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Chapter 10

Ex-Vivo Lung Perfusion: From Bench to Bedside

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Additional information is available at the end of the chapter

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Abstract

Lung transplantation is an established treatment option for eligible patients with end-stage lung disease. Nonetheless, there exists an imbalance between donor lungs considered suitable for transplantation and the ever-growing number of patients dying on the waiting list. This chapter reflects the potential alternative, normothermic ex-vivo lung perfusion (EVLP), which has emerged to address this issue and how it can expand the currently limited donor pool. Normothermic ex-vivo lung perfusion (EVLP), as a novel preservation technique, is capable of assessing, evaluating, and improving lung function prior to lung transplantation. Here, we (1) contrast the various available commercial EVLP available and used around the world; (2) outline the University of Alberta novel EVLP circuit; (3) discuss the limitations present between clinical and laboratory applications; and (4) present what we are currently working on at the laboratory to further improve the assessment techniques used on EVLP.

Keywords: donor lung preservation, donor lung repair, ex-vivo lung perfusion, lung transplantation, lung health index

1. Lung transplantation

1.1. History

Human lung transplantation (LTx) has been widely accepted as a modality of treatment for advanced stage lung disease [1]. The annual report from the Registry of the International Society for Heart and Lung Transplantation (ISHLT) states more than 45,000 LTx cases performed worldwide since the 1990s. In 2012, ISHLT reported that in that year it had the

second highest annual activity, following the highest activity level in 2011, in LTx performed. "The number of adult primary lung transplants in 2012 was 40-fold higher than the number of pediatric primary lung transplants" [1]. The agency for healthcare policy and research in the United States mentioned that "lung transplantation has evolved as a clinical procedure achieving a favourable risk–benefit ratio and acceptable 1- and 2-year survival rates" [1].

In the 1940s and the 1950s, a rise in animal experimentation verified feasibility of LTx procedure [2–4]. However, it was not until 1963 when the first human lung transplantation was performed. The recipient of that first lung transplantation received a left lung, which was donated from a cardiocirculatory death donor (DCD); however, the recipient survived for only 18 days [5]. From 1963 to 1980, almost 44 lung transplantations were attempted worldwide; due to rejections and problems with anastomotic bronchial and tracheal healing, the survival rates were only several days [6, 7].

The introduction of cyclosporine A in the 1980s, a powerful immunosuppressant, generated a renewed interest in organ transplantation, including LTx. In 1983, Dr. Cooper from Toronto performed the first successful human single lung transplantation, while Dr. Patterson performed the first double lung transplantation in 1988 [6, 7]. Despite the relatively short history of thoracic transplantation, there has been significant improvement in post-transplantation mortality rate from only weeks to several months and years. This success can be attributed to the advent of the heart–lung machine, improved preservation solutions, immunosuppression regimes, and specialized patient care by transplant clinics.

1.2. Indications

Lung transplantation is considered for patients with end-stage lung disease. Referral for transplantation is urgent when the lung disease begins to limit basic daily activities and poses a high risk of death in the short term.

According to ISHLT, the most common primary indication for adult lung transplants between January 1995 and June 2013 was chronic obstructive pulmonary disease (COPD, 33%) not associated with α_1-antitrypsin deficiency (A1ATD), followed by interstitial lung disease (ILD, 24%), including idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF, 16%) associated with bronchiectasis, and 6% of COPD associated with A1ATD [8, 9]. For the 45,711 lung transplants that occurred from 1990 to 2012, recipients with COPD not associated with A1ATD, ILD, and CF contributed to the greatest amount of growth in the number of LTx [8, 9].

1.3. Criteria

The appropriate timing for patients to be referred for lung transplantation is when they are believed to have less than 50% of a survival chance in 24–36 month period. An additional consideration is the patient's quality of life. The following are the guidelines for referral for LTx, based on the underlying lung disease [10] (**Table 1**).

Criteria for referral in patients with COPD and alpha1-antitrypsin deficiency emphysema are as follows:

- BODE index > 5
- Postbronchodilator FEV_1 < 25% predicted
- Resting hypoxemia (i.e., PaO_2 < 55–60 mm Hg)
- Hypercapnia ($PaCO_2$ > 50 mm Hg)
- Secondary pulmonary hypertension
- Clinical course marked by rapid rate of decline in FEV_1 or life-threatening exacerbations

The BODE index, a multidimensional 10-point scale, can be used to assess the need for transplantation in patients with COPD. It consists of the following [46]:

- B—Body mass index
- O—Degree of airflow obstruction
- D—Degree of dyspnea, as measured by the modified Medical Research Council dyspnea scale
- E—Exercise capacity (E), which is measured with a 6 min walk test

FEV_1, forced expiratory volume in 1 s; PaO_2, partial pressure of arterial oxygen; $PaCO_2$, partial pressure of arterial carbon dioxide

Criteria for referral in patients with cystic fibrosis are as follows:

- Postbronchodilator FEV_1 < 30% predicted
- Resting hypoxemia, i.e., PaO_2 < 55 mm Hg
- Hypercapnia ($PaCO_2$ > 50 mm Hg)
- Clinical course—Increasing frequency and severity of exacerbations (ICU stays)
- Development of pulmonary hypertension

Criteria for referral in patients with idiopathic pulmonary fibrosis are as follows:

- DLCO < 39%, predicted
- A 10% or greater decrement in forced vital capacity (FVC) during 6 months' follow-up
- FVC < 60–65%, predicted
- Decrease in oxygen saturations <88% during 6 min walk test

DLCO, diffusion capacity of carbon monoxide; FVC, forced vital capacity.

