been encountered when comparing the rat and porcine pancreata during SCS [63]. Rat data must be interpreted with appreciation for oxygen transport limitations, especially when trying to translate outcomes from animal models into the clinical arena.

Finally, more recently, Treckmann built upon the work of his predecessors and published studies that highlight the promising prospects of clinical PSF [96,97]. In 2006, Treckmann et al. used a porcine autotransplantation model to show that R-PSF of kidneys damaged by significant WIT, when compared with conventional SCS, may yield improved survival [97]. Left kidneys from porcine donors were clamped off *in situ* for 60–120 min of WIT, resected, flushed anterogradely, preserved by R-PSF or SCS for 4 h and autotransplanted. They demonstrated that all animals transplanted with persufflated kidneys after 60 min of WIT survived the 7-day observation period, with robust renal function. Animals receiving a similarly-treated kidney but stored by SCS survived in 57.1% of cases. This paired comparison was also performed for both 90 and 120 min of WIT, but did not reveal strong differences between R-PSF and SCS, at least in terms of post-transplant survival. It is important to note that only the persufflated kidneys received a pre-treatment of superoxide dismutase (SOD), which had been determined from their earlier liver work, to help protect against the oxidative damage caused by hyperoxia and reperfusion [49]. A follow-up study compared R-PSF and HMP, using the same porcine autotransplant model; 13 kidneys were resected following 60 min of WIT and preserved by SCS, HMP or R-PSF following pre-treatment with SOD [96]. Results showed that all indicators of renal function were significantly better in persufflated kidneys versus HMP and static cold-stored kidneys at POD 7. Recipient survival after 7 days was 57%, 60% and 100% after transplantation of kidneys preserved by SCS, HMP and R-PSF, respectively. These

**Table 3**

Summary of published work on kidney PSF.

Year	Author [Ref.]	Model	Approach	WIT (min)	Duration of PSF (h)	Gas used	Temp (°C)	Primary endpoint
1961	Talbert [88]	Dog	A-PSF	–	2–4	Carbogen <sup>a</sup>	37	Renal function after reperfusion, gross and microscopic morphology
1971	Denecke [10]	Dog	NS	–	4	O <sub>2</sub>	6	Renal function and metabolic profile after PSF and during reperfusion
1972	Isselhard [28]	Dog	A-PSF, R-PSF	–	2–72	O <sub>2</sub> , Air, Mixture <sup>b</sup>	6, 26	Metabolic profile during PSF
1973	Isselhard [29]	Dog	A-PSF	–	4	O <sub>2</sub>	6	Renal function and metabolic profile after PSF and during reperfusion
1974	Isselhard [32]	Dog	R-PSF	–	4	O <sub>2</sub>	6	Renal function and metabolic profile after PSF and during reperfusion
1975	Flatmark [19]	Human <sup>Tx</sup>	A-PSF	–	1–2	Air	4	Renal function post-allotransplant
1978	Fischer [17]	Dog <sup>Tx</sup>	R-PSF	2, 30	24	O <sub>2</sub>	6	Renal function post-autotransplant (into neck, with no contralateral native nephrectomy)
1978	Isselhard [27]	Dog <sup>Tx</sup>	R-PSF	30	24, 48, 72	O <sub>2</sub>	NS	Renal function post-autotransplant
1979	Ross [70]	Dog <sup>Tx</sup>	A-PSF, R-PSF	30	24	O <sub>2</sub>	NS	Renal function post-autotransplant
1982	Ross [71]	Dog <sup>Tx</sup>	R-PSF, Ureteral		48	O <sub>2</sub> , Carbogen <sup>a</sup> , Air	NS	Renal function post-autotransplant
1984	Rolles [68]	Dog <sup>Tx</sup>	R-PSF	30, 60	24, 48	O <sub>2</sub> , Air, N <sub>2</sub> , Helium	0–6	Renal function post-autotransplant
1986	Stowe [84]	Dog <sup>Tx</sup>	R-PSF	–	48	O <sub>2</sub>	4–5	Renal function post-autotransplant
1989	Rolles [69]	Human (DCD) <sup>Tx</sup>	R-PSF	13–80	15–27	O <sub>2</sub>	0	Renal function post-allotransplant
1989	Pegg [64]	Rabbit	R-PSF	60	24	O <sub>2</sub>	0	Mechanistic evaluation of oxygen utilization and morphology before and after PSF
1996	Yin [101]	Rat (DCD) <sup>Tx</sup>	R-PSF	30	24	O <sub>2</sub>	4	Metabolic profile before and after PSF, renal function post-allotransplant, and evaluation of exogenous adenosine in cold preservation solution
2006	Treckmann [97]	Pig <sup>Tx</sup>	R-PSF	60, 90, 120	4	O <sub>2</sub>	4	Renal function post-autotransplant
2009	Treckmann [96]	Pig <sup>Tx</sup>	R-PSF	60	4	O <sub>2</sub>	4	Renal function post-autotransplant, comparison with HMP

<sup>a</sup> 95% O<sub>2</sub>, 5% CO<sub>2</sub>.<sup>b</sup> 55% N<sub>2</sub>, 40% O<sub>2</sub>, 5% CO<sub>2</sub>; DCD, donation after cardiac death; NS, not specified; Tx, signifies transplant model.

results in the larger animal model suggest that R-PSF is a promising way to maintain organ quality in DCD kidneys, particularly because R-PSF was directly compared against the already clinically-accepted HMP and performed comparably if not better. The collective work performed on kidney PSF has been summarized in Table 3.

### Liver

PSF was tested for the first time in rat livers around 1980 by Fischer's group at the University of Cologne [15], but was not studied in depth (by the same group) until the 1990s. Initial work revolved around establishing that livers damaged by prolonged WIT could be resuscitated using R-PSF. Each liver in the R-PSF cohort was flushed with cold preservation solution and R-PSF was performed by administering gaseous oxygen at 18–30 mm Hg via a hepatic vein, while providing an escape route for the gas by introducing small, pin-sized holes on the liver surface. They reported that 24 h of R-PSF resulted in no detectable lactate accumulation, but a substantial decrease in glycogen content.

It was during this period that anti-oxidants were identified and used to provide additional value in preservation using R-PSF, by conferring improved hepatocellular integrity and function following reperfusion [49]. In the 1990s, the same group extended some of their *in vitro* studies into small animal (rat) and large animal (pig) liver transplant models. They showed that poorer quality livers could be revived to function successfully post-transplant. As recently as 2008, the same researchers showed that clinical PSF is possible; having a cohort of 5 patients transplanted with persufflated livers and showing no adverse effects and strong graft function [94]. Another interesting niche for PSF has been in the conditioning of a liver near the end of the preservation period, in so-called end- or post-ischemic conditioning [56]. As it stands, liver PSF is actively being explored and the preceding 3 decades of work highlights some of the reasons why.

