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REVIEW

Ex-vivo lung perfusion

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Conflicts of interest
DVR is a consultant for Transmedics, Andover, MA, USA; MC and SK are founding members of Perfusix Inc, a company that provides *ex vivo* organ perfusion services.

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Summary

This review outlines the new and promising technique of *ex vivo* lung perfusion and its clinical potential to increase the number of transplantable lungs and to improve the early and late outcome after transplantation. The rationale, the experimental background, the technique and protocols, and available devices for *ex vivo* lung perfusion are discussed. The current clinical experience worldwide and ongoing clinical trials are reviewed.

Introduction

Lung transplantation (LTx) is the ultimate treatment for selected patients suffering from any form of end-stage pulmonary condition to prolong survival and to improve quality of life [1]. This treatment, however, is limited by the low percentage of acceptable deceased donors and transplantable pulmonary grafts (15–30%) when compared to kidney and liver transplantation [2,3]. Various strategies to increase the standard lung donor pool were implemented in transplant centers worldwide during recent years including the use of lungs from extended-criteria donors, living lobar donors as well as donors after circulatory death (DCD) [3–6].

Alexis Carrel and Charles Lindbergh reported in 1935 the first normothermic *ex vivo* organ perfusion demonstrating already that organs could remain viable for several days [7]. Normothermic *ex vivo* lung perfusion (EVLP) was described in historical papers as a method to assess the quality of the pulmonary graft during storage [8] and as a technique to preserve heart and lungs during distant procurement [9]. The concept of EVLP was reintroduced by Steen *et al.* [10] as a technique to evaluate lungs from an uncontrolled DCD prior to transplantation. This unique case report demonstrated for the first time that lungs can be transplanted successfully after a period of warm ischemia, *ex vivo* perfusion and evaluation, and cold storage. Subsequent experimental work in Steen's laboratory in Lund, Sweden [11], stimulated many research groups worldwide including both our teams in Leuven [12–18] and in Toronto [19–23], to further investigate the potential role of EVLP as a method to

increase the number of suitable pulmonary grafts, to reduce the incidence of primary and late graft dysfunction, and to improve outcome after LTx.

The objective of this review on EVLP was to describe the rationale, different techniques and protocols, and available devices. Current and future potential clinical applications as well as the worldwide experience to date and ongoing clinical trials are discussed.

Rationale for EVLP

The rationale for EVLP is to keep the lungs in a physiologic status prior to transplantation. In contrast with cold static lung preservation whereby cell metabolism is slowed down and requirement for oxygen and essential nutrients is reduced to prevent organ deterioration, normothermic EVLP under physiologic conditions allows pulmonary cells and tissues to remain metabolically active and viable for several hours. This period provides a window for prolonged lung preservation, assessment, and reconditioning of previously less than optimal performing pulmonary grafts by several mechanisms: dehydration of lung tissue by the high oncotic pressure in the perfusate, removal of harmful and toxic waste products (blood clots, neutrophils, inflammatory cytokines) with filters and membranes in the circuit, and recruitment of atelectatic areas resulting in better ventilation/perfusion matching. If longer perfusion times (>12–24 h) would prove to be possible, repair of injured grafts by delivered therapies interacting via several mechanisms and pathways could be investigated. Finally, EVLP offers a platform to study avenues for preconditioning and protection of the pulmonary graft against subsequent inflammatory and immune insults following LTx.

EVLP technique and protocols

After cold pulmonary flush and retrieval, lungs are instrumented in the donor hospital or in the recipient hospital (after a period of cold ischemia during transport) for immediate or delayed normothermic perfusion, respectively. A perfusion cannula is inserted in the pulmonary artery and fixed. The left atrium (LA) can be left open for free drainage of the effluent, or a funnel-shaped cannula is sewn to the remnant of the muscular cuff depending on the preferred technique (see below). Finally, an endotracheal tube of an appropriate size is inserted in the trachea and fixed proximal to the bifurcation with the lungs still inflated.

The EVLP system consists of a perfusion circuit with tubing and a reservoir. The system is primed with the perfusate (±2000 ml) and additives and warmed to 32 °C. After mixing of the solutions, a sample of the perfusate can be drawn for biochemical analysis to correct pH, HCO_3^-, and glucose levels as needed. The lungs are then placed in a specially designed organ chamber depending on the preferred equipment. A pump drives the perfusate from the reservoir through a gas exchange membrane, heat exchanger, and leukocyte filter before entering the lungs via the pulmonary artery. Pulmonary effluent from LA drains back to the reservoir and is recirculated.

Upon initiation of perfusion with careful monitoring of pulmonary artery pressure (PAP) maintained below 15–20 mmHg, flow will gradually increase by increasing the pump speed over time according to the institution's protocol (30–60 min). Once the temperature of the outflowing perfusate has reached a preset temperature (32–34 °C), protective lung ventilation is started (tidal volume 5–7 ml/kg donor weight; respiratory rate 7–20 breaths per minute; positive end-expiratory pressure (PEEP) 5–7 cm H_2O; peak airway pressure <25 cm H_2O; gas mixture preset for preservation or testing). Lung temperature will further increase to 37 °C reaching targeted flow. Alveolar recruitment maneuvers with airway pressure up to 25 cm H_2O can then be performed to remove atelectatic areas if any.

The basic principle of EVLP is that lungs remain viable without additional injury reflected by edema formation. Three key elements for successful normothermic perfusion can be identified: (i) controlled gradual perfusion to avoid hemodynamic shear stress [24,25]; (ii) perfusate with an extracellular, dextran 40-based solution with optimal colloid pressure [11]; and (iii) controlled ventilation with low tidal volume and PEEP to protect against ventilator-induced lung injury [26].

Three different EVLP protocols have been reported so far. Basically, these can be summarized as the (i) Toronto protocol; (ii) Lund protocol; and (iii) Organ Care System™ (OCS) protocol (Transmedics, Andover, MA, USA). All these protocols vary in composition of the perfusate, in perfusion and ventilation settings, and in equipment used (Table 1). *Firstly*, the perfusate in both the Toronto and Lund protocols is based on Steen Solution™ (XVIVO Perfusion, Goteborg, Sweden) as originally described by Stig Steen and coworkers from Lund University [11]. This is an extracellular solution with the addition of human albumin to maintain optimal colloid pressure and dextran 40 to protect the endothelium from complement- and cell-mediated injury and to inhibit coagulation and platelet aggregation. The perfusate in the OCS™ protocol is based on OCS™ Solution® (Transmedics) or Perfadex® (XVIVO Perfusion AB, Goteborg, Sweden), both low potassium dextran 40-based solutions without additional human albumin. *Secondly*, controversy persists whether adding red blood cells to these solutions is needed to better mimic the physiologic and rheological conditions once the lungs are transplanted and to reliably assess oxygenation capacity in a more physiologic manner. Red blood cells (either packed cells or full blood) are added in the Lund and OCS™

Table 1. Comparison between different *ex vivo* lung perfusion protocols.

