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Review

Persufflation—Current State of Play

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Abstract: With the ever-increasing disparity between the number of patients waiting for organ transplants and the number organs available, some patients are unable to receive life-saving transplantation in time. The present, widely-used form of preservation is proving to be incapable of maintaining organ quality during long periods of preservation and meeting the needs of an ever-changing legislative and transplantation landscape. This has led to the need for improved preservation techniques. One such technique that has been extensively researched is gaseous oxygen perfusion or Persufflation (PSF). This method discovered in the early 20th century has shown promise in providing both longer term preservation and organ reconditioning capabilities for multiple organs including the liver, kidneys, and pancreas. PSF utilises the organs own vascular network to provide oxygen to the organ tissue and maintain metabolism during preservation to avoid hypoxic damage. This review delves into the history of this technique, its multiple different approaches and uses, as well as in-depth discussion of work published in the past 15 years. Finally, we discuss exciting commercial developments which may help unlock the potential for this technique to be applied at scale.

Keywords: gaseous oxygen perfusion; hypothermic reconditioning; organ preservation; organ perfusion; persufflation; VSOP

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1. Introduction

In the cases of acute or chronic end-stage organ failure, organ transplantation is often the only effective treatment. This is problematic as the number of patients waiting for donation far exceeds the number of available donor organs of suitable quality. The result is that patients languish on ever expanding waiting lists and many will unfortunately run out of time before receiving life-saving treatment.

The number of transplants has been increasing year upon year; however, this is still not enough to match the number of donations required to meet demand [1,2]. This upwards trend in organ donations can be attributed to the increased use of organs donated after cardiac death (DCD) and other expanded criteria donors. In the UK alone, the number of DCD organs used has been steadily increasing, and now accounts for over 40% of donated organs [1]. Historically, DCD organs were excluded from transplantation, as studies have shown that they have a higher chance of primary graft dysfunction and are therefore subject to stricter selection criteria.

In an effort to improve organ availability, legislators from several countries have implemented laws that aim to increase the amount of organ donations and improve the selection criteria for transplant allocation. This includes the introduction of opt-out laws, such as the Max and Keira's law [3] in the UK, where, adults are considered to have given consent for their organs to be donated unless documented otherwise [4]. Another such law introduced in the US aims to revise how organ allocation works, moving away from a region-based approach to a priority-system based on donor-organ range to transplant centres dictated by the organs' preservation time. This aims to address regional inequalities

and provide improved donor organ allocation by facilitating an increase in organ donations and transplantation rates [5]. In conjunction with this, surgeons are constantly pushing the boundaries of what constitutes a suitable donor in an effort to meet the ever-present demand and save patients' lives.

These measures and laws complicate the existing logistics of transportation and preservation. During procurement and preservation, organs experience stresses that could cause life-threatening issues if transplanted [6,7]. Researchers are investigating a variety of methods for enhanced preservation that aim to improve organ health during preservation and transportation, whilst also catering for and supporting these emerging legal requirements.

Currently, the most commonly used method of preservation, static cold storage (SCS), provides a simple and cost-effective method of preserving organs. This technique works by slowing the metabolism of the organ using specialised preservation solutions. However, lack of oxygen for extended periods leads to deleterious effects which may impact on transplantation outcomes. Whilst this technique is acceptable for standard criteria donors over shorter preservation times, it has been shown to be incapable of preserving them for longer durations. This technique's shortcomings become more prevalent when using extended-criteria donor organs. Due to the lack of active oxygen supply, there is an increase in risk with extended preservation times that can lead to hypoxia and the damage associated with it, especially with large solid organs [8,9]. This has driven the need for improved techniques that can cater for these requirements. So called 'active' preservation techniques, which typically perfuse fluids through the existing vasculature, have shown to be able to do just that. Various active preservation techniques have been investigated that have been shown to be better at providing oxygen directly to the organ and thus providing extended preservation times without a degradation in organ viability. Such techniques include hypothermic machine perfusion (HMP) and normothermic machine perfusion (NMP) which perfuse the organ using specialised preservation solution, or persufflation (PSF) which uses humidified oxygen gas directly as the perfusate.

PSF was first described in the early 20th century when it was discovered by R Magus, which went unused for several decades due to lack of possible applications [10]. This technique would see a resurgence in the late 1950's after the first successful transplantation in humans was performed; however, its usage as a preservation technique was overshadowed by the discovery and adoption of SCS in the 1960's [11]. Interest in PSF remained prevalent in the following decades with two different approaches, retrograde and anterograde, being discovered in the 1970's [12]. In recent years, PSF has regained the interest of the scientific community for its ability to aptly provide oxygenation to larger organs, its relative ease of use, and simplicity when compared to other advanced preservation techniques [13]. PSF has shown promise in improving the durations an organ can be safely preserved for, whilst also maintaining organ quality to help improve the outcome of the transplantation.

The aim of this review is to give an overarching view of PSF, its history and the recent developments across multiple different organs. A high-level overview of and comparisons with, alternate techniques is also discussed, as well as concluding by exploring commercial technologies that make use of PSF.

2. Material and Methods: Literature Research

2.1. Search Strategy

Using online search tools, such as those offered by PubMed, literature related to PSF was assessed. Primarily for this review, studies published from 2005 till present were the main focus. The abstracts of the published papers were analysed to determine the nature of the study and its relevance to this work.