In 1993, Minor et al. studied reperfusion injury in the rat liver following an ischemic period during preservation [49]. They procured a number of rat livers, flushed sequentially with lactated Ringers and Euro-Collins solutions via the hepatic vein, and bathed the organ in Krebs–Henseleit solution at 37 °C for 60 min. After the controlled duration of WIT, the liver was submerged in Euro-Collins solution at 4 °C and stored for another 60 min. Following static storage alone, the organs underwent normothermic (37 °C) R-PSF for 30 min and were subsequently flushed with lactated Ringers solution. Persufflated organs received some combination of anti-oxidant pre-treatment, either a bolus injection of allopurinol prior to ischemia and/or the addition of allopurinol or SOD into the flushing solution. At the end of the 2.5-h treatment, the liver was flushed with lactated Ringers solution via the infrahepatic caval vein. The effluent was collected and analyzed for ATP and total adenine nucleotide content. The authors also determined the amount of malondialdehyde accumulated (via free radical-induced lipid peroxidation) and the amounts of liver enzymes released by damaged hepatic parenchyma. It was reported that R-PSF of the liver was capable of partially reversing ATP levels after 120 min of combined warm and cold ischemia. Additionally, it was shown that pre-treatment with anti-oxidants decreased the degree of lipid peroxidation and improved ATP recovery with R-PSF. Samples from persufflated livers pre-treated with allopurinol/SOD revealed that gaseous PSF alone can harm the liver due to oxidative damage, but also highlighted the potential of anti-oxidant administration as an adjunctive therapy.

In a follow-up study, the same group showed that early administration of R-PSF reduces lipid peroxidation and may actually suppress the adverse effects of free-radical damage [46]. The authors speculated that immediate PSF may prevent damage by enabling the preservation of free-radical scavenging activity, which itself can require energy. Two years later this concept was studied in more depth using a rat liver reperfusion model [47,48]. The effect

of R-PSF in suppressing ischemia-reperfusion injury was compared to preservation with 48 h of SCS in UW solution at 4 °C and followed by a 30 min period of re-warming with normal saline at 25 °C. Reperfusion consisted of pre-oxygenated (95% O<sub>2</sub>, 5% CO<sub>2</sub>), re-circulating Krebs–Henseleit solution delivered through the portal vein for up to 45 min. The effluent was analyzed and it was reported that endothelial and hepatic parenchymal damage was lowered and that activation of Kupffer cells was reduced in livers that had been persufflated. These findings suggested that R-PSF may avoid disturbances in perfusion (like higher portal venous pressures) that result from damage to vessels. In addition, ATP concentrations were higher in persufflated livers than in static cold-stored livers and, at different times throughout the reperfusion, comparable to fresh control livers that were not subjected to cold storage. It was interesting to note that livers preserved by R-PSF exhibited significantly greater ATP levels as compared to reference values obtained from native rat liver [47].

These studies were extended to evaluate whether R-PSF in combination with SOD pre-conditioning would resuscitate the DCD rat liver, harvested after 30 min of WIT [50]. Livers are known to be poorly resistant to warm ischemic damage [93]. In fact, outcomes following DCD liver transplant have been strongly tied to the extent of warm ischemic injury [66,67,93]. In this study, DCD livers preserved for 24 h with R-PSF appeared to be healthier when compared with livers preserved by SCS in UW solution. The extent of lipid peroxidation was found to be lower in the R-PSF cohort. Interestingly, bile production and ATP levels were higher, while endothelial damage and portal perfusion pressures were lower than even fresh controls following 45 min of reperfusion with pre-oxygenated Krebs–Henseleit solution. It appeared that the putative, harmful effects of high oxygen concentrations during R-PSF could be prevented with the help of anti-oxidant treatment and resuscitation of livers from DCD was possible using R-PSF.

In 1997, Minor et al. introduced a novel rat liver transplant model [52] in which rat livers were preserved with SCS alone or R-PSF. The hypotheses they tested was the proposal that R-PSF limited proteolytic degradation of the liver, believed to contribute to hepatocellular injury during SCS, and that R-PSF would result in improved post-transplant indicators such as decreased plasma levels of malondialdehyde, decreased alanine aminotransferase, increased bile production and increased hepatic tissue perfusion. Proteolysis was estimated by a measured tissue content of free L-alanine. Twenty-four hours of SCS in UW solution resulted in significantly higher concentrations of free L-alanine than fresh (non-stored) controls, while R-PSF seemed to prevent some proteolysis. Following *in vivo* reperfusion, Minor et al. reported that both the static cold-stored only and persufflated livers experienced decreased hepatic perfusion initially, but that the persufflated organs exhibited an overall better recovery. Furthermore, plasma levels of malondialdehyde and alanine aminotransferase were significantly lower in the livers having undergone R-PSF, but still elevated in both, while hepatic bile production was significantly increased in persufflated livers and comparable to fresh controls. It was concluded that R-PSF may help the ischemic liver obviate some of the ill-effects of hypoxia (like activation of cytosolic proteases and autolysis) by maintaining a more favorable metabolic condition. The authors reiterated that protecting tissue from oxidative damage necessitates sufficient energetic support, fueled by an adequate oxygen supply.

In the same year, Minor et al. examined the effect of lower pressure (9 mm Hg) versus higher pressure (18 mm Hg) R-PSF and the use of both pure gaseous oxygen and air [53]. The effectiveness and homogeneity of the R-PSF was studied by detecting autofluorescence of nicotinamide adenine dinucleotide [54,55,59], accumulated primarily in anoxic tissue. The reason why R-PSF was traditionally performed at 18 mm Hg of pressure was arbitrary; it

had been determined that it was the pressure required for visual detection of bubbles escaping from the surface of the perforated liver. Nonetheless, interrogation at 1 and 24 h after the start of cold preservation revealed that R-PSF at 9 and 18 mm Hg resulted in a comparable and significant decrease in nicotinamide adenine dinucleotide over static cold-stored controls. On the other hand, R-PSF with air at 18 mm Hg did not decrease detected nicotinamide adenine dinucleotide levels below those detected in livers on SCS, suggesting that air may not provide adequate oxygen during liver PSF.

Following the extensive studies in rat livers, translation of PSF into a larger animal model was the next step. In 1998, Minor et al. described work in which recipients were allotransplanted with DCD porcine livers following 1 of 2 preservation protocols [57]. All livers were harvested after 45 min of *in situ* WIT in a non-heparinized donor. Using the first protocol, the donor livers were flushed with heparinized normal saline and UW solution via the portal vein and stored on UW solution at 4 °C for 4–5 h. With the second protocol, the donor livers were also flushed with heparinized normal saline and UW solution, but the last 100 mL of UW solution used to flush the liver was spiked with SOD. Following the flush, R-PSF was initiated via the inferior vena cava for 4–5 h. After the period of cold preservation, the stored livers were orthotopically transplanted into recipients. Shortly following transplantation, it was determined that SCS alone was not able to adequately preserve DCD livers; all 5 recipients died in the early post-transplant course. On the other hand, all livers stored by R-PSF were capable of normalizing ammonia levels by POD 1 and aspartate aminotransferase plasma levels by POD 7.