Parameter	Toronto	Lund	OCS*
Perfusion			
Target flow	40% CO	100% CO	2.0–2.5 l/min
PAP	Flow dictated	≤20 mmHg	≤20 mmHg
LA	Closed	Open	Open
Perfusate	Steen™ solution	Steen™ solution + RBC's hct 14%	OCS™ solution + RBC's hct 15–25%
Ventilation			
Start temp (°C)	32	32	34
Tidal volume	7 ml/kg bw	5–7 ml/kg bw	6 ml/kg bw
RR (bpm)	7	20	10
PEEP	5 cm H_2O	5 cm H_2O	5–7 cm H_2O
FiO_2 (%)	21	50	12

CO, cardiac output; FiO_2, inspired fraction of oxygen; hct, hematocrit; LA, left atrium; PAP, pulmonary artery pressure; RBC's, red blood cells; bw, body weight donor; bpm, breaths per minute; RR, respiratory rate; PEEP, positive end-expiratory pressure; Temp, temperature.
All parameters are listed for perfusion in steady state (preservation); values may vary during monitoring of the graft.
Modified from [35].
*Organ Care System™ (Transmedics).

protocols (referred to as cellular perfusate) up to a hematocrit of ±15–25%, while acellular perfusion is the preferred method in the Toronto protocol. As no comparative clinical data between acellular versus cellular EVLP are available at the moment, further studies are needed to investigate any impact of these cells on preservation, assessment, and outcome after LTx. A *third* difference between these protocols is the target flow during EVLP. This is set at 100% of cardiac output in the Lund protocol, while it is only 40% in the Toronto protocol and 2–2.5 l/min in the OCS™ protocol (Table 1). We believe that full flow is not necessary to adequately assess lung performance. It is important to monitor mean PAP to stay below 15–20 mmHg to avoid development of hydrostatic pulmonary edema. In the Toronto method, most of the time PAP remains below 12 mmHg at targeted flow. A *fourth* major difference between these protocols is whether LA (and thus the circuit) should be left open for drainage of pulmonary effluent (Lund and OCS™ protocols) or closed by suturing the atrial cuff to a specially designed plastic cannula to allow maintenance of a positive LA pressure between 3 and 5 mmHg by adjusting the height of the reservoir (Toronto protocol). There is experimental evidence from isolated rat [27] and rabbit [28] lung perfusion models that a maintained LA pressure plays an important role on pulmonary mechanical properties. This is believed to counteract edema formation by preventing collapse of the pulmonary veins and alveolar wall capillaries and maintaining venous afterload and microvascular recruitment, thereby reducing vascular resistance. *Fifth*, a strategy of protective mechanical lung ventilation with low tidal volume (5–7 ml/kg) and PEEP 5-7 cm H_2O starting when the lung has rewarmed to 32–34 °C is recommended in all three protocols, but gas mixtures (12–50% O_2) and respiratory rate (7–20 breaths/min) during preservation differ (Table 1).

An important discussion remains regarding the best timing for EVLP: in the donor hospital immediately after cold pulmonary flush or in the recipient hospital after transport and a period of cold storage (delayed EVLP). This question was addressed in a single experimental study from the University of Virginia [29]. In a porcine model of hypoxic cardiac arrest followed by 1 h of warm ischemia, three groups of animals were compared: static preservation for 4 h at 4 °C versus immediate EVLP for 4 h at 37 °C versus delayed EVLP with 4 h of cold storage followed by 4 h of EVLP. Superior post-transplant oxygenation, lower levels of inflammatory markers on bronchoalveolar lavage, and less histologic lung injury were seen in the group of delayed EVLP. Further studies are required to confirm these intriguing findings before any firm clinical recommendation can be given on the best timing for EVLP.

EVLP assessment and parameters

Once a steady state is reached, lung performance can be assessed according to the institution's protocol based on preset flow, ventilation, and gas mixture. Different parameters can be evaluated including hemodynamics (PA pressure, LA pressure, flow, and pulmonary vascular resistance), ventilation (lung compliance, airway pressures), and oxygenation capacity ($S_{PA}O_2$, $S_{LA}O_2$, $P_{LA}O_2/FiO_2$, $P_{\Delta LA\text{-}PA}O_2/FiO_2$). In addition, parameters such as loss of perfusate volume in the reservoir and sequential X-rays of the graft can be indicative as markers of lung edema. Perfusate or bronchial lavage samples can be taken for biochemical, immunologic, and microbiologic analyses.

Ex vivo perfusion of porcine lungs with stable parameters up to 12 h has been reported by the Toronto group using acellular Steen Solution™ [19]. The value of effluent PO_2 as a marker for deteriorating function remains unclear. The same group published results demonstrating stable *ex vivo* PO_2 in edematous lungs with decreased compliance and increased airway pressure, but poor PO_2 and increased pulmonary vascular resistance after transplantation [23]. A reduced effect of shunt on the *ex vivo* PO_2 was found to be attributable to the linearization of the relationship between oxygen content and PaO_2 in the acellular perfusate system which does not occur in red blood cellular perfusion whereby O_2 molecules bind to hemoglobin and the oxygenation reflects the more familiar hemoglobin–oxygen dissociation curve. *Ex vivo* PO_2 drop alone in acellular perfusion therefore is a late finding and hence not a reliable indicator

Table 2. Acceptance and exclusion criteria after 4–6 h of *ex vivo* lung perfusion applied by the Toronto team.