Table 1. Guideline criteria for referral to lung transplantation, based on underlying lung diseases [10].

1.4. The burden

The Canadian Organ Replacement Registry (CORR) has reported that in the past decade, the annual number of lung transplants has gradually increased over the years [11]; meanwhile, the waiting list increases at a much faster rate. Therefore, a staggering increase in the morbidity rate and a high waiting list mortality rate have been reported [11]. With the advancements of medical knowledge and specialized patient care over the years, lung disease patients with

other ailments can now have their nonrelated lung conditions managed appropriately and live longer till they require a lung transplant.

Currently, more than 80% of donor lungs are potentially injured and therefore not considered suitable for transplantation [12]. At the University of Alberta, we report that from 2007 to 2011 there have been a total of 681 lungs offered, and only 183 lungs deemed acceptable for LTx. This equates to approximately a 27% utilization rate over the past 5 years. With the University of Alberta/Mazankowski Alberta Heart Institute acting as a catchment for over 6 million Canadians, this institute performs the majority of thoracic transplantations for several provinces in Canada. Unfortunately, with such a low lung utilization rate, there are more than 24 deaths/year for patients waiting for a suitable donor lung. Having said that, various strategies need to be implemented to increase the utilization rate of the current standard lung donor pool.

During recent years, transplant centers worldwide have started to include the use of lungs from extended/marginal criteria donors, living lobar donors, as well as tapping into the unused pool of donors after circulatory death (DCD) [13, 14]. Normothermic ex-vivo lung perfusion (EVLP) emerged as a new and promising platform, with the clinical potential to increase the number of transplantable lungs and improve the early and late outcome post-transplantation. EVLP has the potential to assess, evaluate, and recondition lungs, and eventually expand the limited donor pool. Currently, EVLP is limited to only 4–6 h of a reconditioning window [13]. This narrows therapeutic interventions that can be applied during this short perfusion time. The need for an extended clinical EVLP protocol (≥12 h) is critical to achieve its full potential. Gene therapy and stem cell therapy are promising therapeutic examples. However, their respective delivery techniques using EVLP are yet to be optimized.

2. Normothermic ex-vivo lung perfusion

2.1. Lung preservation

Since the late 1980s, conventional donor lung preservation has been focused around the use of cold static preservation (CSP): placing them on ice for transportation to a recipient site. CSP supports the slowing down of cell metabolism, thus, reducing the demand for oxygen and other substrates [15]. Low metabolic state decreases enzymatic activity related to ischemia and hypoxia, thereby protecting the graft from their deleterious effects. However, the associated decrease in function of vital enzymes such as Na^+/K^+ ATPase causes an ionic imbalance, leading to edema and a rise in intracellular calcium, which causes cellular injury [16]. With the lungs inflated during CSP, studies have shown significant generation of reactive oxygen species, leading to more damage of the donated lungs [17, 18].

Over the years, there has been a predominant effort to optimize retrograde and antegrade flushing solutions, with the compositions representing mostly extracellular characteristics [19]. Further studies reported better results utilizing flush solutions, with temperatures at 10°C, whereas others supported the routine use of solutions in the 4–8°C range [20]. This was

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achieved after flushing the lungs with the respective flush solution and storing them on ice for the duration of the transport of the donor lungs to the recipient site. Cold preservation was thought to benefit the lungs more than other organs, given the ability to store them inflated with oxygen, allowing for efficient aerobic metabolism and maintaining their gas-exchange surface [21].

2.2. Definition and history

Physiological normothermic ex-vivo lung perfusion is a novel method that maintains the organ in a more physiological protective condition, outside the body, during preservation. EVLP will help increase the utilization of donor lungs by allowing trained professionals to accurately evaluate and assess the functionality of lungs (which otherwise would be unutilized) during the transport period. While the lungs are on EVLP, they will be maintained under normothermic physiological conditions to help alleviate the deleterious ischemia reperfusion injury that is observed with CSP, furthermore, permitting the treatment/reconditioning of the lungs prior to transplantation. Currently, with CSP, lungs have no way to be truly assessed for injury that occurs during the transport period which can range from 6 to 8 h. Thus, transplanting lungs that have suboptimal functions can result in poor postlung transplantation outcome and increase the severity of primary graft dysfunction/failure.

Ex-vivo perfusion of organs began with the work of Carrel and Lindbergh in 1935 [22]. They have documented 26 perfusions of whole organs: ovary, thyroid, kidney, and heart. Organs that were perfused were functional for several days with active cellular proliferation. Since the advent of the work of Carrel and Lindbergh on ex-vivo perfusion, ex-vivo systems were limited to the study of organ physiology, including lungs [23]. It was not until 2001 that Stig Steen first described the use of EVLP in clinical lung transplantation. Using a proprietary lung-perfusion solution (STEEN Solution™), put together in Dr. Stig Steen and his team's lab, the group was able to reassess uncontrolled donation after cardiocirculatory death (DCD) lungs [24, 25]. Until then, the majority of donor lungs were from brain-dead donors (BDD). With the help of EVLP, the successful reconditioning of these DCD lungs (an unutilized donor pool) resulted in a cascade of research to revisit the possibility of utilizing donor lungs from the DCD pool.

