



Review

Ex Vivo Lung Perfusion: A Platform for Donor Lung Assessment, Treatment and Recovery

Luke Milross ¹, Chelsea Griffiths ¹ and Andrew J. Fisher ^{1,2,*}

¹ Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne NE1 7RU, UK; luke.milross@nhs.net (L.M.); c.griffiths3@newcastle.ac.uk (C.G.)

² Institute of Transplantation, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne NE7 7DN, UK

* Correspondence: a.j.fisher@newcastle.ac.uk; Tel.: +44-(0)-191-208-7067

Abstract: Lung transplantation offers a lifesaving therapy for patients with end-stage lung disease but its availability is presently limited by low organ utilization rates with donor lungs frequently excluded due to unsuitability at assessment. When transplantation does occur, recipients are then vulnerable to primary graft dysfunction (PGD), multitudinous short-term complications, and chronic lung allograft dysfunction. The decision whether to use donor lungs is made rapidly and subjectively with limited information and means many lungs that might have been suitable are lost to the transplant pathway. Compared to static cold storage (SCS), ex vivo lung perfusion (EVLP) offers clinicians unrivalled opportunity for rigorous objective assessment of donor lungs in conditions replicating normal physiology, thus allowing for better informed decision-making in suitability assessments. EVLP additionally offers a platform for the delivery of intravascular or intrabronchial therapies to metabolically active tissue aiming to treat existing lung injuries. In the future, EVLP may be employed to provide a pre-transplant environment optimized to prevent negative outcomes such as primary graft dysfunction (PGD) or rejection post-transplant.

Keywords: ex vivo lung perfusion; lung transplantation; normothermic machine perfusion



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1. Introduction

For many people with very advanced or terminal respiratory diseases, lung transplantation is the only therapeutic intervention which may prolong survival and improve quality of life. However, a significant and expanding mismatch between supply and demand for donor lungs means prolonged suffering and significant mortality of up to 30% on waitlists [1]. At present, transplantation activity is significantly restricted with only 20% of potential donor lungs being utilised, the rest being excluded due to an assessment of unsuitability, either true or perceived, with an estimated 40% of those declined being potentially suitable in retrospective analysis [2]. An ‘ideal’ donor has traditionally been considered as being less than 55, having a PaO₂/FiO₂ ratio > 300, having no smoking history, clear chest imaging and bronchoscopy and retrieved in the context of minimal ischaemic time [3]. Many potential donors do not fit these criteria or have demonstrated lung injury. Injury is common during the peri-mortem period and includes trauma, aspiration, infection, neurogenic oedema and barotrauma. However, lungs falling outside ‘ideal’ criteria restrictions may not necessarily be contraindicated for donation and injury may be reversible or inconsequential. Such organs are considered ‘marginal’ and expanded donor selection criteria have been implemented to encourage their use where appropriate. Whilst this initiative has been associated with increased primary graft dysfunction (PGD) and higher 30-day mortality, there seems to be no effect on survival found beyond this timeframe and this has profoundly expanded the pool of potential donors [4].

At present, most retrieved lungs are transported using static cold storage (SCS), which although remains an effective preservation tool, provides limited scope for in-depth

assessment prior to implantation. Ex vivo lung perfusion (EVLP) is an organ preservation technique which provides a unique opportunity to thoroughly assess donor lungs in conditions aimed at imitating normal physiology, which may allow for the safe utilisation of more lungs for transplant, especially those considered to be 'marginal'. EVLP may be particularly useful in the setting of lung donation after circulatory death and may increase clinician confidence in utilising organs from this pool in particular by providing more detailed objective assessments [5]. Even further potential for EVLP lies in its scope as a platform for lung recovery via the administration of therapeutics to metabolically active and responsive cells, limiting or even reversing pre-existing lung injuries or those inherent to retrieval, fostering a lung environment best aligned with excellent outcome post-transplantation. This review first outlines the development of EVLP, its role as a lung assessment tool and the current evidence surrounding its use. It then focuses on its great potential as a therapeutic platform in the future.