The main keyword used to find the papers was "persufflation". However, other keywords such as "oxygen perfusion" and "venous systemic organ oxygenation", were used to find other related studies. Furthermore, for particular organs, search terms used included the specific organ as a term as well to help refine the search criteria. Apart from

this, relevant references in these papers and relevant theses were analysed to see whether they satisfy the search criteria.

2.2. Inclusion and Exclusion Criteria

Original studies or commentaries that fit within the search criteria which were published by a peer-reviewed journals were considered for inclusion in this review. Apart from this, completed dissertations relevant to the subject matter were also considered for inclusion. Any other reviews that were published within this period were excluded as they did not provide new insight into PSF or related techniques.

Only work that was published in English was included in the recent works section of this review with no limitation on the number of overall papers to provide a comprehensive look at the last 15 years of developments in PSF.

3. Persufflation

3.1. What Is Persufflation?

Organ preservation and transplantation presents various logistical and technical challenges. Organs are often required to be transported great distances and may face local competition from operating theatres necessitating extended preservation. This frequently leads to operations being performed out-of-hours by tired transplant teams, which has been shown to lead to higher rate of surgical complications [14].

The most common form of organ preservation, SCS, relies solely on reducing metabolism whilst osmotically balancing the extracellular space to match what cells experience intracellularly during hypothermic storage. While this can provide adequate preservation from traditional donor organs exposed to brief periods of cold ischaemia, it can manifest several changes over time, which may damage the organ either during preservation itself or following reperfusion leading to what is known as Ischaemia Reperfusion Injury (IRI) [15,16]. These changes damage organs and may lead to dangerous scenarios if transplanted; resulting in an often overly cautious approach and unfortunately resulting in wastage of transplantable organs. To address this, a variety of preservation technologies have been investigated in recent years.

PSF is one such technology, which aims to restore tissue oxygenation by providing maintenance of bioenergetic status; ameliorating tissue degradation due to hypoxic exposure during ischaemia. This enables a longer period of possible preservation facilitating more successful transplants with better posttransplant function. For PSF, the organ or tissue that is being preserved, is cannulated and connected to a device that perfuses oxygen gas directly into the vasculature of the graft. Multiple studies have demonstrated that, for larger organs, this method provides superior oxygenation to the organs when compared to alternatives such as SCS [17,18]. This has been shown to extend the preservation time as well as providing some restorative benefits to marginal organ grafts.

Hypothermic reconditioning (HR) is also a key interest of PSF studies. This being the process of applying PSF after a period of SCS to reverse ischaemia associated alterations, reduce the risk of IRI, and permanent tissue damage [19].

PSF is typically performed in either of two ways as can be seen in Figure 1. Retrograde Persufflation (R-PSF), is the process of cannulating the vein of the organ to pass the gaseous oxygen through. This method requires that the organ is punctured using multiple pinpricks to gradually let the oxygen escape from the organ. Using a high concentration of oxygen (>95%), gas is perfused through the veinous system in a reversed manner to normal physiological flow. This version requires lower pressures to perfuse oxygen through the organ leading research to suggest that it has no adverse effect on venous endothelium [20]. On the other hand, Anterograde Persufflation (A-PSF), works like R-PSF with the distinction that the flow is reversed to mimic the natural flow of blood in the body, typically entering via an artery before traversing the capillary bed and draining via the vein. Studies have shown that A-PSF has demonstrated the ability to provide more efficient gas exchange when compared to R-PSF due to the higher surface area provided

by the capillary bed and therefore lower concentrations of oxygen (40%) are used to avoid damaging the graft tissue.

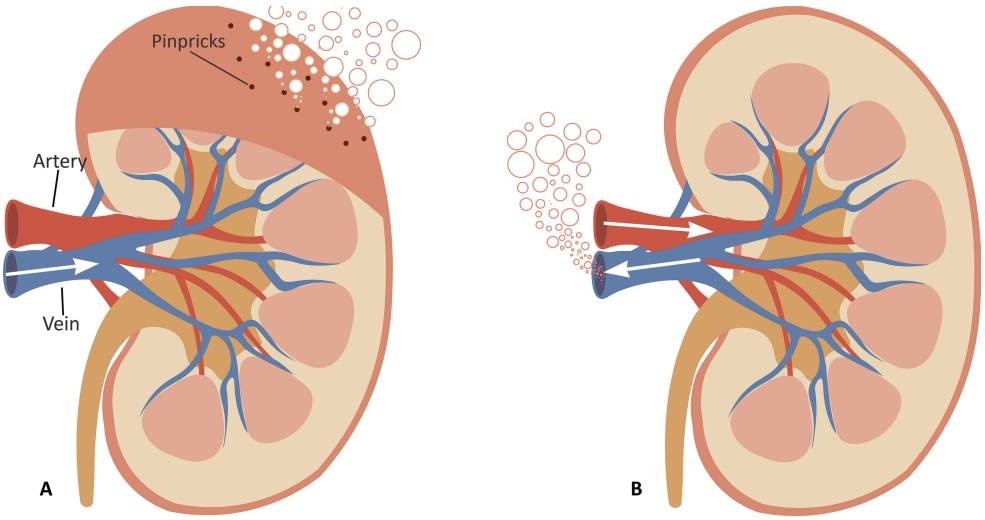

Figure 1. Cross-sectional diagram of the kidney being persufflated using two different approaches: (**A**) Retrograde Persufflation (R-PSF); with the gas being perfused through the vein and bubbling out through the pinpricks. (**B**) Anterograde Persufflation (A-PSF); with the gas being perfused through the artery and exiting through the vein.