It was not until further modifications of the EVLP system and perfusion technique by the University of Toronto group, which allowed perfusion of pig lungs on EVLP from only 4 to 6 h [25] to a prolonged 12-hour ex-vivo perfusion, without damaging the organ [26]. The group went on to determine the impact of prolonged EVLP using injured ischemic donor pig lungs. To mimic the clinical scenario, where lungs undergo a period of cold ischemia during transportation, pig lungs were preserved under CSP for 12 h and subsequently divided into two groups: cold static preservation (the current gold standard) and normothermic EVLP for a further 12 h of perfusion (total 24 h of preservation) [27].

It became evident that unlike CSP, normothermic EVLP demonstrated noticeable improvement with regard to overall lung function: less edema formation post-transplantation, better alveolar–epithelial cell tight junction integrity, enhanced metabolic function, and improved oxygenation [25].

2.3. The circuit

As described in more detail in reference [14], in general, most EVLP platforms utilized around the world (used experimentally or clinically) consist of the same components. The circuit consists of a perfusion circuit with tubing, a reservoir, a pump, membrane gas exchanger, a leukocyte depletion filter, and an ICU-type ventilator [14] (**Figure 1**). The system is then primed with their respective perfusate and additives, and then warmed to 32–34°C. Once this temperature is achieved, careful institutional specific lung ventilation commences, allowing the lungs to continue to reach a perfusate temperature of normothermia (37°C).

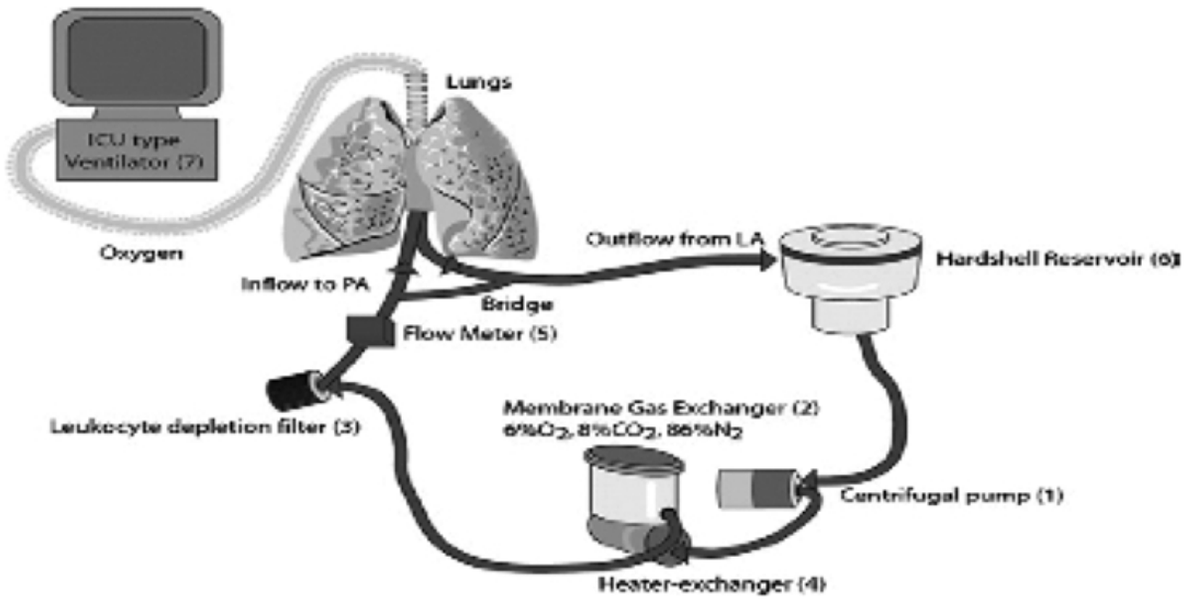

Figure 1. Schematic of the standard ex-vivo lung perfusion circuit [26].

The lungs are placed in a specially designed organ chamber. A pump, roller or centrifugal, circulates the perfusate from the reservoir through a gas-exchange membrane and a leukocyte filter, before entering the lungs via the pulmonary artery. Before entering the leukocyte filter, the gas-exchange membrane is connected to a heat exchanger and a special gas tank: the heat exchanger warms up/maintains the perfusate at normothermic temperatures, while the special gas tank consists of a low oxygen mixture to deoxygenate the perfusate before returning to the lungs (6% O_2, 8% CO_2, and 86% N_2) [14]. The outflow perfusate returns to the reservoir either through a left atrial (LA) cannula or via an open atrium, where it is then recirculated. Catheters or pressure transducers are used to continuously monitor and measure pulmonary artery pressures (PAP) and left atrial pressures (LAP), if it is a closed left atrial system. A temperature probe monitors the circuit temperature throughout the perfusion, and flow probes measure PA and LA perfusate flow (if the circuit has a closed left atrium). Finally, lungs are ventilated with a standard intensive care unit (ICU) ventilator [14].

