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MINIREVIEWS

# Animal models of *ex vivo* lung perfusion as a platform for transplantation research

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## Abstract

*Ex vivo* lung perfusion (EVLP) is a powerful experimental model for isolated lung research. EVLP allows for the lungs to be manipulated and characterized in an external environment so that the effect of specific ventilation/perfusion variables can be studied independent of other confounding physiologic contributions. At the same time, EVLP allows for normal organ level function and real-time monitoring of pulmonary physiology and mechanics. As a result, this technique provides unique advantages over *in vivo* and *in vitro* models. Small and large animal models of EVLP have been developed and each of these models has their strengths and weaknesses. In this manuscript, we provide insight into the relative strengths of each model and describe how the development of advanced EVLP protocols is leading to a novel experimental platform that can be used to answer critical questions in pulmonary physiology and transplant medicine.



**Key words:** *Ex vivo* lung perfusion; Transplantation; Rat; Porcine; Small animal; Large animal; Model; *Ex vivo* lung perfusion

**Core tip:** *Ex vivo* lung perfusion allows for lungs to be assessed for their physiologic and functional parameters prior to transplant. As a tool for experimental research, the technology is an extremely powerful tool that enables isolated organ modification and evaluation. Utilizing small and large animal models have complementary approaches to addressing transplant related questions.

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## INTRODUCTION

### Overview of lung transplantation donor organ shortage

Lung transplants have become a viable option for patients with end stage lung disease. Unfortunately, only about 15% of donor lungs are deemed appropriate for transplant[1], and estimates show that about 50% of patients die while waiting for a lung transplant[2]. Addition-