In a follow-up study, the same investigators again compared SCS and R-PSF/SOD preservation by measuring plasma levels of aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and clotting times at 1-h post-transplant, with the primary endpoint of recipient survival at POD 5 [75]. DCD livers underwent 60 min of WIT prior to 4 h of SCS with UW solution or R-PSF and pre-treatment with SOD. All DCD transplants were compared with control livers resected and transplanted immediately following cardiac arrest. They reported that UW-stored livers fared poorly post-transplant, accounting for significantly higher plasma hepatic enzyme levels, lactate dehydrogenase and prolonged partial thromboplastin time, suggesting that the liver had incurred significant hepatocellular injury during the preservation. Additionally, all of the animals receiving such livers died within 3 h of reperfusion. On the contrary, animals receiving livers preserved by R-PSF/SOD survived the observation period. Impressively, it was stated that persufflated porcine DCD livers performed comparably to fresh livers during the post-transplant course. Since very few DCD livers are transplanted nowadays, making more DCD livers available for transplant would reduce the numbers of patients on liver transplant waiting lists.

Around the same time PSF was being used in the resuscitation of DCD livers, Minor et al. explored an interesting application of PSF in pre-conditioning the long-term, static cold-stored liver for reperfusion [56]. In his first study on this topic, rat livers were subjected to either 48 h of SCS on UW solution or 47 h of SCS on UW solution followed by R-PSF for 60 min. Each of the organs were then warmed and reperfused *in vitro* for 45 min. Following reperfusion, livers conditioned (with R-PSF) fared significantly better – exhibiting decreased parenchymal enzyme levels and portal venous pressures, and improved bioenergetic status. It is believed that the delivery of gaseous oxygen prior to reperfusion may prevent edema formation due to the improvement in metabolic condition of the organ. Additionally, it may be that free-radical scavenging activity has been depressed during SCS and that pre-conditioning for reperfusion using PSF helps provide the oxygen necessary for replenishment of such activity [56,58].

The concept of using PSF to condition for reperfusion was studied more than a decade later for the preservation of fatty rat livers [58]. In these most recent studies, R-PSF for 90 min following 20 h of SCS decreased hepatic parenchymal enzyme release, lipid peroxidation, cellular apoptosis and autophagy, and improved the functional clearance of ammonia, microscopic morphology and overall metabolic status of these livers as compared with unconditioned livers. These preliminary studies illustrate that post-ischemic conditioning of preserved organs prior to transplant is an area meriting further study.

With anti-oxidant therapy having already been explored for use with PSF [49,50,57,75], it became apparent that continued success using PSF in preservation may require protection against reperfusion injury. In 2003, Lauschke et al. studied the effect of administering taurine or SOD prior to R-PSF [38]. These studies employed a DCD rat model in which the livers were resected following 60 min of WIT and preserved either by SCS in UW solution, by R-PSF/taurine, or by R-PSF/SOD for 24 h. Following the preservation period, livers were reperfused *in vitro* using Krebs–Henseleit solution maintained at 37 °C for 45 min. Analysis of the effluent at the end of the reperfusion period revealed that livers treated with anti-oxidant exhibited decreased enzyme release and portal vascular resistance, and increased bile production. Interestingly, taurine and SOD appeared to have a similar protective effect for DCD livers in the face of ischemia–reperfusion. Future work to enhance the successful application of PSF for all organs of interest may require the identification of the appropriate anti-oxidant, dose(s) and schedule.

Most recently, Treckmann and colleagues have taken a major step, by translating R-PSF into the human clinical setting. In 2008, they reported results from a pilot study using R-PSF to resuscitate 5 DCD human livers between April 2004 and March 2005 [94]. These donor cadaveric livers were estimated to have undergone anywhere from 20–60 min of WIT and the donors had expired after failed attempts at cardiopulmonary resuscitation or having experienced prolonged periods of hypoperfusion (<60 mm Hg). It is also important to note that these donor livers were rejected for transplant by at least 3 different centers. The livers were procured off-site, perfused with UW solution and histidine–tryptophan–ketoglutarate solution, and then shipped to the transplant center. After establishing recipient consent, the livers were additionally flushed with UW solution containing *N*-acetylcysteine and were retrogradely persufflated at 18 mm Hg for 70–200 min prior to orthotopic transplantation. The results of the transplants were encouraging in all cases. All patients survived and none of the patients required a re-transplant; all patients were alive, with strong graft function, at a minimum of 2 years follow-up. Histologic evaluation was performed on biopsies taken immediately prior to and after R-PSF and directly following reperfusion. Analysis revealed that R-PSF did not appear to cause any vascular damage to the liver. Additionally, it was shown that PSF had recovered ATP levels by 2–5 times the pre-PSF measurements. These data are highly encouraging, especially considering the potential impact that PSF could have in expanding DCD liver transplantation. Table 4 summarizes the published work on liver PSF presented in this review.

#### Small bowel and pancreas

To date, the pancreas and small intestine have not been studied extensively as targets of PSF. In 1997, Minor et al. published the only known work on luminal gas oxygenation of the small bowel [51]. Rat jejunal segments (15-cm in length) along with the vascular pedicle were harvested and stored in UW solution at 4 °C for 18 h and half of the experimental organs underwent low-pressure, luminal gas oxygenation. Following the experimental storage per-

iod, intestinal absorption was estimated by introducing galactose to the lumen and measuring concentrations in portal venous effluent. Collecting the total luminal effluent and subtracting the known inflow volume was used to measure the net influx of water into the intestinal lumen. Results showed significantly increased accumulation of hypoxanthine in the small bowel segments with SCS alone. ATP, creatine phosphate and total adenine nucleotide content were significantly higher in the group undergoing luminal gas oxygenation versus SCS alone and resembled values from rat intestine *in vivo*. However, intestinal carbohydrate absorption was found to be severely impaired in both static cold-stored and gas oxygenated jejunal segments, even though gas oxygenation significantly improved post-ischemic absorption when compared to SCS. The net secretion of water into the gut lumen was significantly lower following gas oxygenation than SCS, reflecting less damage to intestinal villi. The authors noted that luminal gas oxygenation of the intestine could be improved by the introduction of supplements to the cold preservation solution, such as glutamine, an important substrate for intestinal mucosal cells. Although unique opportunities exist for the preservation of the small intestine using intraluminal gas oxygenation (a variant to intravascular PSF), this area of research has remained largely unexplored.

In contrast to small bowel intraluminal gas oxygenation, pancreas PSF has started to attract more interest in recent years. Currently, it is widely regarded that improvements in organ preservation may have a positive impact on pancreatic islet isolation and transplant outcomes [33]. For some time, the two-layer method (TLM) was considered the state-of-the-art for pancreas preservation before islet isolation. This has recently been challenged by studies showing that islet isolation outcomes are equivalent when comparing TLM and conventional SCS [1,7,78]. It is likely that the inefficiency of oxygen delivery by passive diffusion from the organ surface alone is responsible for the limited efficacy of TLM in preserving larger organs [63].