2. The Evolution of EVLP

Organ preservation by SCS has allowed transplantation to flourish and remains the standard technique used today. Its principal objective is to reduce the metabolic demand of tissues as they undergo an inevitable period of ischaemia between retrieval and implantation using topical cooling and intravascular membrane stabilising perfusates. Despite this harm minimisation strategy, cell injury and death due to cold ischaemia still occurs. A further prominent issue is reperfusion injury as oxygen is reintroduced to deprived tissues during implantation. Lung ischaemia reperfusion injury (IRI) is a leading cause of PGD [6] and is thought to have a potential contributory role in long term graft failure. Whole organ perfusion is preservation technique which may provide a solution to this issue. The history of whole organ perfusion experimentation stretches as far back as the 1930's with Dr Carrel's perfusion of intact animal thyroid glands [7] and normothermic perfusion in the modern era was first explored by Hardesty and Griffith in 1987, who preserved heart-lung blocs in a normothermic blood perfusion circuit and transplanted 14 patients, showing some early success with 10 surviving the peri-operative period [8]. However, it was not until the early 1990s that EVLP would find momentum. At this time, Stig Steen developed a platform on which retrieved lungs could be perfused with fluid designed for optimal preservation and physiological assessment, which led to the first successful EVLP lung transplantation from a non-heart beating donor in 2001 [9]. Steen's group later gave the transplantation community a first glimpse of the potential for EVLP in re-evaluating marginal lungs with a case series of 6 successful transplantations from brain dead donors whose lungs were initially declined [10]. Steen also demonstrated the capacity of EVLP for reconditioning with evidence for reversal of oedema by using Steen solution and atelectasis through positive end-expiratory pressure, which correlated with improvements in gas exchange measurements.

3. The Structure of an EVLP Circuit

Steen's platform laid the foundation for contemporaneous EVLP circuits. Lungs are accommodated in a hard-shell reservoir in which they are ventilated via an endotracheal tube and perfused by cannulation of the pulmonary artery. In a closed circuit configuration, the left atrial cuff is also cannulated whereas in an open circuit the left atrium drains freely. Typically positive airway pressure ventilation is carried out although negative pressure ventilation is being explored [11]. The perfusion fluid consists of highly oncotic dextran-containing Steen solution or equivalent \pm supplementation with blood products [12]. Pulmonary venous output collects in the reservoir and is pumped through the circuit by a centrifugal pump, passing through a heat exchanger which maintains normothermia, then a membrane gas exchanger and leucocyte filter before its pressurised return to the pulmonary arteries (see Figure 1). Presently, there are four commercialised devices in use and a variety of perfusion protocols are used among them. Both human and animal (porcine

and murine) models have been employed experimentally. A lack of standardisation has led to difficulties in data comparison [12].