3.2. History (1902–2005)

PSF was discovered in 1902, when Rudolf Magnus unexpectedly observed a feline heart rhythmically contract, while it was perfused by oxygen gas at sub-normothermic temperatures. Whilst originally perfusing the heart with defibrinated blood, which was being oxygenated by compressed oxygen gas, an over-pressurisation of the blood reservoir caused the oxygen gas to be accidentality perfused directly into the heart. Encouraged by these observations, Magnus continued studying this phenomenon and discovered that it was possible to keep the heart beating for over 1 h using this method [10]. PSF would go largely unstudied for several decades until human organ transplantation was shown to be possible [21].

New techniques for organ preservation were being investigated as means to better preserve organs for a longer time. Between the late 1950's and the late 1960's, most studies centred around the heart and investigating its cardiac activity during PSF and post-reperfusion [22–25]. In the early 1970's focus shifted onto kidney preservation [26]. Isselhard conducted various studies on the effects of PSF on canine kidneys [12,26,27]. Amongst these studies, several compared the usage of R-PSF against A-PSF and analysed the results to determine the efficacy of each method and which yielded the best the results, whilst using pure oxygen as the perfusate. At the time it was determined that R-PSF produced the better result [28,29].

A key pilot study at Cambridge University was performed in 1989 by Rolles et al. who investigated the use of R-PSF on human kidneys. They surmised that even given the slight complexity added due to the use of PSF, it was acceptable to use as it improved both initial function and graft quality compared to SCS [30]. Studies investigating kidneys mainly using R-PSF remained prevalent throughout the following years.

In the 1990's, focus shifted to the liver. Initially, the first liver PSF study was conducted in 1980 by Fischer, however it was not until the mid to late 90's when Minor and colleagues published a series of papers studying the effect of PSF primarily on rat liver grafts [20,31–34]. These studies detailed the efficacy of the method as a means of preserving liver grafts and understanding its metabolic profile during reperfusion. Due to the size of livers, one key aim of these was to investigate the level of oxygenation achieved with the organ both during preservation and afterwards.

Around the turn of the century, PSF studies continued primarily using porcine grafts. Studies on the heart by Kuhn-Regnier and Fischer investigated the effect of A-PSF and its impact on cardiac function and endothelial status [35,36]. Similar studies were conducted by Treckmann using kidneys and Saad and Lauschke for livers [37–39]. Several of these studies used DCD organs; which has since become a growing trend in the field of PSF. These organs require greater care during preservation due to their poorer quality and PSF has been investigated as means to improve their efficacy for transplantation.

4. Recent Works (Post–2005)

This section covers recent PSF developments across three different organs, the liver, the kidney, and the pancreas. It is divided into different subsection for each organ covering research from 2005 onwards, starting with studies for rat models moving to large animal models and finally human investigations and clinical trials.

4.1. Liver

Since 2005, a significant effort has gone into investigating the preservation of livers using PSF. Livers present a unique challenge, as their large size exacerbates the logistics of long-term preservation. Recent PSF studies (as detailed in Table 1) into using the method to achieve longer preservation times and avoid post-transplant complications that may require re-transplantation as well as preservation of DCD livers have been undertaken by various groups.

Table 1. Recent Persufflation work on the liver.

Aim	Year	Author [Ref]	Technique	WIT [a] (min)	PSF Time (h)	TPT [b] (h)	Model
HR of DCD Livers	2006	Tolba [40]	R-PSF	30	24	24	Rat
HR of DCD Livers	2008	Treckmann [41]	R-PSF	20–60	1.5–3.3	7–13	Human
HR after long-term preservation	2009	Stegemann [13]	R-PSF	-	1.5	23.5	Rat
Protection via CO Gas	2010	Koetting [42]	R-PSF	30	18	18	Rat
HR of steatotic livers	2010	Ye [43]	R-PSF	-	6	6	Rat
Protection via CO Gas	2010	Koetting [44]	R-PSF	30	18	18	Rat
Optimal timing for HR	2011	Koetting [45]	R-PSF	-	1, 2, 3	19–21	Pig
HR and Survival rates	2011	Minor [46]	R-PSF	-	2	12	Pig
Oxygen PSF as Adjunct in Liver preservation trial	2011	Minor [47]	R-PSF	-	2	-	Human [c]
HR: Before or after CS for DCD livers	2011	Koetting [48]	R-PSF	30	2–20	20	Rat
Protection via NO Gas	2012	Srinivasan [49]	R-PSF	30	24	24	Rat
HR with A-PSF	2012	Minor [50]	A-PSF	-	2	20	Pig
Protection via NO Gas	2013	Nagai [51]	R-PSF	-	2	3	Rat
Protection via NO Gas	2013	Yagi [52]	R-PSF	-	3	3	Rat
Pulsatile Pressure	2014	Lüer [53]	R-PSF	30	18	18	Rat
Protection via NO Gas	2014	Kageyama [54]	R-PSF	30–60	3	3	Rat
HR of DCD Livers	2014	Khorsandi [55]	A-PSF	15–25	2	15	Human
Protection via NO Gas	2016	Porschen [56]	R-PSF	30	1	24	Pig
HR of hypovolemic shocked livers	2018	Jafari [57]	R-PSF	-	1	18	Rat
Oxygen PSF as Adjunct in Liver preservation trial	2019	Gallinat [58]	R-PSF	-	2	11–20	Human
A-PSF O_2 concentration	2020	Minor [59]	A-PSF	-	2	18	Pig

[a] Warm Ischaemia Time; [b] Total Preservation Time; [c] This is a design for a randomised controlled trial using a human model rather than a study.