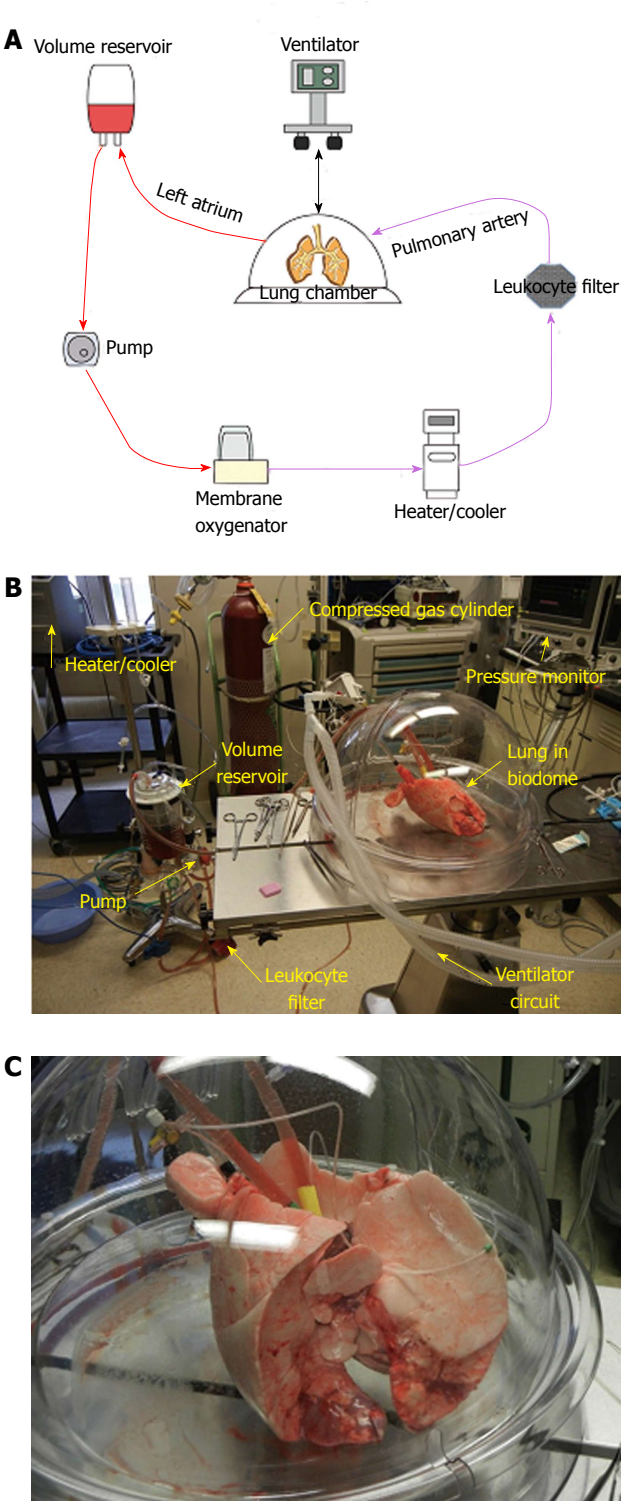


**Figure 1 This diagram depicts a schematic of a large animal (porcine) *ex vivo* lung perfusion circuit (A), the portable stand for the perfusion pump (B), A close up picture of a porcine lung on an *ex vivo* lung perfusion circuit (C).** The ventilator is used to expand the lungs with lung protective ventilation strategies. The volume reservoir contains the perfusates (either sanguineous or acelluar). Centrifugal pumps have the advantage of being able to have the afterload varied and have the circuit clamped easily, which is a challenge with roller pumps. The heater/cooler allows for exact temperature titration. The reservoir, centrifugal pump, membrane oxygenator, and leukocyte filter are all contained on this apparatus. The biodome which houses the large animal lung is visualized with the ventilator circuit attached to the endotracheal tube which directly cannulates the trachea. The inflow and outflow cannulas are at the superior aspect of the lung and the endotracheal tube on image top left.

ally, the average patient who receives a lung transplant waits 412 d for a suitable lung[3]. Because so many lungs do not meet transplantation requirements, the quality of available lungs must be increased in order to increase the amount of lungs available for transplant.

### *Ex vivo* lung perfusion

*Ex vivo* lung perfusion (EVLP) (Figure 1) has the potential to increase the lung donor pool and allows for precise control of important variables including perfusate composition, temperature, tidal volume, positive end expiratory pressure (PEEP), fraction of inspired oxygen, and arterial pressure. EVLP can improve donor lungs that were originally thought to be in too poor a condition to be transplanted and can also be used to determine a lung's condition for donation[4,5]. EVLP also allows for the assessment of donor lungs without having to transplant them to another person.

### Current clinical state of EVLP

Steen *et al*[4] first published their paper on the transplant of a lung that was perfused *ex vivo* in 2007. In 2011, Both Cypel *et al*[6] and Lindstedt *et al*[7] reported that initially rejected lungs that were perfused *ex vivo* performed similarly to lungs that were initially selected for transplant. In 2012, both Aigner *et al*[8] and Zych *et al*[9] reported that EVLP has the potential to improve the quality of donor lungs that otherwise would not be selected for transplant. In 2013, Wallinder *et al*[10] reported the EVLP is a safe method and allows lungs that would have been rejected to be used in transplants. The potential impact of EVLP to expand the available organ donor pool is profound. If the lung donor conversion rate is able to be increased from 17% to 30%, that small incremental increase in donor conversion would ostensibly double the number of transplants able to be performed worldwide annually. Evaluating the mechanisms of lung injury and progression would enable targeted therapies to intervene on these critical set points. EVLP provides an isolated platform where these mechanical traumas can be isolated and evaluated in a mechanistic fashion. Through a combination of lung protective ventilation, reducing airway edema, and targeted therapies we would anticipate that the increase conversion rate would be able to be met.

## EVLP AS AN EXPERIMENTAL PLATFORM

### Evaluation of organ function

While performing EVLP, multiple factors can be assessed in real-time to determine the viability of the lung. These include pulmonary arterial flow, pulmonary arterial pressure and pulmonary resistance, as well as dissolved oxygen concentration in the perfusate before and after passing through the pulmonary circulation. This change in dissolved oxygen corresponds to the oxygen production by the lung. The wet-to-dry ratio of a lung can also be assessed, giving an accurate depiction of how edematous the lung has become.