Acceptance criteria
$P_{LA}O_2/FiO_2 \geq 400$ mmHg
Stable or decreasing pulmonary artery pressure
Stable or decreasing airway pressures
Stable or improving pulmonary compliance
Exclusion criteria
$P_{LA}O_2/FiO_2 < 400$ mmHg
>15% deterioration on pulmonary artery pressure
>15% deterioration on airway pressure
>15% deterioration on pulmonary compliance

$P_{LA}O_2$, partial pressure of oxygen in effluent from left atrium; FiO_2, inspired fraction of oxygen.
Modified from [35].

of edema formation and reduced graft performance. Other physiologic (hemodynamic and ventilatory) parameters should be observed carefully and their trends over time play a greater importance in assessing the *ex vivo* lung.

The acceptance and exclusion criteria as clinically applied by the Toronto group are listed in Table 2.

EVLP technology and devices

Many transplant teams have started to utilize EVLP in the clinical setting using their own homemade circuit assembled with individual components available in the cardiac surgery department for extracorporeal support including a centrifugal pump, heater/cooler, tubing, hard-shell reservoir, hollow-fiber oxygenator, leukocyte filter, in-line gas analyzer, saturation probes, and pressure transducers. Organs are placed in a specially designed plastic chamber to fix the lungs in a stable position during ventilation and to provide a warm and humid environment (Fig. 1A,B). The EVLP circuit for the assessment of rejected human lungs as developed in 2004 in our laboratory at the KU Leuven University is depicted in Fig. 2A [30]. The modern set up of the Toronto EVLP circuit for clinical use is shown in Fig. 2B. The manual set up of the EVLP circuit is time-consuming and requires experienced personnel to monitor the graft during several hours. The whole transplant procedure, including donor assessment, lung retrieval, EVLP, LTx, and safe recipient transport to the ICU can keep various team members busy for nearly a full day. Further standardization of the technology and techniques are therefore needed if we want to simplify EVLP practice. It will also help to compare EVLP selection criteria and post-transplant outcome between centers. The concept of centralizing these procedures in specialized organ repair centers has also been reported in a scenario when an unusable injured donor lung was sent to a distant hospital for EVLP treatment and returned to the original center for successful transplantation [31].

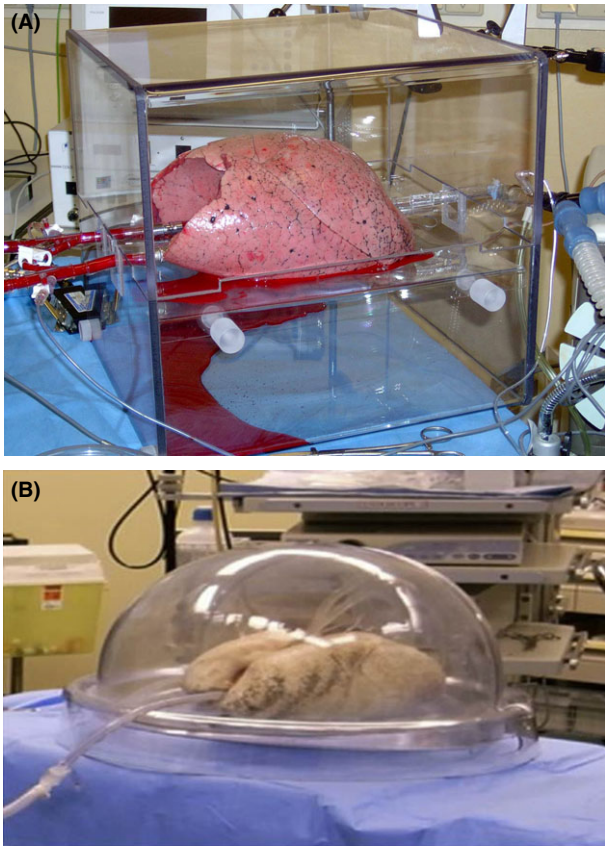

Figure 1 Organ chambers for *ex vivo* lung perfusion. (A) Original "Lung Box" with functioning human lung as used in the first publication on EVLP by Steen *et al.* [10]. Reprinted with permission from Van Raemdonck *et al.* [6]. (B) Modified "Toronto EVLP system" as used in the first clinical series on EVLP reported by Cypel *et al.* [84]. Reprinted with permission from Machuca *et al.* [35].

Several companies have now marketed commercial devices for clinical EVLP (Fig. 3): OCS™ Lung (Transmedics); Vivoline® LS1 (Vivoline Medical, Lund, Sweden); Lung Assist® (Organ Assist, Groningen, the Netherlands); and XPS™ (XVIVO Perfusion AB). There are distinct differences between these devices in terms of technology and design and in concept for clinical use (Table 3). The OCS™ Lung is a portable device on a removable mobile base so that it can be easily transported to the donor hospital. It has all equipment on board including batteries for electrical supply, gas cylinders for preservation and monitoring as well as a ventilator for use during transport of organs from donor to recipient hospital. The piston pump creates a pulsatile-type flow that may be beneficial for perfusion and recruitment of the pulmonary vasculature under physiologic conditions [32]. It offers a platform for normothermic lung preservation eliminating longer periods of cold ischemia, for continuous monitoring and assessment of graft function during storage, and for immediate and sustained

recruitment and resuscitation. The device is CE marked, and FDA approval is pending. It is currently used as platform in ongoing international trials.

Other devices were designed for in hospital EVLP once the donor lung has arrived in the recipient hospital. The Vivoline® LS1 device requires the availability of an external ventilator and gas cylinders to commence EVLP. It has an internal roller pump to create a continuous flow. The Lung Assist® is a less robust device with individual components mounted on a frame designed for EVLP and for in situ evaluation of lungs from uncontrolled DCD at the donor site prior to explanting the organs from the body [33]. Finally, the XPS™ is a fully integrated device that was developed based on the principles of the Toronto technique. The continuous flow is generated by a centrifugal pump. In addition to other devices, it offers X-ray possibilities during EVLP. CE mark approval is pending. Further clinical studies are needed to establish the benefits and risks of these devices to increase the number of transplantable lungs and to compare their added value on the outcome after LTx. Finally, economical studies looking at cost-benefit of these technologies are warranted.

Clinical potential and ongoing trials

Ex vivo lung perfusion was clinically reintroduced by Stig Steen as a method to evaluate lungs from uncontrolled DCD's prior to transplantation [10,11]. It is hoped that this strategy will create other opportunities to expand the lung donor pool and to improve the early and late outcome after LTx. *Ex vivo* lung perfusion is a new promising technique that has imposed a paradigm shift in our current thinking and practice in transplantation today.