Over the last several years, PSF has been identified as a possible improvement to the current pancreas preservation protocol, particularly before islet isolation. Our research group is currently studying PSF of the pancreas to parallel our concurrent interests in pancreas HMP [89,91] and have recently published several works on A-PSF of porcine pancreata for the purposes of improving whole organ and islet quality [78,79]. In one study [79], human and porcine pancreata were preserved using TLM or A-PSF at 4 °C. A-PSF was performed via the superior mesenteric artery and either the splenic artery (human) or the celiac trunk (pig) using a custom-designed, portable electrochemical oxygen concentrator (Giner Inc., Newton, MA). Following procurement, the organs were imaged by conventional MRI and ATP levels and ATP-to-inorganic phosphate ratios were estimated using <sup>31</sup>P-NMR spectroscopy. MRI revealed well-distributed areas of negative contrast throughout all persufflated pancreata, indicating the homogeneous presence of gas within the organ. Rat pancreata preserved by TLM showed relatively high ATP levels, though ATP levels were nearly undetectable in porcine pancreata preserved with TLM. In contrast, human pancreata preserved by A-PSF exhibited ATP-to-inorganic phosphate ratios similar to those observed in the rat pancreata on the TLM. Additionally, when A-PSF was stopped, ATP-to-inorganic phosphate ratios quickly declined to undetectable levels, similar to porcine organs preserved by TLM. When A-PSF was restarted, ATP levels rose again. In another study, DCD porcine pancreata were procured and the splenic lobe was separated from the connecting and duodenal lobes [78] – the anatomy being described previously [12]. The duodenal lobe was isolated following 1.5–2 h of SCS and served as a first control, while the connecting lobe was stored on TLM for 24 h and at 4 °C to serve as a second control. Splenic lobes were submerged in cold preservation solution and preserved by A-PSF via the celiac trunk and superior mesenteric

**Table 4**  
Summary of published work on liver PSF.

Year	Author [Ref.]	Model	Approach	WIT (min)	Duration of PSF (h)	Gas used	Temp (°C)	Primary endpoint
1980	Fischer J [15]	Rat	R-PSF	–	24	O <sub>2</sub>	6	Metabolic profile after PSF
1993	Minor T [49]	Rat	R-PSF	60	0.5	O <sub>2</sub> , N <sub>2</sub>	37	Hepatic injury and metabolic state before and after PSF, evaluation of anti-oxidant treatment after PSF
1994	Minor T [46]	Rat	R-PSF	60	1	O <sub>2</sub>	4, 37	Hepatic injury, oxidative state and metabolic profile after reperfusion
1996	Minor T [48]	Rat	R-PSF	30 <sup>a</sup>	48	O <sub>2</sub>	4	Hepatic injury after reperfusion, endothelial activity and WOOCR
1996	Minor T [47]	Rat	R-PSF	30 <sup>a</sup>	48	O <sub>2</sub>	4	Metabolic profile after PSF and during reperfusion
1996	Minor T [50]	Rat (DCD)	R-PSF	30 <sup>a</sup>	24	O <sub>2</sub>	4	Hepatic injury and metabolic profile during reperfusion
1997	Minor T [52]	Rat <sup>Tx</sup>	R-PSF	–	24	O <sub>2</sub>	4	Assessment of proteolysis after PSF, hepatic function, perfusion, injury and oxidative state post-allotransplant
1997	Minor T [53]	Rat	R-PSF	–	24	O <sub>2</sub> , Air	4	Hepatic oxygenation during PSF
1997	Minor T [55]	Rat (DCD)	R-PSF	60	2	O <sub>2</sub>	12	Hepatic function, injury and metabolic profile after reperfusion
1997	Minor T [54]	Rat <sup>Tx</sup>	R-PSF	–	24	O <sub>2</sub>	4	Hepatic oxygenation during PSF, metabolic profile after PSF, hepatic injury post-allotransplant
1998	Minor T [59]	Rat	R-PSF	–	24	O <sub>2</sub> , Air	4	Hepatic oxygenation before and after PSF
1998	Minor T [56]	Rat	R-PSF	30 <sup>a</sup>	1	O <sub>2</sub>	4	Hepatic function, injury and metabolic profile following post-ischemic conditioning using PSF and during reperfusion
1998	Minor T [57]	Pig (DCD) <sup>Tx</sup>	R-PSF	45	4–5	O <sub>2</sub>	4	Hepatic function, injury post-allotransplant
2001	Saad S [75]	Pig (DCD) <sup>Tx</sup>	R-PSF	60	4	O <sub>2</sub>	4	Hepatic function, injury post-allotransplant
2003	Lauschke H [38]	Rat (DCD) <sup>Tx</sup>	R-PSF	60	24	O <sub>2</sub>	4	Hepatic function, injury and oxidative state after reperfusion, evaluation of anti-oxidant pre-treatment during PSF
2008	Treckmann J [94]	Human (DCD) <sup>Tx</sup>	R-PSF	20–60	1.2–3.3	O <sub>2</sub>	4	Metabolic status before and after PSF, hepatic function and injury post-allotransplant
2009	Minor T [58]	Rat	R-PSF	20 <sup>a</sup>	1.5	O <sub>2</sub>	4	Hepatic function, injury, oxidative state and metabolic profile following post-ischemic conditioning using PSF and during reperfusion

<sup>a</sup> Re-warming period prior to reperfusion; DCD, donation after cardiac death; Tx, signifies transplant model; WOOCR, whole organ oxygen consumption rate.

artery for 24 h and at 4 °C. Biopsies from organs preserved by A-PSF showed distended capillaries and less autolysis and necrosis when compared to organs preserved by TLM. In contrast, TLM-stored pancreata showed frequent pyknotic nuclei, indicating possible irreversible cellular damage. A follow-up study extended the comparison to porcine pancreatic isolation, having shown that 24 h of A-PSF was better than the TLM in preserving islet morphology, viability and post-culture recovery (unpublished results). Collectively, these results illustrate the potential of A-PSF in improving tissue and islet quality in larger pancreata when compared with conventional preservation methods.

These early and promising results have established the need for more extensive studies since pancreas PSF represents a significant opportunity to improve both solid organ and islet preservation over the current preservation techniques used in the field. Additionally, the portable electrochemical oxygen concentrator used in our studies may enable more widespread use of PSF, not just of the pancreas, since it provides a safe method of delivering gas (or gas mixtures) during transportation and especially during air travel. Table 5 summarizes the published work on small intestine and pancreas PSF presented in this review.

A schematic of the key milestone events during the historical development and use of PSF is presented in Fig. 2.

**Table 5**  
Summary of published work on small intestine and pancreas PSF.

Year	Author [Ref.]	Model	Approach	WIT (min)	Duration of PSF (h)	Gas used	Temp (°C)	Primary endpoint
<i>Small intestine</i>								
1997	Minor T [51]	Rat	Luminal	NS	18	O <sub>2</sub>	4	Intestinal function and metabolic profile after PSF
<i>Pancreas</i>								
2010	Scott WE III [79]	Pig (DCD)	A-PSF	<30	<24	40% O <sub>2</sub>	4	Metabolic quality during PSF, comparison with TLM
2010	Scott WE III [78]	Pig (DCD)	A-PSF	<30	24	40% O <sub>2</sub>	4	Histologic assessment after PSF, comparison with TLM

DCD, donation after cardiac death; NS, not specified; TLM, two-layer method.