**Table 1 Dependent and independent variables with *ex vivo* lung perfusion**

| Dependent variables (*i.e.*, what can be measured with *ex vivo* lung perfusion) | Independent variables (*i.e.*, what can be varied in an *ex vivo* lung perfusion) |
|---|---|
| Tracheal pressure | Tracheal pressure |
| End expiratory pressure | End expiratory pressure |
| End inspiratory pressure | End inspiratory pressure |
| Tidal volume | Tidal volume |
| Compliance | Respiratory rate |
| Respiratory rate | Pulmonary artery flow rate |
| Pulmonary artery flow rate | Pulmonary artery pressure |
| Pulmonary artery pressure | Left atrial outflow pressure |
| Left atrial outflow pressure | Perfusate |
| Pulmonary vascular resistance | Ischemic time |
| Lung weight | Temperature of perfusate |
| Wet to dry ratio | Temperature of organ |
| Pre-organ $pO_2$ | Inspired gas concentration and components |
| Post-organ $pO_2$ | |
| Oxygen production | |
| Perfusate pH | |
| Perfusate $pCO_2$ | |
| Perfusate for molecular analysis | |
| Tissue for mRNA, protein, or histologic analysis | |

### *Model of acute lung injury development*

EVLP can also be used as a model of acute lung injury (ALI) development and ventilation induced lung injury (VILI). Currently, there has been only one clinical trial that has resulted in a significant decrease in patient mortality related to ALI and VILI[11]. Although multiple ventilation protocols have been researched, there is little information on how drug treatment might affect lung viability at various tidal volumes and positive-end expiratory pressures. Multiple models of ALI that are typically used for *in vivo* studies (*i.e.*, saline lavage/surfactant dysfunction, acid induced lung injury and LPS induced lung injury) can be easily and quickly implemented using EVLP (Table 1). In addition, precise regulation of tidal volume, PEEP and other ventilator parameters during EVLP allow for modeling the mechanically induced injury that occurs during VILI. Unlike *in vivo* models, EVLP models of ALI/VILI allow for the evaluation of how specific ventilator settings influence lung injury progression without the confounding effects of changes in other physiologic parameters (Table 1). These models can be assessed by measuring pro-inflammatory cytokine secretion and histological characterization of lung tissue. The lungs can also be treated with specific drugs delivered through the perfusate or trans-trachealy to determine if any drug combination results in a minimization of lung damage during ventilation.

### *Pathway to evaluate efficacy of experimental treatments*

**Perfusate:** The selected perfusate should have osmotic and oncotic pressures similar to blood and must also provide an energy source for the cells. Clinically, the perfusate is used to evenly cool the organ tissue and to remove blood, thereby preventing cell injury. It is important to note that the perfusate and all of its components have direct contact with the perfused organs and therefore are an extremely important variable in determining the outcome of EVLP.

Steen *et al*[4,5,12] developed a new perfusion solution and proved EVLP to be a viable method to improve and preserve donor lungs, and it continues to be the most popular perfusion solution used. The Pego-Fernandes group reported that their solution, low potassium dextran-glucose (LPDnac) was comparable to Perfadex (Vitrolife, Goteborg, Germany) but found saline to be inadequate[13,14]. Menezes *et al*[15] also compared Perfadex to Celsior and found lungs perfused with either exhibited similar gas exchange and histopathological findings.

**Gene or molecule delivery:** Multiple groups have shown that gene therapy coupled with EVLP can repair injured lungs before transplantation. Cypel *et al*[16] demonstrated that the delivery of adenoviral vector encoding human interleukin-10 (AdhIL-10) to human lungs showed improvement in arterial oxygen pressure and vascular resistance, concluding that delivery of AdhIL-10 can improve lung function. Yeung *et al*[17] later showed that ex vivo delivery of adenoviral genes to lungs is superior to *in vivo* delivery due to the decreased vector-associated inflammation and improved post-transplant lung function. Emaminia *et al*[18] also showed that delivery of adenosine A2a in the perfusate reduced the inflammatory response in acutely injured pig lungs.