EVLP for donor lung preservation

Cold static preservation to prevent organ deterioration by slowing down cell metabolism and by reducing the need for oxygen and other substrates has traditionally been an important prerequisite for successful outcomes after LTx [34–36]. Normothermic machine preservation is currently being proposed as an alternative and superior preservation method for other solid organs such as kidney [37], liver [38], and heart [39,40]. EVLP could become a technique for prolonged normothermic preservation of lungs. In this way, cold ischemic injury and time constraints related to long transport times can be eliminated so that the transplantation itself can be done safely as a planned procedure. However, continuous warm ischemia in the absence of perfusion (such as during mounting on the device and during implantation of the organ) presents the risk of warm ischemic damage to the lung. Clearly further studies need to be carried out to determine whether continuous warm

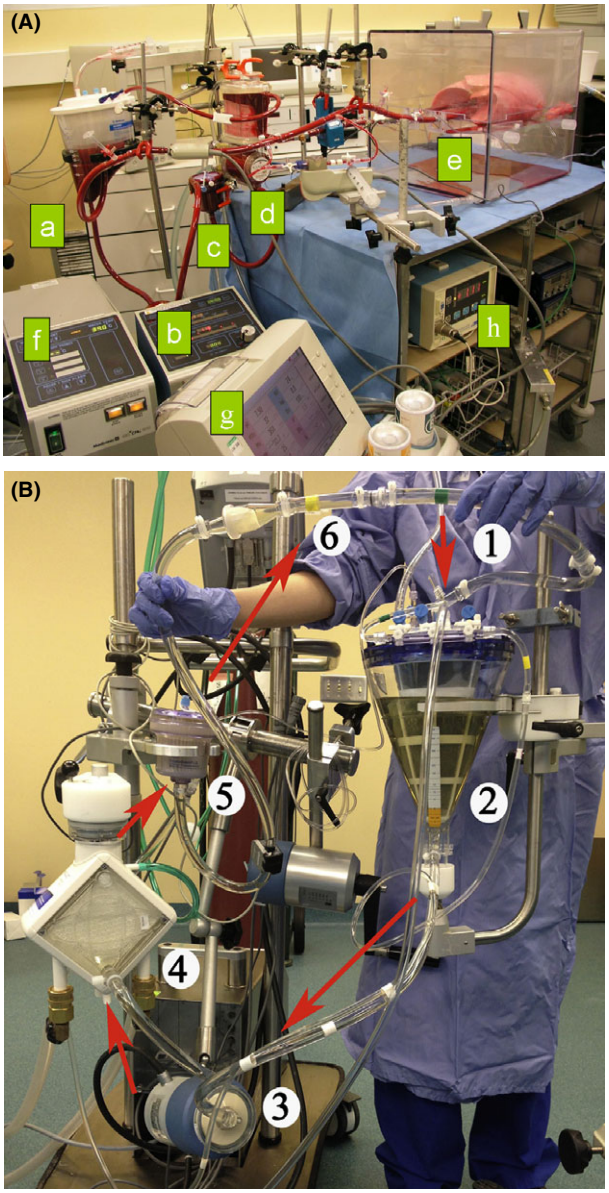

Figure 2 Reperfusion circuits for *ex vivo* lung perfusion. (A) Setup at the laboratory for experimental thoracic surgery at the KU Leuven University. From the hard-shell reservoir (a), the perfusate is recirculated by a centrifugal pump (b) passing a leukocyte filter (c) and a membrane oxygenator (d) before entering the lung block (e). The heater/cooler (f) is connected to the membrane gas exchanger. Blood gases and pulmonary artery flow are continuously measured using an in-line blood gas analyzer (g) and an electromagnetic flow meter (h), respectively. Reprinted with permission from Van Raemdonck *et al.* [94]. (B) The Toronto EVLP circuit: Circuit is primed with 2 l of Steen solution™, heparin, methylprednisolone, and imipenem. (1) Outflow end (green), which will be connected to the atrial cannula; (2) hard-shell reservoir; (3) centrifugal pump; (4) heater/cooler and gas exchange membrane; (5) leukocyte filter; (6) inflow end (yellow), which will be connected to the pulmonary artery cannula. Red arrows denote the direction of flow. Reprinted with permission from Machuca *et al.* [35].

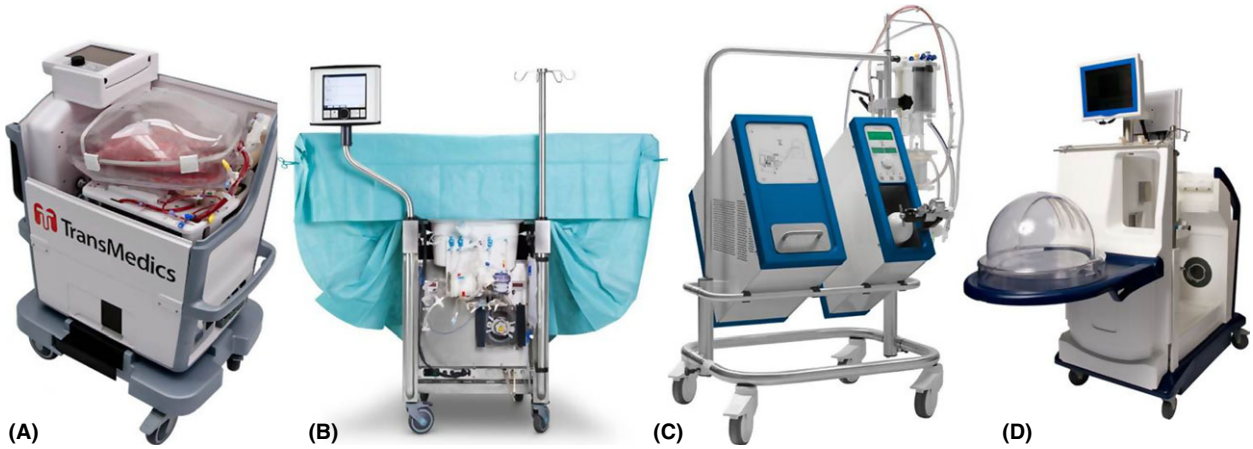

Figure 3 Commercial devices for *ex vivo* lung perfusion. (A) OCS™ Lung (Transmedics); source: www.transmedics.com. (B) Vivoline® LS1 (Vivoline Medical); source: www.vivoline.se. (C) Lung Assist® (Organ Assist); source: www.organ-assist.nl. (D) XPS™ (XVIVO Perfusion AB); source: www.xvivoperfusion.com. Reprinted with permission from Van Raemdonck *et al.* [68].