2.4. EVLP protocols

Reference [14] outlines an in-depth review on the currently utilized EVLP platforms and their protocols. As of today, there currently exist three different EVLP protocols utilized around the world: (1) Toronto protocol, (2) Lund protocol, and (3) Organ Care System™ (OCS) protocol (TransMedics, Andover, MA). These protocols vary in composition of their respective perfusate, in perfusion and ventilation settings, and in the equipment used for their circuits [14] (**Table 2**). In general, after cold pulmonary flush and retrieval using an extracellular fluid (ECF)-type solution (low-potassium dextran solution, known as Perfadex®), the donor lungs will be instrumented in the donor hospital or recipient hospital (after experiencing a period of cold ischemia during transport) and placed on the EVLP platform for either immediate or delayed normothermic perfusion, respectively. Interestingly, reference [28] investigated the best timing for EVLP: at the donor hospital immediately after cold pulmonary flush or at the recipient hospital after transport and a period of cold storage (delayed EVLP) [14, 28]. It was further found that lower levels of inflammatory markers on bronchoalveolar lavage were present, and less histological lung injury and superior post-transplant oxygenation were seen in the group of delayed EVLP (4 h of cold storage followed by 4 h of EVLP) [14, 28].

Parameter	Toronto	Lund	OCS
Perfusion			
Target flow	40% CO	100 % CO	2.0–2.5 1/min
PAP	Flow dictated	≤20 mm Hg	≤20 mm Hg
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

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

Table 2. Comparison among the three different protocols currently used for EVLP [14].

2.4.1. Toronto protocol

The Toronto group uses an acellular perfusate, STEEN Solution™ (XVIVO Perfusion, Goteborg, Sweden), which was originally described by Stig Steen and coworkers from the Lund

University [25]. This proprietary solution is an extracellular solution, with the addition of human albumin, which maintains optimal colloid pressure, and dextran-40, which protects the endothelium from complement- and cell-mediated injuries and inhibits coagulation and platelet aggregation [14, 25]. Once the LA cannula is filled with STEEN Solution™ (XVIVO Perfusion, Goteborg, Sweden), perfusion commences at 10% of the calculated cardiac output flow, and is incrementally increased till the final 40% cardiac output flow for the remainder of the perfusion run, by 50 min from the start of perfusion [12–15]. Ventilation is initiated once the perfusate temperature reaches 32°C at an immediate 7 ml/kg tidal volume, positive end-expiratory pressure (PEEP) of 5 cm H_2O, respiratory rate (RR) of 7 breaths/min, and with an inspired fraction of oxygen (FiO_2) of 21% [12–15] (**Table 2**). Unlike the other two protocols, the Toronto method elects to have a closed left atrium and has the height of the reservoir adjusted manually to maintain a positive LA pressure between 3 and 5 mm Hg [12–15]. Finally, the Toronto group carefully monitors and maintains the mean pulmonary arterial pressure (PAP) to stay below 15–20 mm Hg, which is flow-dictated. This is believed to avoid development of hydrostatic pulmonary edema [14, 15].

2.4.2. Lund protocol

The Lund group utilizes a cellular perfusate, STEEN Solution™ (XVIVO Perfusion, Goteborg, Sweden), mixed with packed red blood cells (pRBCs) to obtain a hematocrit of 14% [14, 25] (**Table 2**). In the Lund technique, ventilation begins at a tidal volume of 3 ml/kg at 32°C and gradually increases by 1 l/min, for each degree, until it reaches 5–7 ml/kg at 37°C [14]. Other parameters that differ from the Toronto protocol are the open LA system at 100% cardiac output flow, respiratory rate (RR) of 20 breaths/min, and a FiO_2 of 50% [14, 25, 29] (**Table 2**).

2.4.3. OCS (transMedics) protocol

The OCS™ protocol is based on a cellular perfusate like the Lund protocol; however, in this protocol, the perfusate is composed of an OCS™ Solution® (TransMedics) or Perfadex® (XVIVO Perfusion AB, Goteborg, Sweden) and pRBCs to achieve a hematocrit between 15 and 25% [14, 30]. Both of these solutions are low-potassium dextran-40 based solutions, without the addition of human albumin (unlike STEEN Solution™) [14]. Perfusion flow is set to 2–2.5 l/min, PAP maintained less than 20 mm Hg, with an open LA system, initiating ventilation at 34°C and 6 ml/kg, a RR of 10 breaths/min, PEEP of 5–7 cm H_2O, and an FiO_2 of 12% [14, 15, 30]. The variations among these protocols have been summarized in **Table 2**.

2.5. EVLP application

2.5.1. Commercial application of EVLP

There are several commercially available EVLP platforms, under different stages of development. Today, there exist four EVLP platforms used commercially that differ in their technology and perfusion protocol, and in the concept for clinical use.

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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.co.

Table 3. Comparison between commercially available devices for EVLP [14].

1. OCS™ Lung (TransMedics) is a portable device that uses a cellular-based perfusate, piston pump (creating a pulsatile-type flow), LA open system, with all the required equipment on board: batteries, gas cylinders for preservation and monitoring, and a ventilator for use during transport of organs from donor to recipient hospital [14]. Whether there is any benefit for pulsatile versus nonpulsatile flows has been a topic of controversy over the years; however, some document that the presence of a pulsatile-type flow may be beneficial for recruitment of the pulmonary vasculature, while being perfused under physiological conditions [14, 31].