**Optimize the nutrients needed to sustain the lungs:** Using an acellular perfusate can avoid mechanical damage to the lung over long lung perfusions[1,19] and is more widely used over cellular solutions. Pro-inflammatory cytokines can accumulate in the perfusate over time so the perfusate should be replaced periodically to avoid increased inflammation.

**Trans tracheal and aerosolized agent delivery:** Drugs cannot only be delivered through the perfusate, but also as an aerosolized drug trans-tracheally. Pulmonary delivery of aerosolized drugs has been modeled using an EVLP system by many groups. Dong *et al*[20] showed that administration of aerosolized chitosan-coated poly (lactide-co-glycolide) based nanoplexes containing antisense 2'-O-Methyl RNA (OMR) resulted in a significantly higher uptake of OMR in the respiratory epithelium compared to administration of OMR alone using an EVLP model. Beck-Broichsitter *et al*[21] also used an EVLP model to show that delivery of biodegradable nanoparticles may be a viable approach for drug delivery.

## LARGE ANIMAL MODEL OF EVLP

### *Advantages*

Porcine EVLP has a direct translation to the human clinic (Figure 1). In general, the advantages of this large animal model of EVLP can be broadly grouped into the following 4 categories.

**Table 2 Physiologic *ex vivo* lung perfusion parameters**

| | Rat | Pig |
|---|---|---|
| Tidal volume | 4-10 mL/kg | 6-8 mL/kg |
| Positive end expiratory pressure | 2-6 cm $H_2O$ | 5 cm $H_2O$ |
| Flow rate | 5-30 mL/min (estimated cardiac output: 25-50 mL/min per 100 g) | 40% cardiac output/min (estimated cardiac output: 100 mL/min per kilogram) |
| Pulmonary artery pressure | 13.6 cm $H_2O$ | 10-15 mmHg |
| Perfusate albumin concentration | 2%-4% | 5%-7% |

**Size appropriate:** The swine model offers very appropriate size comparisons to humans. Because of this, comparable tidal volumes, PEEP, and perfusion times can be used for the EVLP. As a result, information obtained in this large animal model of can be rapidly and directly transferred to settings for clinical trials. This direct transfer of information to clinical trials is typically not possible when using smaller animal models. There are variations in physiologic parameters based on animal model sized (Table 2).

**Similar immune system and biology:** The pig has a greater similarity to humans in gene sequence and physiology compared to mice and rats which makes it a superior model[22]. This results in a simpler comparison to humans and therefore a more direct path to clinical relevance.

**Allows for opportunity to perfect scale up to human size and clinic setting:** Because of the pig's larger size, the opportunity exists to experiment with the exact same equipment that would be used in clinical trials[12]. The amounts of perfusate needed as well as ventilator settings are more closely related to clinical settings compared to smaller animal models. The amount of time a pig lung can be perfused is comparable to humans.

**Accepted transplant model:** All animals should receive care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research, the Guide for the Care and Use of Laboratory Animals.

Generally, protective settings for mechanical ventilation are used during EVLP. Typically, pigs are sedated with 40 mg/mL ketamine and anesthetized with 8 mg/kg pentobarbital. A period (about 60 min) of warm ischemia is usually implemented before starting EVLP to mimic a donor's lung. Volume-controlled ventilators are used and tidal volumes of about 4-6 mL/kg, a PEEP of about 5 cm$H_2O$, and a respiratory rate of about 17 breaths per minute are used. The fraction of inspired oxygen ($FiO_2$) usually ranges from 100% to 40% (Table 2).

### *Limitations*

While pigs offer one of the best parallels to humans, their cost to purchase and to care for is much higher than small animals. The amount of perfusate used on an isolated pig lung is much higher than with small animal, making each experiment much more expensive. This also makes having a high amount of replicates in an experiment very difficult. Because of the large size of an isolated pig lung, the perfusion circuit itself is custom built and requires the same equipment that would be used for a clinical perfusion.