4.1.1. Liver Persufflation in a Rat Model

One of the main objectives of using PSF being investigated is its use to 'resuscitate' the organ during the preservation period; improving its viability. Long waiting lists of patients who urgently require a liver transplant has necessitated the use of extended criteria donors including the use of DCD organs. However, many of these organs still go unused due to concerns over post-transplant complications. Application of PSF can re-oxygenate these organs under hypothermic conditions reversing some of the changes, which otherwise may lead to severe IRI post-transplantation.

DCD organs are the subject of many studies involving various methodologies including PSF. Studies by Tolba et al. in rat models have produced favourable results when a DCD organ was persufflated for the full preservation period of 24 h [40]. It was demonstrated that the livers preserved with PSF achieved similar function upon reperfusion as organs donated after brain death (DBD). This was an encouraging result especially when compared to the clinical maximum for liver of 12 h when using SCS [60].

In a later study by Stegemann et al., male Wister-rat DCD livers were persufflated for 90 min after 22 h of SCS. These were then compared to other livers who either received no treatment at all or 90 min of HMP. DCD livers with 6 h of SCS were used as a control. The HR organs demonstrated significantly improved functional markers upon reperfusion when compared to the 22 h SCS cohort, displaying similar values to those recorded by the short-term SCS preserved control livers [13].

Given the two varying approaches in the work performed by Tolba and Stegemann above, a study by Koetting et al. using a rat liver model, was conducted comparing the merits of continuous PSF versus brief use of PSF prior to reperfusion as a HR protocol. It showed that there were no disadvantages to using short-term PSF for 2 h following long-term SCS when compared to continuous active preservation using the same method in the liver [48].

Similarly, Jafari et al. investigated the potential application of PSF to recondition rat livers that have experienced a 30 min period of hypovolemic shock [57]. As a comparison for the results, DCD and heart-beating donor grafts were preserved using SCS alone or preserved and reconditioned using R-PSF for one hour. Reconditioning hypovolemic shocked livers proved futile to improving their condition, with the persufflated cohort showing increased signs of deterioration; an effect experienced by none of the other reconditioned groups [57]. The authors note however, that they cannot conclude whether this was but a metabolic snapshot of a prolonged recovery process for 'shocked' livers and would require further research with a longer interval.

4.1.2. Adjuvant Approaches during Liver Persufflation

Certain processes that occur as a result of warm ischaemia can cause cell damage which exacerbates the risk of IRI and may lead to primary graft dysfunction and primary non-function. These events increase the production of oxygen-free radicals, which cause cell injury or death. This had led to researchers experimenting with the use of antioxidant agents to ameliorate free radicals during PSF and exert their protective effects on the organ. The usage of mechanical stimulation was also explored as a method to provide further protection to the organ's cells.

In a 2010 study, Ye et al. [43], investigated using R-PSF with high-dose reduced-glutathione to recondition steatotic male Wister rat livers. This short-term treatment provided a remarkable improvement in viability by restoring hepatic glutathione and adenosine triphosphate (ATP) levels as well as reducing hepatocyte necrosis [43].

In a pair of studies by Koetting et al., DCD rat livers were persufflated with carbon monoxide (CO) gas; evaluating the potential for CO to suppress the early inflammatory reaction and serve as a cryoprotective mediator in the case of IRI [42,44]. In [44], it was noted that using low dose CO (50 ppm) dissolved in nitrogen provided additional protection to the graft tissue and increased functional integrity while reducing cellular enzyme loss when compared to SCS. In a second study by the same group, the hypothesis of using CO dissolved in oxygen (at a concentration of 250 ppm) for PSF was tested against standard PSF using pure oxygen and SCS for an 18 h preservation period. By evaluating liver function and tissue integrity it was observed that whilst the $CO+O_2$ provided significantly improved results when compared to SCS, they were similar to those exhibited by PSF using only oxygen [42]. Thus, no improvement in protection was offered by adding CO.

More recently, a set of studies on PSF using 40 ppm Nitric Oxide-doped (NO) oxygen on rat livers have been published [49,51,52,54,56]. The antioxidant or vasodilative properties of NO were hypothesised to have beneficial effects on the graft. When PSF was supplemented with NO, livers showed significantly improved viability when compared to SCS. When compared to oxygen-only PSF, the NO-supplemented group exhibited a statistically significant improvement in graft quality and structural integrity [49]. When used to preserve standard or mildly-steatotic partial livers, a positive impact was observed as the grafts exhibited reduced hepatocellular damage and improved circulation [51,52]. For whole DCD graft HR, similar results were observed [54]. These beneficial effects of using NO-enhanced PSF were demonstrated to be more pronounced when the HR was performed within the first hour of perseveration rather than the last, which contrast from standard oxygen only PSF and Koetting's findings above [48,56].