Table 3. Comparison between devices for *ex vivo* lung perfusion.

Equipment	OCS™ Lung	Vivoline® LS1	Lung Assist®	XPS™
Pump type	Piston	Roller	Centrifugal	Centrifugal
Flow	Pulsatile	Continuous	Continuous	Continuous
Ventilator	Yes	No	No	Yes
Monitor	Yes	Yes	No	Yes
Gas cylinder	Yes	No	Yes	Yes
Gas analyzer	Portable	No	No	In-line
Real time X-Ray	No	No	No	Yes
Portability	Yes	No	Yes	No

OCS™ Lung (Transmedics); source: www.transmedics.com.
Vivoline® LS1 (Vivoline Medical); source: www.vivoline.se.
Lung Assist® (Organ Assist); source: www.organ-assist.nl.
XPS™ (XVIVO Perfusion AB); source: www.xvivoperfusion.com.

perfusion is superior and safe in the clinical lung transplant setting. Past attempts at prolonged machine preservation of lungs have largely failed due to the inability to maintain the integrity and normal barrier functions of the vasculature and epithelial membranes leading to progressive deterioration in vascular flow and concurrent development of edema [9]. The modern success of EVLP without edema formation is in part due to the use of a buffered, extracellular solution with an optimal colloid osmotic pressure.

While normothermic *ex vivo* lung perfusion preservation was studied and even carried out clinically in the 1980s [9], it was abandoned due to lack of superiority and clinical impracticality using current technology available at the time. The current era experiments on blood-based EVLP were carried out in Steen's laboratory in Lund [11]. In more recent years, much experimental work was carried out at the University of Toronto. Studies in pig lungs demonstrated that 12 h of EVLP at physiologic temperature using an acellular perfusate was achievable and maintained the donor lungs without inflicting significant added injury [19–22]. This long period opens the possibility to preserve and to treat donor lungs for a longer period of time. Further studies in pig lungs after 12 hours of cold storage demonstrated that ongoing lung injury was prevented during 12-h EVLP when compared to a control group with further 12-h cold storage (20).

The INSPIRE trial (Clinical Trials.gov number NCT 01630434) is an ongoing, prospective, international, multicenter, randomized controlled, noninferiority clinical study comparing normothermic preservation of standard donor lungs using the OCS™ Lung perfusion device (treatment group) to cold storage (control group) [41]. A total of 264 patients will be randomized in both treatment arms (132 patients each). This will be the largest clinical trial in lung preservation performed to date. The primary effectiveness endpoint is a composite of patient and graft survival at day 30 and absence of primary graft dysfunction grade 3 (PGD3) within the first 72 h post-transplantation as defined by the International Society for Heart and Lung Transplantation (ISHLT) [42]. Secondary effectiveness endpoints are incidence of PGD2-3 at 72 h post-transplantation and patient and graft survival at day 30 post-transplantation. Interim results were presented at the 2013 annual ISHLT meeting [43]. The trial is expected to be completed early 2014 (Table 4).

EVLP for donor lung assessment and reconditioning postretrieval

Ex vivo lung perfusion offers a platform to (re)assess questionable lungs for transplant suitability under better

conditions compared with the *in situ* situation. The graft can be inspected, palpated, and evaluated bronchoscopically and radiographically enabling the transplant surgeon to carefully exclude the presence of tumors, areas of contusion, edema, infection, emboli, bullae, or interstitial parenchymal abnormalities. Graft performance including gas exchange, hemodynamics, and ventilatory mechanics can be assessed during several hours with the lungs on the perfusion circuit. In addition, bronchoalveolar lavage and lung tissue specimens can easily be obtained for further microbiologic, molecular, and morphological analysis. These noninvasive objective indices of donor lung injury may further help to rationalize the selection process of suitable organs in the future [44,45].

Ex vivo lung perfusion can be very helpful in assessing lungs recovered from DCD's with uncertainty about their function after LTx. Lungs from controlled DCD's with a long agonal phase and sustained injury and those from uncontrolled DCD's with previously unknown function could be thoroughly evaluated prior to acceptance and transplantation. Much research on the role of EVLP to predict the quality of DCD lungs after transplantation was carried out in Steen's laboratory in Lund [11], in our laboratory in Leuven [12–16], in Groningen [46], and in Melbourne [47]. We demonstrated both in a rabbit and in a porcine DCD model that the length of tolerable warm ischemia after circulatory arrest was about 60–90 min [12,14]. Furthermore, we found that the quality of lungs retrieved from DCD pigs 1 h after myocardial fibrillation was superior compared with lungs from brain-dead donors [15]. Finally, we compared with EVLP the impact of the mode of death in the DCD and concluded that the quality of lungs from donors with a long agonal phase (those dying from hypoxic or hypovolemic cardiac arrest) was inferior to those with sudden death from myocardial fibrillation [16].

In the clinic, lungs from uncontrolled DCD's (Maastricht Categories I and II) represent a higher risk of severe PGD3 (38%) and early mortality (17%) as previously reported [48]. These pulmonary grafts are therefore ideally suited for EVLP assessment prior to transplantation as currently performed in Madrid [49]. At this moment, it remains unknown whether lungs from controlled DCD's (Maastricht Categories III and IV) would benefit from systematic EVLP as graft function can be assessed in the donor in the hours prior to withdrawal of life support. Some of these lungs may have suffered damage in the near brain-dead donor or get injured during the process of withdrawal of life support in donors with a long agonal phase (>1 h), prolonged hypotension, or unsuspected aspiration after extubation.

The role of EVLP for assessment and reconditioning of questionable donor lungs is currently being investigated in

Table 4. Ongoing multicenter trials investigating the role of *ex vivo* lung perfusion in clinical lung transplantation.