OCS™ Lung (TransMedics) was included in an international INSPIRE trial used to compare normothermic preservation versus cold static preservation, ending its trial in January 2014 [14, 30, 32, 33]. The University of Alberta Hospital being one of the centers involved in this trial, we demonstrated the feasibility of prolonged EVLP using the OCS system. Our results revealed how complications, postoperatively, in regards to primary graft dysfunction (an acute lung injury that can occur in the first 72 h after transplantation), were resolved after 30 days. Moreover, the patient/recipient demonstrated excellent pulmonary function at 1 year post-transplantation, despite getting reconditioned extended criteria lungs that otherwise would have been discarded [33].

2. Vivoline® LS1 (Vivoline Medical, Lund, Sweden) is a nonportable device that uses the Lund technique, requires the availability of an external ventilator and gas cylinder, and has an internal roller pump to create a continuous flow (nonpulsatile). It was utilized in the United Kingdom under the "Donor Ex-Vivo Lung Perfusion in United Kingdom" (DEVELOP-UK) trial to assess reconditioned extended criteria lungs versus standard-criteria lungs; the trial ended in October 2015 [34].

3. Lung Assist® (Organ Assist, Groningen, the Netherlands) is deemed "a less robust device with its individual components fixed 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" [14, 35].

4. XPS™ (XVIVO Perfusion AB) utilizes the Toronto protocol and only differs by the addition of various in-line monitors to streamline organ assessment [32]. It contains a centrifugal pump that delivers a continuous flow (nonpulsatile); it is a fully integrated device, and unlike the other commercially available devices, it offers X-ray possibilities during EVLP [14]. XPS™ (XVIVO Perfusion AB) has been involved in the FDA NOVEL lung trial: "Normothermic Ex-Vivo Lung Perfusion as an Assessment of Extended/Marginal Donor Lungs," since May 2011–May 2014 to compare the reconditioned extended criteria lungs versus standard-criteria lungs in the United States [14, 36]. A summary of the various commercially available devices and their technological differences is described in **Table 3**.

Figure 2 provides a visual representation of the four commercially available devices previously mentioned.

Figure 2. Commercially available ex-vivo lung perfusion devices. (**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 [37].

2.5.2. Potential applications of EVLP

As described in more detail in reference [38], there are a few applications that can benefit from the platform of EVLP:

Transplantation

- Extended donor lung preservation
- Functional assessment prior to transplantation

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- Biological assessment before implantation
- Enabling organ's natural recovery processes
- Active repair of injured lungs using various therapies
- Active molecular treatments for organ preparation
- Xenotransplantation studies

Regenerative medicine

- Bioreactive for lung de-cellularization and regeneration
- Stem cell and gene therapy for lung injury

Respiratory medicine

- Study of acute lung injury
- Functional studies for endobronchial interventions for chronic obstructive pulmonary disease
- Study of lung physiology

Cancer

- Study of chemotherapeutic agents to evaluate lung toxicity and antitumor activity
- Lung cancer treatments

2.6. EVLP and lung transplantation

The first clinical use of EVLP was in 2001 by Stig Steen [39]. Steen evaluated lungs from DCD donors and six extended criteria donor lungs for 60 min on EVLP before transplantation. It was observed that the mean time in the intensive care unit (ICU) was longer for the perfused lungs with EVLP compared to the standard criteria lungs. However, the 30-day survival rate post lung transplantation from the perfused groups with EVLP was 100% [39–41].

Human ex-vivo lung perfusion (HELP) trial in 2011 was the first prospective clinical trial done at Toronto General Hospital. Of the 23 lungs from high-risk brain death (BDD) and cardiac death donors (DCD) that underwent 4 h of EVLP, 20 were considered suitable and later transplanted [12, 26, 38, 42]. The criteria to terminate perfusion and discard lungs included pulmonary vascular resistance (PVR), dynamic compliance (C_{dyn}), and peak inspiratory pressure (PIP) decline by more than 15%, and also a change in partial pressure of oxygen/fraction of inspired oxygen ratio ($\Delta PaO_2/FiO_2$ or P/F ratio) of less than 350 mm Hg. Again, there were no significant differences in primary graft dysfunction (PGD) trends, extubation time, ICU/hospital stay, and 30-day mortality rate, compared to the standard criteria lungs [12].

In Europe, Zych et al. [43], from Hartfield, evaluated 13 sets of rejected lungs, of which 6 improved during EVLP and were later implanted: no difference in ICU stay and in 3 and 6

months survival compared to the standard criteria lungs [43]. From Vienna, Aigner et al. [44] perfused and reassessed 13 sets of lungs, of which 9 showed improvement after EVLP.

Currently, FDA mandated multicenter clinical trial (the NOVEL lung trial) to approve the clinical use of EVLP for assessment of extended/marginal donor lungs. Eight centers using a nonrandomized, controlled, clinical study in the United States were involved in the trial using inclusion/exclusion criteria for perfusion on EVLP, described in the HELP trial [29, 38] (**Table 4**). The trial began in May 2011 and ended in May 2014; first report of 30 patients who received EVLP lungs were comparable to 31 control groups of non-EVLP transplants. The 2014 updates described 76 EVLPs yielding 42 transplants [45]. No significant difference was present between transplanted lungs after EVLP reconditioning and the 42 non-EVLP perfused controls in regards to the 1-year survival rates.