As a means of improving PSF, a study on the possibility of using pulsatile pressure to provide mechanical simulation to the vein structure of the graft was performed. Following 18 h of preservation the rat livers exhibited improved portal vascular resistance and organ integrity similar to that exhibited by standard PSF. Ultimately no significant improvement was found by using pulsatile over constant pressure during PSF [53].

4.1.3. Liver Persufflation in a Large Animal and Human Preclinical Models

These works explore the usage of varying approaches and parameters of PSF for the preservation of DCD porcine livers.

Further research was performed into the parameters used during organ HR or preservation. Using porcine livers subjected to 18 h of SCS and no warm ischaemia time (WIT), it was shown that whilst a significant improvement in function markers (including ATP, bile production, and hepatic arterial flow) were observed after 1 h of PSF, 2 h provided the most consistent and optimal results, with no notable improvements observed for longer periods of HR [45].

A study by Minor et al. into analysing the survival rates of persufflated DCD livers demonstrated that this method provided an 83% 7-day survival rate for a porcine liver model, in stark contrast to the 0% produced using organs preserved solely by SCS [46]. The high survival rate obtained for the experimental group was supported by the significantly improved liver pre-transplant energy charge and initial graft function [46].

R-PSF presents the issue of introducing pinpricks into the liver in order to dispel the oxygen from the vasculature. To prevent introducing further trauma to the organ, save time and effort, Minor et al. used a minimally invasive variant of R-PSF on porcine livers in an attempt recondition them [50]. This method involves perfusing the oxygen using suprahepatic caval vein instead of the portal vein and which allows the usage of R-PSF without pinpricks. The livers showed no signs of damage to their vascular endothelium and experienced significant improvement in hepatic function when compared to their SCS counterparts [50].

A-PSF has gained more popularity in recent years as it facilitates the use of PSF without the need to introduce pinpricks. In order to find the optimal parameters when using A-PSF, Minor et al. attempted HR using pure and 40% humidified oxygen [59]. The porcine livers improved more consistently when the pure oxygen was used showing increased ATP levels and bile flow leading the study to conclude that for HR purposes, A-PSF using pure oxygen promotes better early graft function [59].

Steatotic livers are often rejected by transplantation centres as they may increase the risk of IRI [61]. A study by Khorsandi et al. using A-PSF to persufflate rejected steatotic human livers was published [55]. These grafts suffered from varying degrees of steatosis that made them unsuitable for transplantation. Using A-PSF for HR, mildly steatotic (<30%) livers demonstrated a significant increase in cellular energy charge and improved hepatocyte function when compared to the those preserved using SCS. For livers suffering from moderate to severe steatosis (>30%), no beneficial effects were exhibited [55].

4.1.4. Clinical Liver Persufflation

Clinical translation of this technique has also been evaluated. The first human trial of using PSF for the purposes of HR livers was performed by Treckmann et al. in 2008 [41]. Using marginal organs which had suffered from ischaemic damage (with WIT varying from 20 to 60 min) and had been rejected by at least 3 transplantation centres, the non-steatotic livers were transplanted into patients who were unlikely to get a graft due to waiting time or poor MELD score ([62]). Prior to implantation, livers were treated with R-PSF using a constant pressure of 18 mmHg for between 60 to 200 min. Significant improvements were observed for the ATP level post-PSF compared to pre-PSF; with all patients experiencing good primary function for the first week postoperatively [41].

Owing to the successful results published in [41], Minor et al. set up a procedure for a randomised controlled PSF trial for livers [47]. The Oxygen PSF as Adjunct in Liver preservation trial (OPAL) detailed how their planned clinical trial would be carried out as well as the intended parameters to be used, participant criteria and data to be gathered for primary and secondary endpoints. In 2019, OPAL was used for a study involving 116 patients. Using participants who are unlikely to receive a liver transplant based on their poor MELD score (same as in [41]); marginal DCD organs are split into 2 groups, with 57 reconditioned for 2 h using R-PSF and the other left untreated. The post-operative readings demonstrated that the treated group showed a small non-significant improvement in peak-aspartate aminotransferase levels and a positive effect on early allograft dysfunction. Both groups displayed similar rates for 30-day mortality and post-operative complications. Upon subgroup analysis of the 5-year survival rates of the patients, it was demonstrated that a significant difference existed between the two groups for recipient patients over the age of 70. For patients aged less than 70, the 1 to 5 year survival rate was a constant 80% for the treatment group with 85% and 75% for the control. For those aged over 70, the treatment group saw the rates of 75% to 65% for 1 to 5 year rates respectively and the control showing a significant decrease with 70% to 48% [58].

This trial exhibits that PSF offers a clear clinical benefit when used to recondition organs in specific subcategories as well as demonstrating its safety in a clinical setting. A second observation of note was that for younger subgroup, even without treatment, the recorded survival rates were high (85% to 75%) and the rates of complication were low which suggests that the organs used were viable for transplantation even though they were rejected for usage [58]. This serves as a cause of concern as equally viable organs are being discarded as they do not meet the current criteria for organ donation which can lead to patients dying rather than taking advantage of them.

Further investigation with a larger cohort of patients, especially in the younger subgroup, could be beneficial as they may lead to better observations and more significant differences might become apparent. We look forward to more studies similar to this, as they explore the usage of marginal organs which might ultimately help increase the donor pool and the usage of such organs in transplantation.