Trial [ref.] Objective	Design	Comparison	Subjects	Primary endpoint	Location	EVLP protocol	EVLP device	Start date	Estimated completion date
INSPIRE [41] Preservation	Randomized	Normothermic preservation (OCS™) versus cold standard of care (SOC)	264 (132 OCS™ vs 132 SOC)	Composite patient + graft 30-day survival and PGD3 T0–T72	USA Europe Canada Australia	OCS™	OCS™ Lung	Nov 2011	January 2014
NOVEL [50] Reconditioning	Nonrandomized	Reconditioned extended-criteria lungs versus standard-criteria lungs	84 (42 EVLP versus 42 controls)	30-day mortality	USA	Toronto	XPS™	May 2011	May 2014
DEVELOP [52] Reconditioning	Nonrandomized	Reconditioned extended-criteria lungs versus standard-criteria lungs	408 (102 EVLP vs 306 controls)	1-year survival	UK	Lund	Vivoline® LS1	April 2012	October 2015
EXPAND [53] Reconditioning	Noncontrolled	Reconditioned extended-criteria lungs	55 OCS™	Composite patient + graft 30-day survival and PGD3 T0–T72	USA Europe	OCS™	OCS™ Lung	January 2014	December 2015

three trials (Table 4). The NOVEL trial (Clinical Trials.gov number NCT 01365429) is a prospective, nonrandomized, controlled, clinical study in 84 recipients in eight US centers comparing 30 days post-transplant mortality as primary endpoint between standard donor lungs (42 cases) versus extended-criteria donor lungs (42 cases) after EVLP reconditioning according to the Toronto protocol using the XPS™ device [50]. The trial started in May 2011 and the estimated completion date is May 2014. Preliminary results on the first 28 cases in each arm were presented at the 2013 Annual ISHLT Meeting [51]. The DEVELOP-UK trial (Controlled Trials.com number ISRCTN44922411) is another prospective, nonrandomized, controlled, noninferiority clinical study in all five lung transplant centers in UK (Newcastle, Harefield, Papworth, Manchester, Birmingham) [52]. One-year survival as primary endpoint in 408 LTx recipients is compared (1:3 ratio) between standard donor lungs (306 cases) versus extended-criteria donor lungs (102 cases) after EVLP reconditioning according to the Lund protocol using the Vivoline® LS1 device. The trial was started in April 2012. The estimated completion date is October 2015. The study was initiated as a national trial funded by the National Institute for Health Research Technology Assessment Programme after initial good clinical experience at several UK transplant centers. Finally, the EXPAND trial (Clinical Trials.gov number NCT 01963780) is a prospective, international, multicenter, nonrandomized, single arm clinical study that proposes to examine the safety and effectiveness of the OCS™ Lung perfusion device for recruiting, preserving, and assessing expanded-criteria donor lungs for transplantation [53]. A total of 55 patients will be included. Donor lung eligibility criteria are donor $PaO_2/FiO_2 \leq 300$ mmHg, expected ischemic time >6 h, DCD's, and donor age ≥55 years old. The primary endpoint is a composite of patient and graft survival at day 30 and absence of ISHLT [42] PGD3 within 72 h post-transplantation. The trial is expected to start early 2014 and to be completed by the end of the next year.

EVLP for donor lung repair

Many donor lungs get injured in the hours before and after the onset of brain death as a result of contusion, atelectasis, aspiration, infection, or neurogenic edema formation. Much research is currently ongoing to investigate whether the quality and performance of these nonacceptable lungs can be adequately improved so that some of these can still become transplantable. Firstly, EVLP allows for recruitment of atelectatic lung areas, cleaning of bronchial secretions, and removal of clots from the pulmonary circulation. In addition, the *ex vivo* system provides an excellent environment to repair lungs with targeted injury-specific treatment during EVLP before LTx. Direct pharmacological interventions to the lungs in the *ex vivo* circuit is possible via an endotracheal or intravascular route. The easiest strategy to deliver drugs directly to the organs is by adding these to the perfusate or by injecting them into the afferent tubing running to the vasculature of the graft. Theoretically different drugs according to the type of injury or even a combination ("cocktail approach") could be administered at high doses and repeated intervals: antibacterial, antiviral, and antifungal agents to treat infection, cytokine inhibitors to block pro-inflammatory responses, bronchodilating and vasodilating agents to improve ventilation–perfusion matching, fibrinolytic agents to dissolve microthrombi, high osmotic agents to remove interstitial edema etc. An advantage of this isolated setting is that these drugs could be given at higher doses than *in vivo* as there is no risk to harm other organs. A restriction, however, may be that certain drugs cannot be metabolized in the *ex vivo* circuit and therefore active components would have to be given. On the other hand, toxic metabolites may accumulate over time. Therefore, repeated renewal of the perfusate or insertion of filters in the *ex vivo* circuit may become necessary.

Perfusates with a high oncotic pressure gradient or ß-adrenergic drugs were found to accelerate removal of lung edema [54]. Our group in Leuven has previously investigated the prophylactic role of the antioxidant *N*-acetyl cysteine in DCD pig lungs subjected to 3 h of warm ischemia. Functional performance [55] and inflammatory response [56] assessed during EVLP was attenuated compared with the nontreated control group. The Zurich group investigated the role of EVLP in reconditioning pig donor lungs that were injured by acid aspiration [57]. *Ex vivo* administration of surfactant via lavage resulted in improved graft function when compared to a control group. Investigators at the University of Hamburg demonstrated that pig lungs damaged by acid aspiration could be repaired during EVLP [58]. Our group in Leuven developed a similar model of gastric content aspiration leading to higher PVR and worsened pulmonary mechanics on the EVLP circuit compared with sham animals [17]. In a subsequent experiment, administration of IV steroids and macrolides prior to the caustic injury on EVLP performance was investigated. Gas exchange was better in animals treated with steroids, but no differences could be seen in pulmonary mechanics, pro-inflammatory cytokine levels, cell count in bronchoalveolar lavage, and in lung histology compared with controls [18]. The Zurich group found that adding the fibrinolytic drug urokinase to the reperfusion solution resulted in improved graft function with decreased pulmonary vascular resistance and better oxygenation [59]. A clinical case report with the use of tPA during EVLP of a donor lung affected with massive pulmonary emboli was recently reported by the Toronto group. Functional, histologic, and biochemical evidence of clot lysis was observed, and the lungs were