### Comparison between anterograde and retrograde persufflation

Earlier, we outlined that 2 main modes of PSF have been introduced and evaluated as a means of delivering oxygen to an organ. The relative merits of R-PSF versus A-PSF were not clearly established by earlier studies and it was not until the early 1970s that studies were undertaken to directly compare the 2 methods in a single system (canine kidney) [28,29,32]. Isselhard et al. developed an *in situ* ischemia model to study the effects of SCS, A-PSF or R-PSF on canine kidney preservation. Initially, they claimed that adequate preservation of kidneys required oxygen gas pressures of at least 60 mm Hg for A-PSF and 30–60 mm Hg for R-PSF. Because the authors did not present data at lower PSF pressures, it is unclear what criteria were used to determine adequate oxygenation – whether it was visual detection of gas escaping the renal vein or puncture holes in the capsule, or whether it was based on measured ATP. Nevertheless, they showed that an increase from 60 to 100 mm Hg in A-PSF did not improve ATP levels. The investigators did not comment on whether these pressures were sufficient to maintain ATP levels at control levels. On the other hand, the metabolic profile improved with an increase from 30 to 60 mm Hg in R-PSF. After 8 h of R-PSF at 60 mm Hg, the ATP levels were near that of healthy controls. When directly comparing A-PSF at

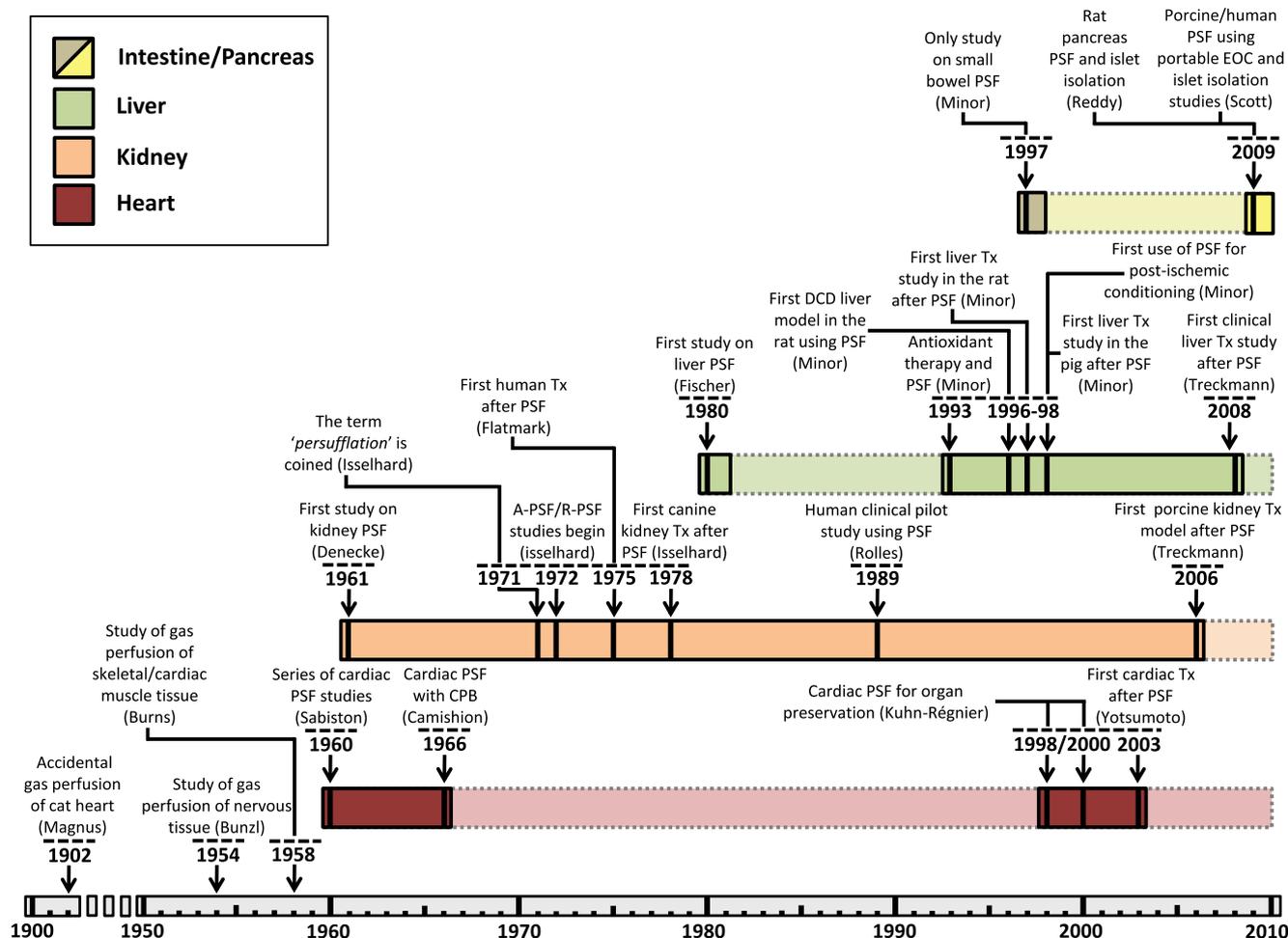


Fig. 2. Historical timeline of significant contributions to the development of persufflation as a method of tissue and organ preservation.

60 mm Hg and R-PSF at 30 mm Hg, the authors illustrated that ATP levels were maintained at similar levels and at similar points during cold preservation.

Subsequently, this research group extended their comparison between A-PSF and R-PSF to the reperfusion period. They showed that A-PSF (at 90–100 mm Hg) for 4 h resulted in deterioration of renal function upon reperfusion, despite being able to maintain adequate levels of ATP throughout preservation. These results were in contrast to those obtained for R-PSF (at 30 mm Hg); R-PSF for 4 h with reperfusion exhibited no such deterioration in function and resulted in faster restoration of normal kidney function when compared with static cold-stored kidneys. Additionally, glomerular filtration rate and renal plasma flow dropped in most kidneys preserved by A-PSF, whereas they had normalized by the second day after reperfusion in kidneys preserved by R-PSF. The authors noted that systemic blood pressures immediately increased for both sets of animals following reperfusion, regardless of the preservation technique employed. However, in animals having a kidney preserved by A-PSF, the systemic blood pressures increased abruptly – until they normalized again after 40–60 min. Dramatic blood pressure increases were not seen with kidneys preserved by either SCS or R-PSF. It was unclear how A-PSF would cause such disparate change in systemic blood pressure – yet these physiologic changes may be attributable to either vascular spasm following the mechanical stresses of surgical manipulation or PSF, reflexive responses of systemic vessels to decreased renal perfusion (via the renin–angiotensin system) or vascular damage and dysfunction with possible thrombosis. In further investigations, these researchers decided to

use a A-PSF pressure of 90–100 mm Hg, even though it was determined that lower pressures would suffice. It is very possible that the elevated PSF pressures may have damaged the renal vasculature. Aside from mechanical damage, elevated oxygen levels throughout the glomerular capillary beds may have created a favorable setting for free radical damage. This may not be the case during R-PSF, where the resistances to gas flow are 2–3 times lower and the regional oxygen concentrations are lower (as gas circumvents capillary beds and exits capsular veins). The authors have cited that histologic evidence points to more noticeable changes in the glomerular structure during A-PSF than R-PSF, but these data were unpublished and thus inconclusive.