## SMALL ANIMAL MODEL OF EVLP

### *Overall advantages*

Small animal models that have been employed in EVLP include rat, mouse, guinea pig and rabbit (Figure 2). These systems offer several distinct advantages compared to their larger counterparts. Overall, their cost is much lower. This includes initial startup cost, such as in surgical and perfusion equipment as well as the animals themselves. Because of the smaller cost, one can complete more perfusion experiments with less money and in less time than if the same study were completed using a porcine or canine model system. Additionally, one can capitalize on the higher sample size in order to aid in achieving statistical significance. Most small animal experiments use 5-8 animals per group and up to 50 animals in total per study[23-26]. These numbers are simply not feasible in larger systems and helps increase confidence in experimental data.

An inherent advantage of EVLP is the isolation of the lungs from the rest of the body. This has helped elucidate differences in the immune response of resident lung cells compared to the systemic immune response during ischemia/reperfusion (I/R) in a mouse model[26,27]. More generally, this characteristic of EVLP can be exploited to more easily vary experimental components and limit confounding factors. One avenue of research that has been pursued extensively is in the optimization lung perfusate solutions. This is an area of critical importance in the development and refining of EVLP procedures for clinical use and is a current topic of controversy.

Basic properties, such as the optimal electrolyte composition of the perfusate itself are not agreed upon. Current data are unclear as to which currently available solutions perform best[15]. Perfadex, a solution developed specifically for lung preservation, may not offer better preservation than Celsior, a heart preservation solution[15,25]. One group in Brazil compared Perfadex to a locally produced generic solution, LPDnac and found it to preserve lungs just as well[13]. The potential benefits of varying perfusate temperature and introducing vasodilators has also been studied[28]. Despite the disagreements over perfusate composition, small animal EVLP systems provide an excellent platform for further perfusate development and testing.

### *Rat/rabbit/guinea pig models*

Of the different small animal systems used for EVLP experiments, each offers their own advantages and draw-

