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

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**Abstract**

*Ex vivo* lung perfusion (EVLPL) is a powerful experimental model for isolated lung research. EVLPL 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, EVLPL 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 EVLPL have been developed and each of these models has its strengths and weaknesses. In this manuscript, we provide insight into the relative strengths of each model and describe how the development of advanced EVLPL protocols is leading to a novel experimental platform that can be used to answer critical questions in pulmonary physiology and transplant medicine.

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**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.


**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-
ally, the average patient who receives a lung transplant waits 412 d for a suitable lung\textsuperscript{4}. 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\textsuperscript{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*\textsuperscript{4} first published their paper on the transplant of a lung that was perfused *ex vivo* in 2007. In 2011, Both Cypel *et al*\textsuperscript{6} and Lindstedt *et al*\textsuperscript{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*\textsuperscript{8} and Zych *et al*\textsuperscript{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*\textsuperscript{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

<table>
<thead>
<tr>
<th>Dependent variables (i.e., what can be measured with ex vivo lung perfusion)</th>
<th>Independent variables (i.e., what can be varied in an ex vivo lung perfusion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheal pressure</td>
<td>Tracheal pressure</td>
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<tr>
<td>End expiratory pressure</td>
<td>End expiratory pressure</td>
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<tr>
<td>End inspiratory pressure</td>
<td>End inspiratory pressure</td>
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<tr>
<td>Tidal volume</td>
<td>Tidal volume</td>
</tr>
<tr>
<td>Compliance</td>
<td>Respiratory rate</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>Pulmonary artery flow rate</td>
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<td>Pulmonary artery flow rate</td>
<td>Pulmonary artery pressure</td>
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<tr>
<td>Pulmonary artery pressure</td>
<td>Left atrial outflow pressure</td>
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<tr>
<td>Left atrial outflow pressure</td>
<td>Perfusate</td>
</tr>
<tr>
<td>Pulmonary vascular resistance</td>
<td>Ischemic time</td>
</tr>
<tr>
<td>Lung weight</td>
<td>Temperature of perfusate</td>
</tr>
<tr>
<td>Wet to dry ratio</td>
<td>Temperature of organ</td>
</tr>
<tr>
<td>Pre-organ $pO_2$</td>
<td>Inspired gas concentration and components</td>
</tr>
<tr>
<td>Post-organ $pO_2$</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 Physiologic *ex vivo* lung perfusion parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rat</th>
<th>Pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tidal volume</td>
<td>4-10 mL/kg</td>
<td>6-8 mL/kg</td>
</tr>
<tr>
<td>Positive end expiratory pressure</td>
<td>2-6 cm H2O</td>
<td>5 cm H2O</td>
</tr>
<tr>
<td>Flow rate</td>
<td>5-30 mL/min (estimated cardiac output: 25-50 mL/min per 100 g)</td>
<td>40% cardiac output/min (estimated cardiac output: 100 mL/min per kilogram)</td>
</tr>
<tr>
<td>Pulmonary artery pressure</td>
<td>13.6 cm H2O</td>
<td>10-15 mmHg</td>
</tr>
<tr>
<td>Perfusate albumin conc.</td>
<td>2%-4%</td>
<td>5%-7%</td>
</tr>
</tbody>
</table>

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. 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. 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 cmH2O, and a respiratory rate of about 17 breaths per minute are used. The fraction of inspired oxygen (FiO2) 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. 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. 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. Perfadex, a solution developed specifically for lung preservation, may not offer better preservation than Celsior, a heart preservation solution. One group in Brazil compared Perfadex to a locally produced generic solution, LPDNaC and found it to preserve lungs just as well. The potential benefits of varying perfusate temperature and introducing vasodilators has also been studied. 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-
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[29] 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[33]. 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[29-32]. 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[40] or could interfere with other areas of lung function[41]. Maxey et al[35] 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 mouse op-
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.[25] 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-osmotic, albumin-based solution is best. The acellular 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 °C 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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