4.2. Kidney

Most works during this period investigated (Table 2) the performance and efficacy of PSF to preserve and recondition against other established types of preservation such as machine perfusion and SCS. During the period of interest for this review there was no reported data for small animal or human models.

Kidney Persufflation in a Large Animal Model

Given the encouraging results observed when PSF was used to preserve porcine livers after warm ischaemia, Treckmann et al. investigated a similar setup using pig kidneys instead. Having multiple cohorts exposed to varying lengths of WIT, the grafts were preserved using R-PSF and SCS. For limited WIT of up to 1 h, the kidneys showed significant improvement when preserved with PSF. For longer WIT, no benefits were observed when compared to SCS [39].

Several studies have attempted to compare various kidney preservation techniques to elucidate the most effective one [18,63]. A 2009 study by Treckmann et al. explored the effects of different preservation techniques on porcine kidneys exposed to WIT of 1 h [18]. Using SCS, R-PSF or HMP to preserve the organs for a 4 h period resulted in the PSF group yielding the highest survival rate (100%) with HMP and SCS survival rates being much lower (60% and 57% respectively). Recorded laboratory parameters including urine production and creatine in plasma were also improved by R-PSF compared to SCS and HMP [18]. Similar results were echoed by a more recent study that attempted to preserve the pig kidneys for a longer period of time [63]. In this study, the WIT of the kidneys was 45 min, whilst the attempted duration of preservation was 24 h. R-PSF provided the best protection for the ischaemically damaged kidneys and superior oxygenation whilst having all parameters recorded not significantly differ from the control, non-damaged kidneys [63].

Table 2. Recent Persufflation work on the kidney.

Aim	Year	Author [Ref]	Technique	WIT [a] (min)	PSF Time (h)	TPT [b] (h)	Model
HR of DCD kidneys	2006	Treckmann [39]	R-PSF	60,90,120	4	4	Pig
HR of DCD kidneys	2009	Treckmann [18]	R-PSF	60	4	4	Pig
HR after long-term preservation	2012	Minor [64]	R-PSF	-	2	20	Pig
HR of DCD kidneys	2016	Moláček [65]	R-PSF	20	2	2	Pig
HR of DCD kidneys and comparison of methods	2016	Kalenski [63]	R-PSF	45	24	24	Pig
HR of DCD kidneys and comparison of methods	2018	Moláček [66]	R-PSF	20	1	1	Pig
HR of DCD kidneys and comparison of methods	2018	Min [67]	A-PSF	30	24	24	Pig

[a] Warm Ischaemia Time; [b] Total Preservation Time.

In a similar fashion to the liver studies, PSF has been attempted on kidneys to recondition damaged or marginal organs to increase the donor pool. Minor et al. in 2012 investigated this technique on porcine kidneys and compared the resultant parameters against SCS counterparts. Following 18 h of SCS, a group of kidneys were persufflated using R-PSF for 2 h. These grafts displayed significantly improved urine flow, creatinine clearance, and better renal morphology [64].

Machine perfusion has also been demonstrated to have the potential to recondition organs a similar manner. In [65,66], porcine kidneys are used to compare and contrast both methods as well as evaluate resultant kidney quality against a SCS control. In these studies, the kidneys were perfused for 120 and 60 min respectively and resulted that under the test conditions, no significant difference was seen between grafts preserved using HMP and R-PSF. However it was noted that PSF was able to protect and restore the parenchyma relative to SCS [65].

In Arizona, Min explored a different approach for assessing domestic female juvenile pig's kidney quality following preservation using SCS, HMP, and A-PSF (with 40% oxygen concentration) [67]. The organ quality was evaluated by measuring whole organ oxygen consumption rate (WOOCR) and glomerular filtration rate (GFR) following preservation. The A-PSF treated kidneys yielded significantly higher values for GFR and WOOCR compared to those exhibited by SCS alone. These functional markers were then tested again after the temperature of the organ was brought up to a sub-normothormic level (25 °C). The WOOCR had doubled from the hypothermic readings for both the SCS and the A-PSF groups whilst the GFR fell significantly [67]. During this project, the researchers further examined the effects of A-PSF on porcine kidneys as opposed to HMP using the same functional markers of WOOCR and GFR. No significant difference was noted between the two groups of preserved livers [67].

Through personal communications, the authors are aware of ongoing work in this field of PSF and look forward to more peer-reviewed work being published in the near future.

4.3. Pancreas

Most of the works published focuses on the potential for PSF to improve islet isolation and transplantation outcomes. A table summarising the works during the relevant period for the pancreas can be found in Table 3.

4.3.1. Pancreas Persufflation in a Rat Model

The subject of 2014 study by Reddy et al. was the viability of using PSF on DCD rat pancreas and how effective it is compared to other methods such as HMP and SCS. After a 6 h preservation period, the pancreas underwent islet isolation and the islet isolation yield and viability assessed. Whilst still poorer quality then those extracted from the control non-DCD pancreas, the PSF group exhibited the most improved quality and yield when compared the other preservation methods. HMP however, did not show significant improvement when compared to SCS [68].