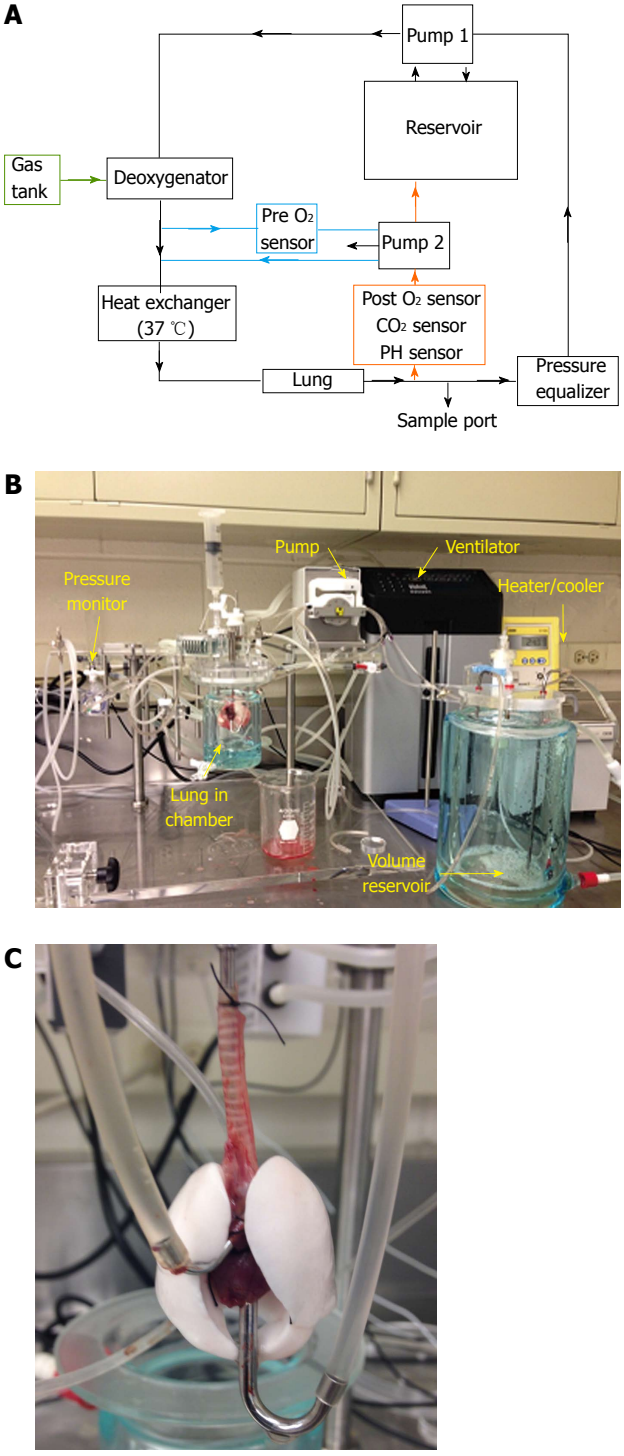


**Figure 2 This diagram depicts a schematic of a small animal (rat) *ex vivo* lung perfusion circuit (A), the small animal perfusion circuit (B); a close-up of a rat lung undergoing *ex vivo* perfusion (C).** Many of the same characteristics that are in the large animal circuit are present. This particular circuit has the ability for fine measurements of pressure, flow, and weight. The image back right shows the thermoregulator and the ventilator. The perfusates reservoir is in the image front right. The small animal circuit is analogous to the large animal circuit. However, due to the relative scale of the organ to the circuit, the perfusate volume needed for a complete perfusion is less. In addition, the ability to perform positive as well as negative pressure ventilation is possible. This varied ventilation an mimic both the mechanical breathing as well as natural intrathoracic breathing. The tracheal cannulation is top-center. The inflow cannula going into the pulmonary artery is from top-left and the outflow cannula going across the left atrium through the left ventricular apex is on screen right.

backs. Rat, guinea pig and rabbit models have a larger thoracic cavity than mice, making surgical procedures easier. Owing to their larger size, initial cannulation (Figure 2) is relatively simple and can be done with or without the aid of a surgical microscope[29,30]. Moreover, a rat left lung transplantation (LTX) technique has been developed and used in multiple studies[29-32].

Recent improvements have increased the success rate of this LTX technique to greater than 95%[29]. Inokawa *et al*[32] used this procedure to create a specific model of transplantation as it relates to EVLP and designed it to closely mimic clinical conditions. Rat donor lungs are explanted, stored on ice for 1 h, perfused, stored on ice again for 2.5 h and finally transplanted. This model has been used to demonstrate the therapeutic potential of low concentration carbon monoxide ventilation during perfusion[23]. Although less common, rabbit[33] and guinea pig[34] models have been used to study the onset of ischemia-reperfusion injury.

One challenge, however, with the use of these three animals as model systems is the relative scarcity of species-specific commercially available antibodies and molecular reagents. Because of this, protein studies are limited in these systems, though Fehrenbach *et al*[35] demonstrated in a rat model of EVLP that the concentration surfactant protein A (SP-A) increased following I/R using a polyclonal antibody against SP-A.

### *Murine models*

Murine models of EVLP offer considerable advantages over rats because of the greater number of species-specific antibodies and gene probes available for experiments. This has facilitated development of a much greater body of scientific literature with regard to these types of studies. For example, the murine immune response to EVLP has been studied for over 15 years[36]. More recently, Barrenschee *et al*[37] used toll like receptor (TLR) agonists to mimic the response during infection and characterized levels of key cytokines/chemokines such as interleukin (IL)-1β, IL-6 and TNF-α. Siegl and Ulrig studied the inflammatory response of mice in high and low ventilation scenarios, including quantification of the phosphorylation of key enzymes involved in the inflammatory response[38].

An additional advantage of the mouse model is the availability of knock out (KO) lines. Deficient genes could be related to the inflammatory response, including TLR-4 deficient[39] and TNF-α deficient mice[27] or could interfere with other areas of lung function[40]. Maxey *et al*[27] used the TNF-α deficient mice in EVLP to demonstrate the importance of TNF-α in initiating the inflammatory response following I/R.