Inclusion	Exclusion
Best PaO_2/FiO_2 < 300 mm Hg	Pneumonia
Pulmonary edema	Severe mechanical trauma
Bilateral infiltrates	Contusion more than one lobe
Chest radiograph	Gross gastric acid aspiration
Transplant team evaluation (poor lung deflation/inflation)	
Blood transfusion (>10 units)	
Donation after cardiac death	
(PaO_2/FiO_2) ratio—partial pressure of arterial oxygen/fraction of inspired oxygen	

Table 4. Inclusion and exclusion criteria for the HELP trial [29].

The end of the trial compared data from 84 recipients regarding their 30-day post-transplant mortality as the primary endpoint between standard donor lungs (42 cases) and extended criteria donor lungs (42 cases) after EVLP reconditioning (Using Toronto protocol and XPS™ device) [14]. The secondary endpoints included PGD, days before extubation, need for extracorporeal membrane oxygenation (ECMO) after transplant, ICU stay, and 1-year survival [29].

The Gothenburg group published a study where 11 EVLPs were done over the course of 18 months period. Eight double and three single post-EVLP transplants were done. Despite the reported 100% survival of the EVLP cohort, ICU stay and ventilation time were longer in perfused lungs compared to that in controls [45].

2.7. University of Alberta experience with EVLP

The University of Alberta Hospital Transplant Program is the most geographically isolated lung transplantation program in the world. Due to this geographical isolation and the large catchment area served to Canadians, compounded by the shortage of suitable donor lungs, we began experimenting with EVLP on a large porcine model in 2014. Our laboratory effort thus

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far has primarily focused on one of the most prevailing questions in literature regarding EVLP: which perfusate, acellular or cellular-based, is more optimal for perfusing the lungs and how can we overcome the current limitation we observe clinically to extend EVLP from merely 4–6 h to >12 h safely?

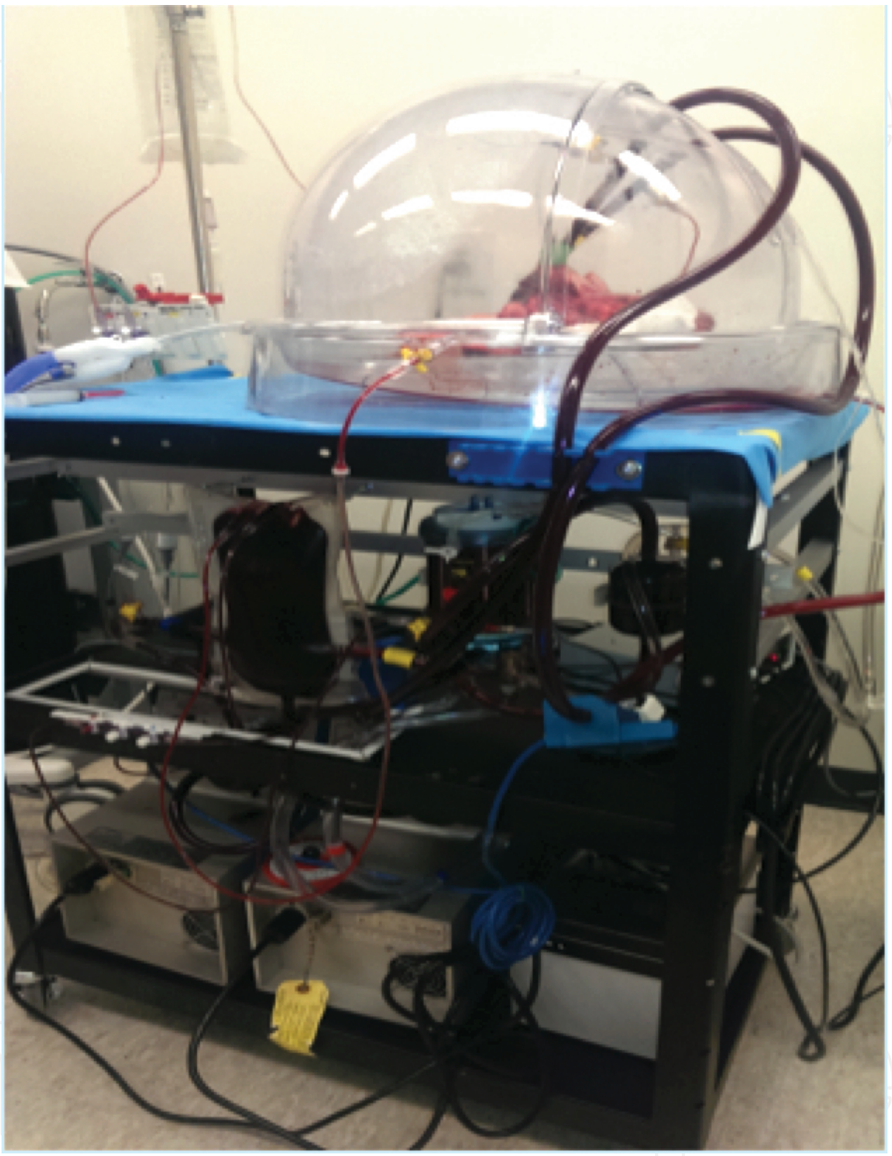

Figure 3. The fully automated and mobile EVLP circuit at the U of A.