4.3.2. Pancreas Persufflation in a Large Animal and Human Model

In 2010, Scott et al., reported on the effects of PSF against the two-layer method (TLM) (a version of advanced SCS described in [69]) in porcine and human DBD pancreata [17]. Following preservation, the organs were examined by a variety of techniques as detailed below. The efficacy of PSF was first investigated using MRI to examine the gas distribution in the vascular system. Here, they observed the presence of gas (negative contrast) in 90% of the vasculature. ^{31}P-NMR spectroscopy was then used to non-invasively asses the techniques' ability to maintain ATP levels. The ATP levels as exhibited by the PSF organs were maintained in excess of 24 h whilst those resulting from the usage of a static method such as the TLM (as mentioned above) were consistently low [17]. The pancreatic ATP levels were assessed following cessation of PSF after 8 h. In this single case, a rapid drop in ATP was observed to an undetectable level. Upon resumption of PSF 3 h later, a partial restoration of ATP was observed [70]. This clearly demonstrated that PSF was driving the ATP maintenance.

When biopsies from porcine pancreata were interrogated for histological assessment, significantly improved organ health was reported following 24 h of PSF when compared to TLM [71].

Table 3. Recent Persufflation work on the pancreas.

Aim	Year	Author [Ref]	Technique	WIT [a] (min)	PSF Time (h)	TPT [b] (h)	Model
Comparison to TLM	2010	Scott [71]	A-PSF	-	24	24	Pig
Oxygen Delivery to DCD pancreas	2010	Scott [17]	A-PSF	-	-	-	Pig/Human
Comparison to TLM	2012	Scott [70]	A-PSF	-	-	-	Pig/Human
HR of DCD pancreas and comparison of methods	2014	Reddy [68]	R-PSF	30	6	6	Rat
HR of DCD Human pancreas	2019	Kelly [72]	A-PSF	-	5	15	Human
Commentary on previous works	2019	Hosgood [73]	A-PSF	-	-	-	Human

[a] Warm Ischaemia Time; [b] Total Preservation Time.

Building upon their findings, when islet isolation was performed on porcine pancreata, PSF pancreas exhibited significantly better outcomes relative to paired TLM preserved tissue in terms of islet isolation yield, quality, oxygen consumption rate, and post culture recovery [70]. In 2012, Scott et al. reported improved islet isolation quality upon preservation using A-PSF compared to TLM [70]. Initial islet isolation yield was also improved demonstrating a significant increase compared to TLM, however, the impact on islet yield only trended towards significance following 2 days of culture with a high variability observed between islet isolations. The same could not be said about the improvement in islet quality, as the oxygen consumption rate and the DNA islet equivalents per gram digested tissue remained significant following two days post-preservation for the PSF group [70].

Similar to the pigs, a 24 h A-PSF preservation period of human pancreata resulted in significantly higher islet yield and comparable quality (as measured in oxygen consumption rate) compared to 10 h of TLM preservation [70]. In a more recent study by Kelly et al., islet

viability and purity were assessed after extraction from human pancreata preserved using SCS and A-PSF. These results were compared to islets extracted from grafts preserved solely by SCS. Whilst the preservation time for the PSF group was longer overall, less time was spent using SCS. The study concludes that the PSF group recorded no adverse effects on the islets and that beginning PSF earlier and limiting SCS time prevents harmful pro-inflammatory signalling and improves insulin secretion [72]. It was also noted that prolonged SCS of pancreas of greater than 12 h adversely effects islets yield and insulin secretion [73].

Through personal communication, the authors are aware of additional research into PSF of the pancreas and look forward to more information becoming publicly available in the years to come.

5. Other Applications

Historically, the heart was a mainstay of PSF studies, however, in recent times interest seems to have subsided. Whilst we are aware of several abstracts being presented during this period, we were unable to find any publications to report in this review.

We are also aware of several abstracts investigating the use of PSF for composite tissue preservation such as for limbs. However, we were similarly unable to find publication to report in this review. These abstracts demonstrate that there is some interests in other applications and we look forward to seeing more publications emerging moving forward .

We are presently unaware of any other applications of PSF beyond those mentioned above.

6. Alternate Techniques

Several other techniques for 'active' organ preservation exist. They aim to address the same challenges faced by PSF using different mechanics with the aim of improving organ preservation and increasing donor organ supply. To date, some of these techniques have seen more commercial exposure than PSF.

One such technique that has shown promise both clinically and commercially is hypothermic machine perfusion (HMP). This technique, originally pioneered in the late 60's, has shown promise is preserving DCD organs and limiting IRI. This technology perfuses the organ with a variety of perfusates and keeping the organ at a low temperature (4 °C to 10 °C) to reduce metabolism and oxygen consumption [74,75]. The preservation fluid being perfused through the organ varies on application, with some of them containing a provision of oxygen. Whilst HMP has exhibited improved preservation times and organ maintenance capabilities compared to SCS, PSF has been shown to be superior at properly oxygenating the organ and resuscitating marginal organs [18,63,66]. Commercially, this technique has seen some usage with devices such as Organ Recovery Systems LifePort suite of devices which offer a portable preservation for the kidney and liver [76,77].