In contrast, Fischer et al. described that A-PSF may not cause functional damage to the vasculature of an organ during preservation [18]. They described a study in which porcine hearts were subjected to 16 min of WIT and stored for 3.3 h at 0–1 °C by either SCS or coronary A-PSF and then orthotopically transplanted. It was shown that nitric oxide production by coronary endothelium was not adversely affected by A-PSF (as compared with SCS) and the ability of the coronaries to dilate and contract was preserved during reperfusion. Notable differences between this study and past studies involving A-PSF were that A-PSF was performed on porcine hearts (rather than canine kidneys) and under lower PSF pressures (45 mm Hg). Clearly, differences between the organ models may have contributed to varying results using A-PSF.

Despite somewhat conflicting results between the experimental models, it is reasonably certain that optimizing the PSF technique for both perfusion pressures and oxygen concentrations is impor-

**Table 6**  
Potential advantages and disadvantages of A-PSF and R-PSF.

Preservation technique	Advantages	Disadvantages
Anterograde persufflation (A-PSF)	Follows physiologic flow path May directly deliver gas to a greater part of the organ	Elevated driving pressures may damage vascular function or endothelium May cause spastic or reflexive vascular changes, reducing blood flow to kidney and increasing systemic blood pressure May damage renal microvasculature and has resulted in poorer transplant outcomes
Retrograde persufflation (R-PSF)	Has been shown to sufficiently oxygenate and preserve various organs  May require lower driving pressures	Flow is in the opposite direction of physiological flow and highest pressures localized to thin-walled veins May require small punctures on surface of organ which could lead to bleeding upon reperfusion May not fully deliver oxygen-rich gas to all regions of the organ

tant in achieving the best preservation possible. It is also likely that the optimal PSF technique may be different for different organs. It may be that A-PSF under lower pressures and lower oxygen tension could yield comparable, if not better, outcomes than R-PSF. However, this scenario has remained largely unstudied. In the case of the kidney and liver, it appears that the Cologne group may have maximized success using R-PSF, but it seems A-PSF has not been fully optimized and may prove to be the better approach for certain organs or applications.

In a very recent review, Fischer recommends that the coronary oxygen PSF should be carried out by A-PSF via the coronary arteries with outflow of gas from the coronary sinus. Retrograde gas flow through the aorta to reach coronary arteries does not establish a retrograde PSF of the myocardium. In contrast, Fischer argues that PSF in organs like liver and kidney should not be established in an anterograde manner because entry of gas into the microvessels may block any reperfusion. Hence, R-PSF is recommended in these organs because gas never reaches the microvessels and leaves the organ via openings in capsular veins [16].

Table 6 summarizes some of the potential advantages and disadvantages with A-PSF and R-PSF.

### Comparison between hypothermic machine perfusion and persufflation

HMP is a method of organ preservation that has recently seen a resurgence of interest and shown clinically to have significant benefits over conventional SCS of kidneys [60,90,95]. Kidneys preserved using HMP have shown better early graft function when compared with SCS [44,61]. HMP has recently been recommended as the preferred preservation method for DCD and extended criteria donor kidneys [60,85,90,95,100]. Recently, data is emerging for clinical use of HMP in other organs. Guarrera et al. have shown excellent outcomes, including decreased length of hospital stay with the first human trial of HMP-preserved livers [23]. The scientific basis behind HMP is largely based on rapidly reducing and maintaining the core organ temperature during ischemia. The potential for delivering nutrients, removing harmful waste products, extending cold preservation times, maintaining a patent vascular bed and being able to prospectively monitor whole organ viability during preservation, are all potential benefits of HMP [90]. Potential disadvantages of HMP may include excessive damage to the vascular endothelium as a result of fluid shear and hydrostatic pressures, inadequate oxygen solubility of the perfusate, the possibility that vital or protective substrates of metabolism are continually removed via the circulation, the development of edema detrimental to the organ and the increased cost relative to SCS and possibly PSF. A summary of the advantages and disadvantages of SCS, HMP and PSF can be found in Table 1.

Very few studies have directly compared HMP and PSF, yet some work exists in this regard. Within the last 10 years, So and

Fuller compared SCS, HMP, and R-PSF in the preservation of rat livers [82]. The organs were harvested and divided into 3 groups, all of which were stored at 4 °C. Group 1 livers were preserved by SCS in non-oxygenated UW solution alone, Group 2 employed R-PSF with livers bathed in non-oxygenated UW solution, and Group 3 livers were preserved by HMP bathed in oxygenated UW solution. Tissue samples were obtained from livers at 2 and 24 h of cold preservation and samples were analyzed for adenine nucleotide levels and glucose, lactate, ketone and alanine contents. At 2 h, ATP levels were elevated in livers preserved by HMP and R-PSF, but were only statistically different from static cold-stored livers in the case of HMP. At 24 h, the situation was different revealing that both HMP and R-PSF had the effect of significantly increasing ATP levels as compared with SCS. Lactate levels were initially elevated during SCS and R-PSF, but after 24 h the lactate levels were comparable in livers preserved by HMP or R-PSF. Measured glucose contents were significantly higher during SCS and R-PSF than with HMP. Additionally, alanine levels were significantly elevated under R-PSF and ketone bodies were significantly lower with HMP at both time points. The authors concluded that both HMP and R-PSF could oxygenate a liver during long preservation times. Differences in early lactate measurements between the 2 groups were attributable to lactate being flushed out continuously during HMP. These are very relevant findings, because it may be that lactate is a beneficial substrate utilized by tissue during cold preservation. The authors noted that R-PSF did not appear to completely reverse the low ATP state of early ischemia as quickly as HMP, but these assertions are debatable. They believed that the metabolic resuscitation of organs following a period of hypoxia would require over 2 h of PSF.