We began with constructing a circuit that should help us relieve our main issue here at the University of Alberta—geographical isolation. As seen in **Figure 3**, our circuit contains all the universal components that are present in the commercially available circuits discussed earlier and illustrated in **Figure 1**: centrifugal pumps, a reservoir, tubing, deoxygenator/heat exchanger, a leukocyte filter, pressure/flow probes, and an ICU-type ventilator. However, our's is the only laboratory in the world that currently uses a circuit that is fully automated and does not require constant monitoring and/or manual manipulation throughout the perfusion.

Unlike the EVLP circuit utilized at the University of Alberta, the Toronto group using the XPS™ (XVIVO Perfusion AB) and the OCS™ Lung (TransMedics) are not fully automated. Our circuit is capable of controlling and manipulating the flow, pulmonary arterial (PA) and left atrial (LA) pressures, in real time without the need for manual alterations. Our software-driven microcontroller (**Figure 4**) receives PA/LA pressures and flow in real time, while adjusting the centrifugal pumps' RPMs accordingly to maintain desired constant PA flow/pressure control (user-selectable) and constant LA pressure. This is unlike that in the OCS™ Lung (TransMedics), where the desired flow would need to be manually changed by an attendee, or that in the XPS™ (XVIVO Perfusion AB), where the LA pressures are manually altered by the use of gravity (adjusting the height of the reservoir).

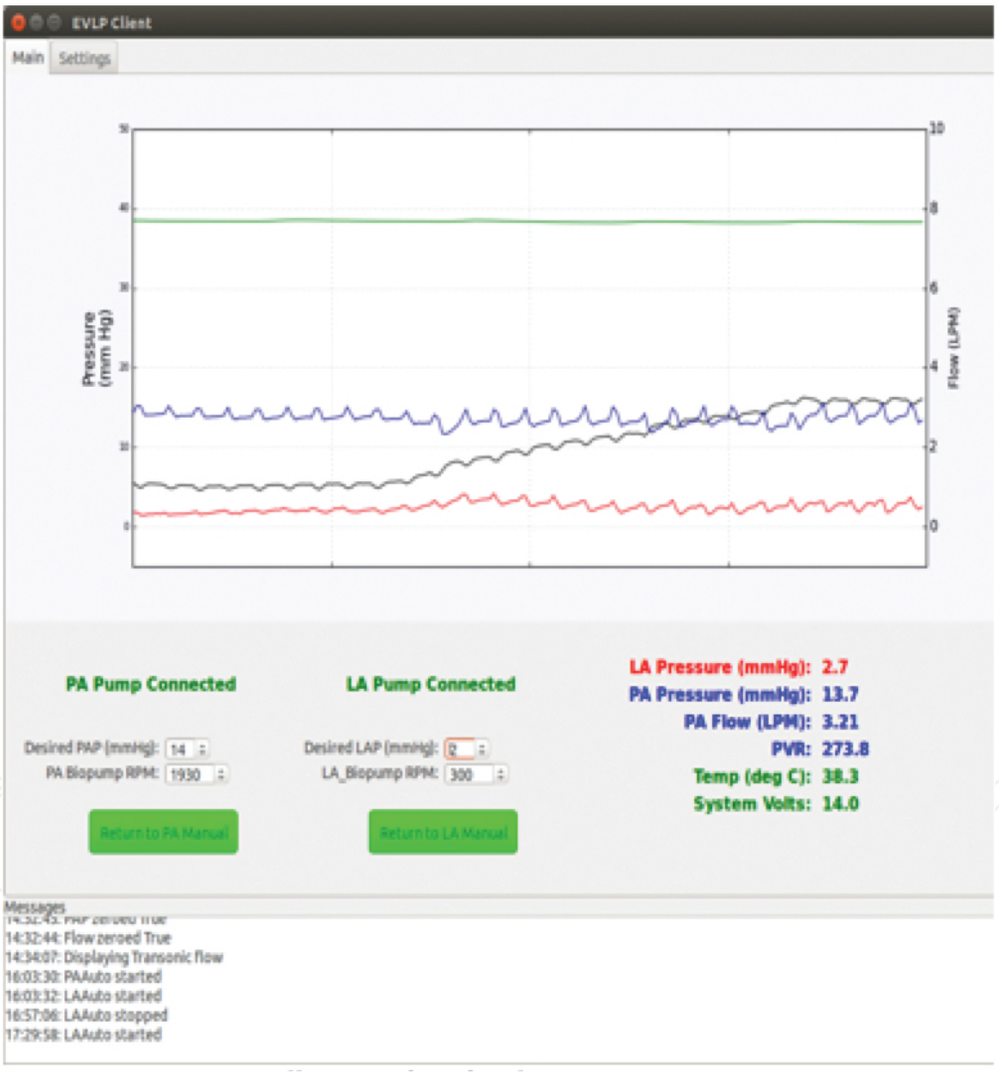

Figure 4. Microcontroller interface for the EVLP circuit parameters.