Another technique that has been explored as a means of providing active organ preservation is normothermic machine perfusion (NMP). This technique shares some similarity to HMP, with the distinction that this technique keeps the organ operating at close to normal (35 °C to 37 °C) or subnormal (20 °C to 34 °C) temperatures [78]. This allows for the perfusion of packed red cells related to Gelofusine or blood plasma replacements to allow for oxygen and nutrients to be provided to the organ in a 'safer', immune-depleted environment [79]. This technique attempts to avoid a common issue with hypothermic techniques called rewarming injury that is caused by large changes in temperature experienced during reperfusion [15]. An additional benefit of NMP is that it enables the organs to be functionally measured prior to transplantation which none of the hypothermic techniques allow due to their usage of low temperatures. NMP has seen some clinical usage and trials for organs such as the liver or kidney demonstrating improved outcomes especially when using DCD organs compared to SCS [80]. Furthermore, investment in this area has led to several relatively new commercial products including the OrganOX *metra* that offers a portable device providing 24 h liver preservation [81]. Another

notable company operating in this space is TransMedics® with their portable Organ Care System (OCS™) designed to use NMP to preserve the lungs, heart, and liver [82].

Some studies have delved into combining these two methods together, where sequential use of oxygenated HMP and NMP has exhibited favourable results. Studies such as [83], demonstrate that when NMP was applied after a period of HMP using an oxygenated perfusate on DCD human livers, the functional markers (including bile production, ATP, and portal vein flow) exhibited an improvement. This presents an interesting avenue for further exploration of this technique in conjunction with PSF given that it is tailored for hypothermic oxygenated perfusion.

7. Market Potential

PSF offers several key challenges to implement widespread use including the need for bespoke rigs and trained personnel to use and accompany them, which has limited its commercial viability. Several products have existed that attempt to leverage PSF to have a commercial product which can offer active preservation whilst also catering for the aforementioned limitation. The introduction and availability of devices such as these will potentially allow for PSF as a technique to be realised in the clinic.

In [59], a device called Minox is used to persufflate a liver using A-PSF. This device uses oxygen from commercial oxygen bottles to perfuse the organ with it. It contains pressure and flow controls as well as other safeguards such as a pressure relief valve to protect the organ. The only use case observed of this device to date was in this study on a porcine liver.

Another company, Giner Life Sciences developed a portable device aiming to use PSF to preserve and transport pancreas. Their P3S™ focuses on the portability as they aim to be able to preserve the organ whilst on the move between the donor and the recipient. The system uses a temperature-controlled chamber to contain the organ and an electrochemical gas concentrator [84]. Using in-situ gas generation technology, the P3S™ system aims to use an electrochemical oxygen concentrator as a means to generate the oxygen for preservation [85]. No recent updates have been provided regarding this system or what timeline is being targeted for its release.

ScubaTx is a new commercial entity in the organ preservation space. Their device aims to utilise PSF as the basis for its preservative capabilities. The device is aimed to be small, portable, and versatile in its aim to cater for multiple organs through the means of organ-specific consumables. The PSF performed by the device is supported by an automated system aimed at monitoring parameters in real-time and constantly adjusting them to safeguard the organ. Following preservation, the device issues a simple and guided report detailing the preservation period [86].

The increase in support for widespread adoption through the introduction of commercially available PSF devices increases the viability and usage of this technique. Only by commercially viable equipment becoming available will the true potential for these various advanced preservation modalities be realised. Ultimately this may help improve donor organ supply whilst also supporting the changes in legislation.

8. Conclusions

PSF, as a method has a long history spanning back to the early 1900s, however, widespread study on the subject only started in the latter half of the 20^{th} century. More recently, many of the studies detailed the methods effectiveness at revitalising marginal organs such as those that have suffered from ischaemic damage and improving their viability for transplantation. In conjunction with these, the method has been investigated as means to diffuse other gases into the organ and attempt to benefit from their protective antioxidant effects.

Over the past decade and a half, many PSF studies have been conducted looking at different aspects of the technique and its effect on the organs. Whilst numerous studies in

this period were dedicated to the liver, other organs such as the kidneys and pancreas have been reported.

Notably, the landmark OPAL study successfully demonstrated the method clinically as a HR tool for marginal livers prior to transplantation. OPAL demonstrated the efficacy and safety of this approach in a clinical setting using a large cohort of patients who would have otherwise been unsuitable to receive treatment.

These encouraging recent developments in PSF has seen commercial entities attempt to develop devices that leverage the use of PSF and its relative simplistic and cost-effective nature to preserve organs. Whilst currently none are wildly available, growth in this regard shows the general acceptance and progress of the technique for more extensive usage. Compared to other active competing techniques, PSF is still a relatively unexploited market, however, with the recent developments in this field this is starting to change. This is a positive development as more advanced preservation techniques become available for wider use, the better it is for the patients as each device aims to achieve the same goal of increasing the donor organ supply.

PSF seems to have a bright future as there is currently a lot of ongoing work centred around multiple organs including clinical translation of the technique. Apart from this, the increasing commercial exploitation of PSF is positive sign of things to come.

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Abbreviations

The following abbreviations are used in this manuscript:

A-PSF	Anterograde Persufflation
ATP	Adenosine Triphosphate
CO	Carbon Monoxide
DBD	Donated after Brain Death
DCD	Donated after Cardiac Death
GFR	Glomerular Filtration Rate
HMP	Hypothermic Machine Perfusion
HR	Hypothermic Reconditioning
IRI	Ischaemia Reperfusion Injury
NMP	Normothermic Machine Perfusion
NO	Nitric Oxide
OPAL	Oxygen Persufflation as Adjunct in Liver preservation
PSF	Persufflation
R-PSF	Retrograde Persufflation
SCS	Static Cold Storage
TLM	Two-Layer Methods
WIT	Warm Ischaemia Time
WOOCR	Whole Organ Oxygen Consumption Rate

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