successfully transplanted into a cystic fibrosis recipient [60]. In a series of human donor lungs determined to be unsuitable for transplantation by the Toronto group, five lungs were subjected to 12 h of normothermic EVLP and treated by transbronchial therapy with the gene coding for anti-inflammatory cytokine interleukin IL-10. Improvements in oxygenation capacity, restoration of alveolar barrier integrity, and attenuation of lung inflammation were noticed compared with the untreated group [21,61]. *Ex vivo* lung perfusion as a platform may also have a role in diagnosing and treating donor lung infection. Administration of antibiotics during EVLP perfusion in human lungs has proven its effects in reducing bacterial load in BAL and subsequently decreasing colony-forming units without systemic side effects [62,63]. Boffini *et al.* [64] reported on a case in which EVLP allowed detection of a previously undiagnosed pneumonia in a donor lung. Wallinder and coauthors reported on a successful case of reconditioning edematous donor lungs using a filter in the circuit for hemoconcentration to preserve the high oncotic pressure of the perfusate. This may become an alternative to intermittent replacement of the expensive hyperoncotic solution [65]. Kakishita *et al.* [66] reported on the use of an adsorbent membrane in the circuit to suppress the levels of inflammatory cytokines during *ex vivo* lung perfusion.

EVLP for immune modulation of pulmonary allograft

Primary graft dysfunction is a major cause of morbidity and mortality in the first month after LTx. It is a form of acute lung injury manifesting upon reperfusion after a period of cold ischemia. PGD is believed to be linked to chronic allograft dysfunction (CLAD), the major limiting factor for long-term survival after LTx. *Ex vivo* lung perfusion is hoped to become a technique that may help to decrease the incidence of both PGD and CLAD through different pathways.

The group in Manchester together with colleagues in Lund reported interesting findings from a small comparative study. Less acute rejection and infection was seen in the group of eight recipients transplanted with lungs reconditioned after EVLP compared with 12 patients in the standard group [67]. The authors speculated that the lack of acute rejection in EVLP patients may be the result of reduced donor organ stress and the mechanical removal of passenger leukocytes which directly contribute to alloresponsiveness.

There is an increasing interest in the potential prophylactic and therapeutic effect of mesenchymal stem cells (MSC's) [68]. Much research was carried out by the group of Michael Matthay at the University of California San Francisco in a model of acute lung injury comparable to donor lung injury. In cultured human alveolar type II cells damaged by a mixture of cytokines, these investigators demonstrated the ability of allogeneic human MSC's to restore epithelial permeability that is needed to limit edema formation after LTx [69]. In one study from the same research group, allogeneic MSC's were administered directly into the airways of human donor lungs declined for transplantation in a model of acute lung injury to study their treatment potential [70]. Several basic anti-inflammatory and antibacterial properties have been attributed to MSC's that may be beneficial to restore lung injury in patients with acquired respiratory distress syndrome [71]. The spectrum of possible MSC's-based therapies for acute lung injury includes both targeted intrapulmonary and intravascular administration during EVLP.

Gene therapy provides the exciting potential to immunologically prepare the donor lung prior to exposure to the recipient immune system response and induce tolerance in the recipient reducing the need for immunosuppression and its attending complications (toxicity, infection, and malignancies). No experimental data have been published so far using EVLP to precondition the allograft to prevent acute of chronic allograft rejection.

Decellularization techniques to build "new" immunotolerant lungs are emerging although still preliminary with technical limitations [72,73]. The role of EVLP to repopulate these lungs with pulmonary endothelial and epithelial cells specific to the recipient remains to be investigated.

Clinical experience with EVLP

The first case report of successful LTx after EVLP was published in 2001 [10]. A left single lung was transplanted into a 54-year-old female recipient with chronic obstructive lung disease after previous lung volume reduction surgery. The donor was a Maastricht Category II DCD declared death after unsuccessful resuscitation following myocardial infarction. The lungs were topically cooled in the intact body for 3 h initiated 65 min after death. The heart–lung block was removed, and functional performance of both lungs was assessed in an *ex vivo* reperfusion system for 1 h, then cooled and further stored for 12 h prior to transplantation. The function of the transplanted lung was good throughout the first five postoperative months until death from CMV infection.

Several research groups worldwide have previously reported on the feasibility of EVLP in human discarded lungs in preparation of a clinical EVLP transplant program [21,30,74–77]. Clinical single-center series on transplantation after EVLP are listed in Table 5.

After an initial report in 2007 on the first case [78], the Lund group reported in 2009 on a series of six successful transplantations with lungs from heart-beating donors previously declined by other Scandinavian transplant teams

Table 5. Reported clinical single-center series on *ex vivo* lung perfusion.

Transplant group	First report [ref.]	Update [ref.]	Protocol	Perfusate	Number EVLP	Number LTx	Utilization rate (%)	Outcome survival/Mortality
Lund	2009 [79]	2011 [80]	Lund	Cellular	9	6	66	100% at 3 months 66% at 2 years
Harefield	2009 [82]	2012 [83]	Toronto	Acellular	13	6	46	100% at 3 months
Toronto	2011 [84]	2012 [85]	Toronto	Acellular	58	50	86	4% 30-day mortality 87% at 1 year
Vienna	2012 [86]	/	Toronto	Acellular	13	9	69	0% 30-day mortality 78% at 1 year
Goteborg	2012 [87]	2014 [88]	Lund	Cellular	11	11	100	100% at 3 months
Newcastle	2012 [89]	/	Toronto	Acellular	18	7	39	100% at 3 months
Milan	2012 [90]	/	Lund	Cellular	NR	2	NR	0% 60-day mortality
Turin	2013 [91]	/	NR	NR	9	7	78	NR
TOTAL					113	89*	79	

EVLP, *ex vivo* lung perfusion; LTx, lung transplantation; NR, not reported.
*Excluding two cases from Milan group.

[79]. The donor lungs were reconditioned *ex vivo* with Steen Solution® mixed with erythrocytes to form a hyperoncotic solution to dehydrate edematous lung tissue. Functional evaluation was performed with deoxygenated perfusate by changing the gas mixture to the oxygenator in the circuit. After reconditioning, the lungs were kept immersed at 8 °C in the perfusate on the EVLP circuit until the time of transplantation. There was no difference in early outcome (time on ventilator, ICU, and hospital stay) when compared to 15 lung recipients transplanted with conventional donor lungs in the same time period [80]. The outcome in this clinical series was updated in 2011 with longer follow-up [81]. Two of the six transplanted patients have died (one from sepsis after 95 days and one from rejection after 9 months). The remaining four patients were alive 24 months after the transplant (Table 5).