The current design of our circuit provides us with the freedom of portability, with full automation, to decrease the amount of cold ischemia the lungs experience when performing our porcine experiments. That being said, so far our lab has been capable to demonstrate a

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reproducible technique to successfully perfuse these large porcine lungs up to 12 h. With preliminary unpublished data demonstrating that an acellular based perfusate results in 50% more edema formation after 12 h of perfusion, compared to perfusing with either cellular based perfusate – whole blood or packed red blood cells (pRBCs) ($p < 0.01$). Here, edema formation corresponds with the deteriorating lung vasculature and integrity. We believe that despite the lungs showing stable physiological parameters during EVLP, especially with acceptable lung oxygenation (*P*/*F* ratios) of >300 mm Hg, lung oxygenation is not a sensitive parameter of lung health, even though it has been a widely accepted modality for evaluating lung integrity. Our data confirms what others have shown, that the focus when assessing lung integrity/health after EVLP should be with the trends of compliance over the duration of EVLP than oxygenation of the perfusate (*P*/*F* ratios) [27, 47–49]. Moreover, we believe that the blunting we observe in lung vasculature tone throughout the duration of the perfusion (with serial hypoxic challenges) can be another more sensitive physiological index of lung health. The decrease in magnitude in hypoxic pulmonary vasoconstriction (HPV) response likely correlates with the diminishing lung quality during EVLP, as supported by an ongoing cytokine profile that accumulates over time.

3. Conclusion

Lung transplantation has shown over the years to be a life-saving therapy for patients that are suffering from end-stage lung disease. However, despite the improvements in techniques, lung donor grafts have the lowest graft acceptance rate of any solid organ [50]. With only 15–25% of lungs from multiorgan brain death donors (BDD) currently deemed suitable for clinical transplantation, the rest acquire too much injury during brain death, ICU-related complications, or the onset of a prolonged cold ischemic time, rendering the donor lungs unusable. Therefore, as observed at the University of Alberta, the mortality rate on the waiting list continues to grow as clinicians must remain conservative in their donor selection to avoid post-transplantation primary graft dysfunction (PGD). The advent of normothermic ex-vivo lung perfusion (EVLP), as a novel donor preservation and reconditioning technique, has demonstrated over the years results that are positive if not different between lungs deemed unsuitable (marginal/extended) and standard (unperfused) criteria lungs, after lung transplantation [12, 41].

Normothermic ex-vivo lung perfusion (EVLP) has the capability, as a platform, for real-time functional assessment, evaluation, and reconditioning through administration of targeted therapies, prior to lung transplantation—a capability that clinicians were unable to perform, prior to the establishment of this platform, in 2001. Moreover, EVLP has permitted us to re-explore other donor pools: marginal lungs, extended criteria lungs, and cardiocirculatory death (DCD) lungs. As more research goes into developing the technology and improving the current evaluative/assessment techniques, simplifying EVLP will help more centers around the world to utilize its beneficial attributes and save lives: by expanding the currently limited donor pool. Our transplant program at the University of Alberta serves as a massive catchment area for the majority of thoracic transplantation, spanning 6 million km^2 for more than 7 million

Canadians. Being the most geographically isolated transplantation program in the world, our continuous research to further develop our program one of a kind fully automated circuit and to make it truly portable is imperative. We can save 24 human lives per year, if EVLP is used just twice a month to recondition lungs that otherwise would be discarded because they incurred too much damage or came from an unusable donor pool.

There is still much to investigate with EVLP and to refine. As we continue to seek out EVLP techniques that will allow us to safely extend the limited clinical perfusion of human lungs from merely 4–6 h to >12 h, it will open up more avenues for therapeutic interventions such as cell and gene therapies. Normothermic ex-vivo lung perfusion is the future, and it will help usher in a new era in medicine and lung transplantation, sooner than we think.

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Abbreviations

1. LTx – Lung Transplantation
2. ISHLT – International Society for Heart and Lung Transplantation
3. DCD – Cardiocirculatory Death Donor
4. BDD – Brain-Dead Donor
5. COPD – Chronic Obstructive Pulmonary Disease
6. A1ATD – α_1-antitrypsin deficiency
7. ILD – Interstitial Lung Disease
8. IPF – Idiopathic Pulmonary Fibrosis
9. CF – Cystic Fibrosis
10. PaO_2 – Partial Pressure of Arterial Oxygen

11. $PaCO_2$ – Partial Pressure of Arterial Carbon Dioxide
12. CORR – Canadian Organ Replacement Registry
13. CSP – Cold Static Preservation
14. LA – Left Atrial/Atrium
15. PA – Pulmonary Arterial
16. PAP – Pulmonary Arterial Pressure
17. LAP – Left Arterial Pressure
18. ICU – Intensive Care Unit
19. ECF – Extracellular Fluid
20. PEEP – Positive End-Expiratory Pressure
21. RR – Respiratory Rate
22. FiO_2 – Inspired Fraction of Oxygen
23. pRBCs – Packed Red Blood Cells
24. PVR – Pulmonary Vascular Resistance
25. C_{dyn} – Dynamic Compliance
26. PIP – Peak Inspiratory Pressure
27. P/F or PaO_2/FiO_2 – Partial Pressure of Oxygen/Fraction of Inspired Oxygen
28. ECMO – Extracorporeal Membrane
29. HPV – Hypoxic Pulmonary Vasoconstriction

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