Recently, a model of mouse lung transplantation has been developed for further study of obliterative bronchiolitis. The procedure is very similar to the rat model of LPX from a technical standpoint, but to our knowledge, has not yet been used as an EVLP model[41,42]. This may be due to increased technical difficulties during mice op-

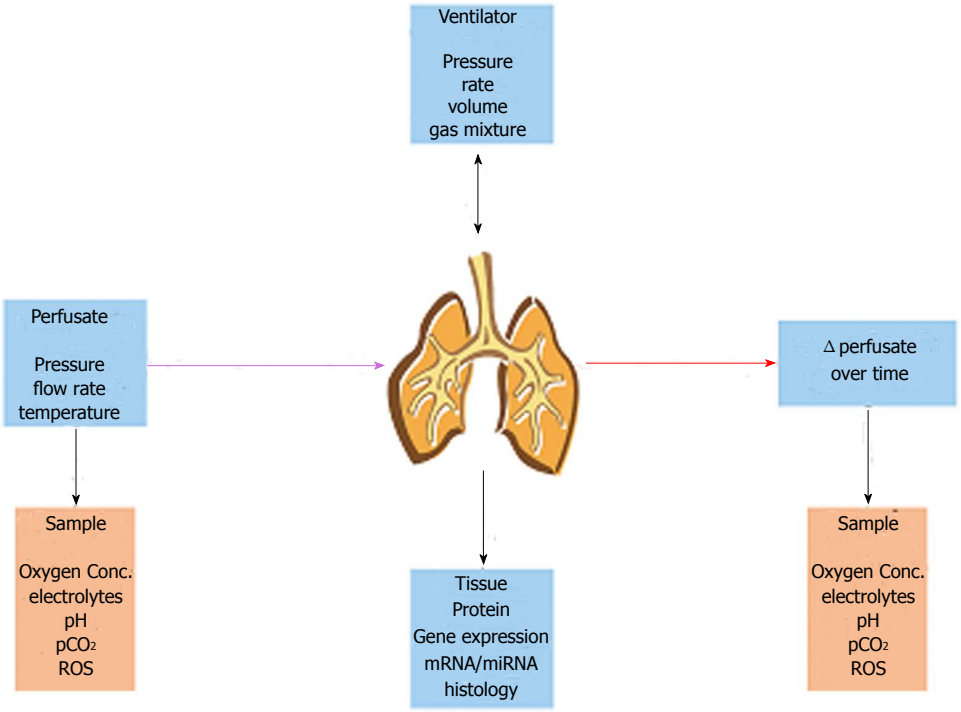


**Figure 3 Diagram of what is able to be able to be measured and varied with the *ex vivo* perfusion circuit.** This figure directly correlates with Table 1.

erations because of their smaller size. However, it is likely that once some initial sets of experiments combine this mouse LTX technique with EVLP, the scope of possibilities of what EVLP platforms can study will be widened.

The greatest challenge in solely relying on the murine model of EVLP is the technical difficulties involved during surgery. Mice have a smaller thoracic cavity and smaller organs than rabbits, rats or guinea pigs. Often, a surgical microscope is required to identify and isolate key structures during the heart-lung block explant[26,27]. Another drawback of this mouse model is the increased difficulty of training personnel on more technical mouse surgery procedures, which can create bottlenecks in experimental plans and ultimately slow down data acquisition. For this reason, it is likely that future studies will still utilize all small animal models, with mouse models of transplant used when necessary (for protein and gene studies) and lung mechanics studies primarily completed using a rat model.

### *Limitations*

Owing to their small size and cost effectiveness, small animal models of EVLP are extremely convenient. When considering their use, however, several key differences need to be taken into account. Mice and rats have much shorter perfusion times than human or pig lungs. One rat model of lung transplant includes 15 min of perfusion time[32]. Other studies perfuse for 50 min[15,25,35] or 60 min[15]. One needs to keep in mind the mismatch in times scales, as murine lungs after 15 min of perfusion/ventilation are closer in damage to pig/human lungs perfused for a much longer time (4-24 h depending on the lung injury model being studied).