The group at Harefield Hospital in London, UK, reported in 2009 on their experience with EVLP using the Toronto technique. Of five unacceptable lungs (low PO_2: $n = 3$; secretions: $n = 2$), 2 (40%) were successfully reconditioned and one was finally transplanted into a 32-year-old cystic fibrosis patient who died 7 months later from a respiratory infection [82]. The series was updated in 2012 with 13 cases of EVLP of which six pairs of lungs (46%) were successfully transplanted with a 3-month survival of 100% [83]. Of interest, three pairs of lungs were not transplanted because of pulmonary edema at the end of EVLP despite good oxygenation.

A prospective, nonrandomized, controlled clinical trial of Human *Ex vivo* Lung Perfusion (HELP) was conducted at the University of Toronto to assess the feasibility and safety of LTx of high-risk donor lungs. Lungs from 23 extended-criteria donors were placed in the *ex vivo* circuit (Toronto *ex vivo* perfusion system) and perfused normothermically with acellular Steen™ Solution for 4 h. Twenty (86%) of these lungs (11 DBD and 9 DCD with median PaO_2/FiO_2 of 275 and 420 mmHg, respectively) fulfilled the criteria of good physiologic function (PVR, C_{dyn}, PIP) and improved oxygenation capacity (from initial 335 to 414 mmHg at 1 h and 443 mmHg at 4 h of reperfusion) and were subsequently transplanted. The outcome was compared with a control group of 116 conventional lungs transplanted during the same time period. Remarkably, the incidence of PGD at 72 h after transplantation was 15% in the EVLP group and 30% in the control group ($P = 0.11$) [84]. No significant differences were observed for any secondary endpoint, and no severe adverse events were directly attributable to EVLP. The series was recently updated with the first 50 transplantations (28 DBD and 22 DCD) after EVLP in 58 cases (yield of 86%) [85]. PGD3 at T72 was 2% in the EVLP group and 8.5% in the control group ($P = 0.14$) with similar early outcome and survival (Table 5).

The Vienna group reported in 2012 their initial experience with LTx after EVLP using the Toronto technique [86]. Thirteen lungs with a median donor PaO_2/FiO_2 of 216 mmHg were evaluated. Nine lungs (69%) improved to a delta PO_2 on the circuit of 350 mmHg, and all were transplanted. None of the recipients developed PGD2-3 within the first 72 h. Thirty-day mortality was 0%, and 1-year survival was 78% (Table 5).

The group at the University of Goteborg reported in 2012 their experience with cellular EVLP of six pairs of initially rejected donor lungs because of low (mean

156 mmHg) PaO_2/FiO_2 ($n = 5$) or infiltrate on chest radiograph ($n = 1$) [87]. Oxygenation during EVLP improved in all cases (mean improvement 255 mmHg), and hemodynamic and respiratory parameters were stable. Two single lungs were not used because of subpleural hematoma or edema. Six recipients (100%) underwent single ($n = 2$) or double ($n = 4$) LTx with one patient presenting with PGD2 at 72 h. Thirty-day survival was 100%. The series was recently updated with 11 EVLP cases, all transplanted successfully (single lung: $n = 3$; double lung: $n = 8$) with 100% hospital survival [88]. Time to extubation ($P = 0.05$) and ICU stay ($P = 0.01$), but not hospital stay ($P = 0.21$) were longer in the EVLP group when compared to 47 patients transplanted with conventional lungs during the same time period. Three-month survival was 100% in EVLP group and 94% in control group with a FEV_1 of 79% in double-lung recipients and 40% in single-lung recipients versus 85% and 55%, respectively (Table 5).

The Newcastle group reported their initial experience at the 2012 ISHLT meeting [89]. Of 18 EVLP cases, only seven patients (three single-lung and four double-lung recipients) were transplanted. One patient presented with PGD3 at T72. Ninety-day survival was 100%. The lung transplant group in Milan reported their experience with successful transplantations after EVLP in two recipients with similar hospital outcome and no mortality at 60 days compared with six patients transplanted with conventional lungs during the same time period [90]. The total number of EVLP cases in this series, however, is unknown. The transplant team at the University of Turin reported in 2013 seven lung transplants after nine EVLP cases (using the Toronto EVLP technique) leading to a 30% increase in their transplant activity [91]. No information on the EVLP protocol and technique and no outcome data were available in the report (Table 5).

The experience with EVLP at the Hôpital Foch in Paris was reported at the 10th International Congress on Lung Transplantation, Paris, 20–21 September 2012, but this single-center data are yet to be published. The combined experience of Toronto, Vienna, and Paris using the Toronto protocol was presented at the 2013 ISHLT meeting [92]. A total of 125 clinical EVLPs were performed (72% DBD and 28% DCD). One hundred and three lungs were subsequently transplanted (utilization rate 82.5%). Incidence of PGD3 at T24 and T72 was 7% and 5%, respectively. Median time to extubation was 2 days and median hospital stay 23 days. Thirty-day mortality was 4% and 1-year survival 88%. Six patients (6%) developed airway complications requiring intervention.

The Hannover and Madrid group have reported their combined experience using the OCS™ protocol and OCS™ Lung device in 12 patients with 100% survival at 30 days [93]. Other lung transplant groups worldwide have successfully transplanted patients after EVLP reconditioning of unacceptable donor lungs according to various different protocols.

From the experience above, one can conclude that transplantation of questionable or unacceptable lungs after assessment and reconditioning with EVLP is feasible and safe with an overall utilization rate approaching 80% in experienced centers and good early outcomes comparable to recipients of conventional, non-EVLP lungs.

Conclusions

Ex vivo lung perfusion is a new promising tool that allows preservation, assessment and reconditioning of ideal, questionable, and unacceptable donor lungs prior to transplantation. Further research is needed to investigate how EVLP can serve as a platform for more advanced therapies and what interventions are possible to repair severely injured lungs and how the pulmonary graft can be modulated to inhibit the immune response once transplanted.

Ex vivo lung perfusion holds significant promise as a method to maximize the number of available donor lungs and to improve the early and late outcome after transplantation by decreasing the incidence of PGD as well as CLAD. Technological developments with lung devices and further research on the optimal technique and solution for long-term *ex vivo* perfusion are needed. Results of ongoing clinical trials are awaited before EVLP will find its definitive place in our daily LTx practice.

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