Along similar lines, Stegemann et al. recently published a study directly comparing the 3 modalities of cold preservation with DCD livers [83]. Following 30 min of WIT, rat livers were harvested and preserved for 18 h using SCS, HMP or R-PSF. Organ viability was evaluated following *in vitro* reperfusion for 120 min with warm, oxygenated Krebs solution. Portal venous pressures were estimated during reperfusion and alanine aminotransferase, lactate/glutamate dehydrogenase levels were measured in the effluent. The degree of lipid peroxidation, metabolic status and cellular morphology were also studied. Hepatocellular damage was found to be greater during reperfusion in HMP-preserved livers versus those preserved by R-PSF. Glutamate dehydrogenase, an enzyme normally found within mitochondria, was shown to be elevated only during HMP. Histologic analysis of tissue biopsies paralleled the enzyme leakage data. Evidence of lipid peroxidation was similar between HMP and R-PSF, while the metabolic status of persufflated livers was better – as evidenced by significantly higher ATP levels during reperfusion. Finally, only after HMP did the portal venous pressures rise during reperfusion. In contrast, bile production rose significantly only after R-PSF. These data suggest that R-PSF may be a superior method of cold preservation for DCD livers. Longer term recovery of function was not studied during these

experiments, yet it begs the question what happens to the organs following 2 h of reperfusion. A limitation of this study was the use of a reperfusate that was not blood and did not contain any of the proteins (including clotting factors) typically found in plasma. It must be emphasized that there are a number of variables directly affecting the oxygen delivery to tissue by either HMP or PSF, including the fluid dynamic parameters, perfusate oxygen solubility and the patency of the intravascular flow path.

We reviewed earlier the most recent study by Treckmann et al., in which they compared SCS, HMP and R-PSF in porcine kidneys using an autotransplantation model [96]. Recipient survival at POD 7 was 100% after re-transplant of kidneys preserved by R-PSF, while only 57% by SCS and 60% by HMP. In animals re-transplanted with kidneys preserved by either SCS or HMP, plasma creatinine levels remained significantly elevated above baseline, whereas animals with persufflated kidneys maintained normal creatinine clearance. Significant proteinuria and increased lipid peroxidation was noted only after re-transplantation of HMP-preserved kidneys. Microscopic evaluation of tissue from explanted organs revealed that only persufflated kidneys were indistinguishable from healthy controls, which was not the case for kidneys preserved by SCS or HMP. Mild interstitial nephritis characterized static cold-stored kidneys, while HMP-preserved kidneys exhibited tubular protein deposits with signs of interstitial inflammation. The authors acknowledged certain limitations of their study, including the use of UW solution as the HMP perfusate instead of the gold-standard, Belzer machine perfusion solution. Another limitation of this study, as noted by the authors, were the relatively short preservation (4 h) and monitoring (7 days post-operatively) times, which are not representative of standard practice. Future research might consider repeating this study using an allotransplant model to provide additional information, as acute rejection episodes have been linked to delayed graft function [62,65], which in turn has been shown to be influenced by ischemia time and the method of preservation [62,90]. It is also noteworthy that only kidneys preserved by R-PSF received the antioxidant SOD, whereas the other 2 groups did not. This difference may have contributed to differences between HMP and R-PSF. Nevertheless, the authors concluded that R-PSF enhanced organ viability and function following a period of WIT in procured kidneys.

It remains difficult to appreciate the true benefit of PSF over HMP (or vice versa), given the conflicting results amongst studies directly comparing the 2 techniques. What is clear is that the 2 modalities exhibit at least comparable potential, particularly with resuscitation of DCD kidneys and livers. Further studies are needed to better reveal the relative utility of each technique with regards to standard and expanded criteria organ preservation.

### Prospective implications for persufflation in transplantation

There is a clear longstanding discrepancy between the numbers of donor organs available for transplant and the numbers of prospective recipients on waiting lists. With the field of allotransplantation having come far over the last 60 years, an indisputable and persistent reality has been the shortage of donor organs. Many approaches have been levied in order to make more organs available for the donor pool, including the responsible expansion of acceptance criteria and improvements in organ preservation strategies. Some of these actions have yielded fruitful results and have helped prevent amplification of the problem. Data compiled by the United Network for Organ Sharing (UNOS) between 2001 and 2009 has illustrated both the promising and concerning trends (UNOS Data as of April 30th, 2010). Though the overall numbers of recipients on waiting lists have steadily decreased for heart, liver and simultaneous pancreas-kidney transplantation, the numbers of prospec-

tive kidney and pancreas transplant recipients have increased since 2001 by 38.9% and 22.3%, respectively. In the case of the kidney, the mean percent growth of the waitlist was 6.35% per year during this time span. In addition, most data indicate that the mean percent increase in the number of new patients added to a waitlist per year has increased during every year of this era, with the number of new kidney transplant candidates added at a mean rate of 5.2% annually (with a range of 0.2–11.0% per year). With kidney transplantation being the definitive treatment option for end-stage renal disease, it is no surprise that the candidate list is getting longer every year.

Due to this increased demand for transplantable organs, the number of DCD transplants has been steadily increasing for both kidney (3.1% per year) and liver (3.5% per year). Fig. 3 depicts the trends in DCD transplants for liver, kidney, pancreas and simultaneous pancreas-kidney. The stark increase in the numbers of DCD kidney transplants over the last 6 years results from the more wide-spread utilization of HMP during preservation, illustrating that continued acceptance of newer preservation strategies can be a successful approach to make more organs available for transplant. Despite these efforts, many more organs could still be retrieved. In 2009, for example, the total number of DCD transplants only amounted to 8.2% of all performed solid organ transplants. Of all organs recovered for DCD transplant since 2001, 30.1% of livers, 21.4% of kidneys and 50.4% of pancreata were never transplanted. According to UNOS records, 17.2% of livers, 3.9% of kidneys and 11.5% of DCD pancreata that had been procured were discarded due to WIT beyond what was considered acceptable. In addition, many more organs were classified under an 'other' category, which suggests that some organs may have been discarded from consideration after having undergone unknown periods of WIT. Many of these consented organs could have been salvageable. It could be argued that the room for improvement is limited (based on these numbers alone). However, it is likely that many potential DCD organs are never procured because it is perceived that their poor quality does not merit the investment of resources required for their recovery. It is conceivable that improved preservation techniques could result in a lengthening of allowable ischemic times (particularly for heart, liver and pancreas), possibly making previously unsuitable organs suitable for transplant. In other words, advancements in organ preservation may in fact accompany the expansion of donor organ criteria. The opportunity to resuscitate organs damaged by prolonged WIT and to better prevent their deterioration during storage

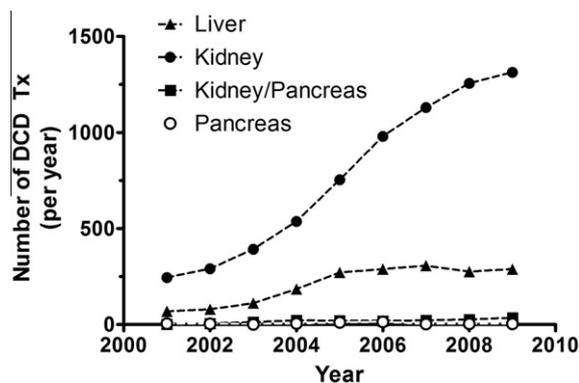
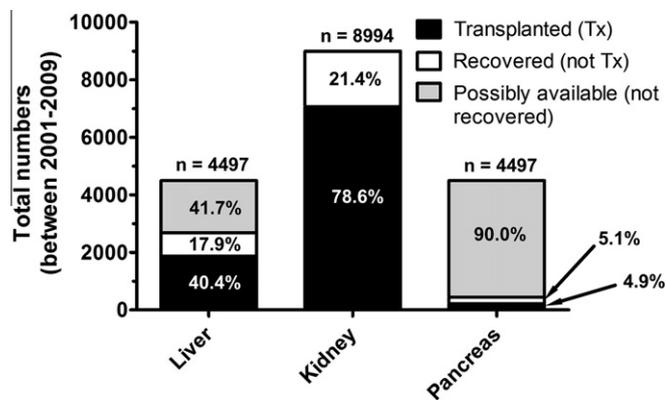