Yet another difference is that rodent lungs are significantly more susceptible to atelectasis. As a result, during the "ischemic" periods of a mouse model of EVLP, the lungs are still ventilated, albeit at a lower rate and in a hypoxic environment[26,27] This is unavoidable though, since without ventilation the lungs would not remain viable long enough to complete the study. Previous investigators have demonstrated that atelectasis and the subsequent reopening of fluid occluded regions can damage the lung epithelium[43,44] and exacerbate inflammation[45,46]. Therefore, it is extremely important to prevent lung damage, atelectasis and pulmonary edema because, unlike human and large animal models, a bronchoscopy cannot be performed to clear fluid from the lungs. Assuming all of these major differences are taken into account, small animal models are excellent starting points for the development of EVLP for clinical use and for the testing of therapeutics against I/R injury.

## TECHNICAL CONSIDERATIONS

### *Perfusate*

Steen solution is the most popular solution used to date and acellular solutions are much more common than cellular solutions. Studies indicate a hyper-oncotic, albumin-based solution is best. The acelluar solutions have the potential benefit of not adding an exogenous antigen source and the red cells are not lysed through the mechanics of the perfusion. The acellular solutions have the potential benefit of helping to support metabolic demands. In the lung this is not as critical as in other organs since the lung itself provides the oxygen. The perfusate needs to be buffered and provide glucose and electrolytes.

### *Ventilator settings*

Ventilator settings should be protective during EVLP for best results. In the large animal model this means a tidal volume of 4-6 cc/kg. From time to time, 10 cc/kg is used. In the rat model, a protective tidal volume is 4 cc/kg with 10 cc/kg being potentially deleterious. Depending on the hypothesis being tested and the animal model used, multiple variables can be changed on the ventilator including tidal volume, PEEP, breaths per minute, and fraction of inspired oxygen (Figure 3).

### *Temperature*

Perfusate temperature is usually either increased temporally or based on current temperature. The perfusate temperature is usually increased until 37 ℃ is achieved. An in-line thermoregulator or perfusion heater/cooler is used to titrate the temperature. A cold or warm ischemic period may precede the actual perfusion depending on the hypothesis being tested.

### *Duration of perfusions*

Small animal perfusions usually run between 30 min-3 h. Pig EVLP have been run for up to 14 h. The times vary greatly depending on the animal model used and the hypothesis being tested.

### *Pulmonary artery flow rates and pressures*

Perfusate flow rates are usually set to achieve a specific pulmonary pressure or a specific pulmonary resistance. A typical experimental set-up is to have the perfusion flow rate increase incrementally over the duration of the perfusion (15-30 min time period). Once full flow (40% cardiac output) is achieved, the pulmonary artery and left atrial pressures are measured. The pulmonary vascular resistance is calculated as a function of the pressures and flow rates. In a well-functioning organ, pulmonary vascular resistance decreases over time. In a poorly functioning organ, the resistance increases. Increased resistances often mirror poor oxygenation.

## CONCLUSION

EVLP has great potential to increase the lung donor pool by providing a platform for improving and evaluating lungs initially thought to be inadequate. Multiple groups across the globe are developing promising models to achieve a greater donor pool. EVLP is also being used as a model for acute lung injury to better understand how the complex mechanical forces applied to the lungs influence injury development and inflammation and to develop strategies that limit the amount of tissue damage/inflammation. EVLP is also being explored as an opportunity for administering therapeutic agents. This idea is unique in that it bypasses the patient's immune system and allows for a higher acceptance rate compared to drugs administered *in vivo*.

Both small and large animal models are advancing our knowledge on EVLP and each has their own specific advantages and disadvantages. While small animal models do not usually run for more than 1-2 h, they are economical and allow for many experiments in a short period of time. Swine models are very expensive but allow for the closest model to human lungs available and use the same equipment that would be used clinically. Since nearly 50% of patients die while waiting for a lung transplant, it is crucial to expand the donor pool. EVLP holds the most promise towards achieving this goal.

The ability to keep organs alive and perfused for extended periods of time will enable the "culture" of organs. This prolonged, perfusion will be the basis for immunomodulation and change of the endothelium through nanoparticle, gene bases, or antibody based delivery of therapeutic agents. This will be the dawning of customized medicine to tailor the transplanted organ to the individual recipient and their biology.

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