Fig. 3. Relative trends comparing number of donation after cardiac death (DCD) liver, kidney, simultaneous kidney/pancreas and pancreas transplants performed in the United States between 2001 and 2009. Data illustrates the increase in DCD liver and kidney transplants over the last six years, with the increase in the number of kidney transplants being due largely to improved preservation protocol, like hypothermic machine perfusion. Data was prepared by the United Network for Organ Sharing (UNOS) on April 30th, 2010.



**Fig. 4.** Total numbers of donation after cardiac death (DCD) transplants performed in the United States between 2001 and 2009, further segregated into transplanted and recovered (but not transplanted) fractions. Additionally, DCD donor livers and pancreata are often not recovered with DCD donor kidneys due to their true or perceived poor quality; these organs (represented by gray bars) are possibly available for recovery and transplant, and may represent target organs for resuscitation via PSF. Data was prepared by the United Network for Organ Sharing (UNOS) on April 30th, 2010.

should provide sufficient impetus to pursue the development of promising preservation strategies – like PSF. Fig. 4 illustrates the total DCD transplants been performed between 2001 and 2009 and further segregates them into the transplanted, recovered (but not transplanted) and possibly available (but not recovered) fractions. The numbers of organs that are possibly available but are never recovered have been estimated by assuming that each DCD kidney donor exhibits the potential to donate a liver and pancreas.

Even if improvements in preservation strategy do not lead to an immediate improvement in the number of transplantable organs, an incremental improvement in this area should be welcomed. Ultimately, the number of patients that die while waiting on transplant lists is the most important statistic. For instance, on an annual basis, 6.4% and 10.4% of potential transplant candidates for kidney and liver, respectively, do not survive long enough to make it to the operating room. Despite the recent strides made by the field of transplantation, many patients still never receive an opportunity to accept a potentially life-saving organ. In this light, seeking better ways to recover and preserve a greater number of suitable organs should continue to be a primary objective.

PSF has the potential to lengthen the allowable WIT and cold ischemia time for any organ, as supported by some of the studies

reviewed herein. As described earlier in this section, there remains a unique opportunity to maximize the number of accepted DCD donors by rescuing these organs from incurring intolerable amounts of ischemic damage. The case has been made that PSF may benefit heart, liver, kidney and pancreas transplant. A potential application that was only briefly discussed in this review is pancreas PSF before islet isolation. In addition to the unique susceptibility of the pancreas and the islets of Langerhans to ischemia [9,11,39,80], islet cell transplantation poses additional challenges that are not seen in solid organ transplant. For instance, due to the complexity and expense associated with islet isolation, very few centers have the capacity to produce therapeutic preparations. Consequently, procured pancreata may need to be transported further in order to be processed for their islets. These additional travel considerations and distances may require longer preservation times (>8–10 h). Studies performed at our institution have shown how pancreas PSF could be used to preserve human islets [78,79]. Given the therapeutic promise of clinical islet cell transplantation [4,13,20,25,26,72–74,81,98], exploring the utility of pancreas PSF is one of many efforts that are worthwhile.

To expand the acceptance and utilization of PSF in organ preservation, the technique must be developed further. Future work in PSF will involve: (1) optimization of technique and/or operational parameters so they are tailored to the tissue/organ-of-interest; (2) exploration of its use in conjunction with other preservation techniques (such as with HMP); or (3) as a method to condition organs prior to reperfusion; (4) direct comparison with other well-accepted preservation techniques; (5) development of portable PSF systems (like the electrochemical oxygen concentrator); (6) the identification of single or multiple pharmacologic agents used to prevent or reduce oxygenation and/or reperfusion injury; and (7) persuasion of the clinical community that *ex vivo* PSF is not the same as *in vivo* gas embolization – the two are fundamentally different from each other and (if performed properly) PSF should not cause embolization. Table 7 summarizes some of the keys areas of future work that may accompany an advancement of PSF.

We have attempted to demonstrate by the work presented in this review that oxygen gas delivered by PSF was found to be useable by a number of different types of tissues during hypothermic preservation. Hypothermic PSF has been shown to be capable of extending the allowable WIT and cold ischemia time and to be better in maintaining organ quality when compared with SCS and possibly HMP. The basis behind the intervention of PSF is to provide an adequate oxygen supply to an organ during preservation. Data collected over decades has confirmed that improved oxygenation is

**Table 7**  
Areas of future work in PSF.

Optimization of technique for specific tissue or organ, including <ul style="list-style-type: none"> <li>• Development of surgical procurement protocol(s)</li> <li>• Identification of appropriate approach (A-PSF, R-PSF or other)</li> <li>• Minimization of required pressures</li> <li>• Identification of appropriate gas or gas mixture (i.e., pO<sub>2</sub>)</li> </ul>
Direct comparisons between PSF and the state-of-the-art in preservation for a specific application
Exploration of utility in non-traditional applications, such as <ul style="list-style-type: none"> <li>• Combination of PSF with other preservation strategies</li> <li>• Use of PSF in post-ischemic conditioning</li> </ul>
Continued development of a portable oxygen generator for PSF
Identification of appropriate strategy for the prevention of injury due to enhanced oxygenation during preservation or ischemia–reperfusion injury, including <ul style="list-style-type: none"> <li>• Type of anti-oxidant(s), anti-apoptotic agent(s), or other drug(s)</li> <li>• Dose of treatment(s)</li> <li>• Schedule of treatment(s)</li> </ul>
Elucidation of differences between PSF and gas embolization, which will include efforts to <ul style="list-style-type: none"> <li>• Demonstrate negligible presence of gas following reperfusion of persufflated organ</li> <li>• Maximize benefit to transplant community (e.g., extended allowable WIT and CIT)</li> <li>• Maximize benefit to recipient of persufflated organ (e.g., lowered risk of delayed graft function)</li> <li>• Establish that transplantation of persufflated organ carries limited risk of adverse clinical sequelae</li> </ul>

better for maintaining the quality of an organ and, in some cases, enables the recovery and resuscitation of reversibly-damaged tissue. Most of the studies presented in this review have demonstrated that PSF exhibits the capacity to improve the metabolic quality of tissue, as measured using a number of methods and in a variety of organs, and is poised for more research and clinical application.

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