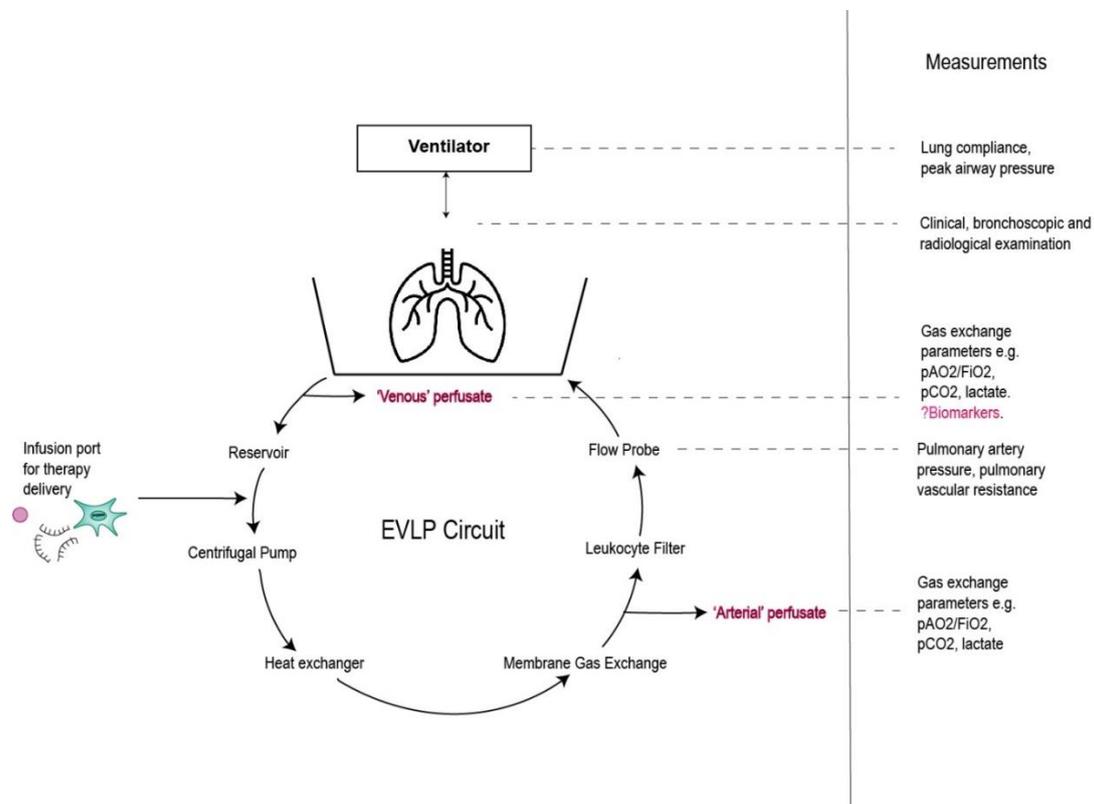


Figure 1. The structure of an EVLP circuit including points for clinical, biochemical and physiological assessment. Key: pAO₂ = partial pressure of oxygen; FiO₂ = fraction of inspired oxygen; pCO₂ = partial pressure of carbon dioxide.

4. Lung Assessment during EVLP

EVLP circuits imitate physiological conditions and provide a window during which a full suite of lung assessments are possible (Figure 1). Assessments can include basic visual and tactile inspection, radiography and bronchoscopy for diagnosis of macroscopic issues such as atelectasis, oedema and secretions. Haemodynamic measurements are taken by the flow probe and parameters obtained include pulmonary artery pressure and pulmonary vascular resistance. Lung compliance, resistance and peak airway pressures are measured by the ventilator. The capacity for gas exchange may be analysed by taking 'arterial' and 'venous' perfusate gas samples from the circuit. Beyond these lie a multitude of potential novel predictive factors, several of which have already been suggested but not yet widely utilised. For example, levels of myeloperoxidase, a product produced by active local innate immune cells, has been suggested to correlate with lung viability [13]. Endothelial activation has also been an area of interest with Hashimoto et al. (2017) noting that soluble intercellular adhesion molecule-1 (ICAM-1) and soluble vascular cell adhesion protein-1 (VCAM-1) was significantly higher in EVLP treated lungs that developed grade III PGD within 72 h [14]. Levels of pro-inflammatory cytokine interleukin-1 β (IL-1 β) within the perfusate have been shown to be predictive of in-hospital mortality and 1-year survival, thus is suggested as a potential therapeutic target [15]. Gene expression changes significantly during EVLP with an upregulation of a range of acute lung injury-related and inflammatory genes being observed [16,17]. There is great scope for further exploration of novel prognostic biomarkers and therapeutic targets related to inflammation, injury and repair differentially expressed in the EVLP lung microenvironment.

5. Clinical Outcomes Associated with EVLP Use

Establishing at a minimum non-inferior safety of EVLP compared with SCS has been the first step to promulgating its use. Several systematic reviews and meta-analyses have collated the clinical efficacy results from EVLP trials reporting short-, mid- and long-term outcomes and adverse events. One such collation of 15 studies comparing 586 recipients of EVLP treated lungs with 1985 standard protocol lung transplant recipients reported comparable short-term outcomes with no statistically significant difference in 30-day survival (RR 1.69, 0.93–2.30 with 95% CI, $p = 0.541$), which was also true at 90 days and 1 year [18]. Interestingly, this study found a reduction in grade III PGD in EVLP treated lungs compared to standard protocol (RR 1.70, 0.64–4.53 with 95% CI, $p = 0.003$). The EXPAND trial which assessed clinical outcomes of EVLP treated extended criteria lungs showed 44% had grade III PGD at 72 h but despite this 99% went on to survive at 30 days [19]. Notably, this trial had a lung utilisation rate of 87%, far surpassing the utilisation rate found in current clinical practice. Other short-term outcomes explored by the aforementioned meta-analysis included no statistically significant difference in post-operative use of extracorporeal membrane oxygenation (ECMO), time to extubation, PaO₂/FiO₂ ratio, peak pulmonary function or hospital stay although there was higher intra-operative ECMO use and longer stays in intensive care units in the EVLP group [18]. With respect to longer term outcomes, a meta-analysis collating 13 trials comparing 407 recipients of EVLP treated lungs with 1765 standard protocol lung transplant recipients reported no statistically significant difference in mid- to long-term survival (HR 1.00, 95% CI 0.79 to 1.27, $p = 0.981$). Survival at 12, 24 and 36 months was 84%, 79% and 74% in the EVLP cohort and 85%, 79% and 73%, respectively, in the standard protocol group [20]. Importantly, a retrospective cohort study of 230 EVLP treated lung recipients with 706 standard protocol recipients showed no statistically significant difference in time to chronic lung allograft dysfunction (CLAD) (70% compared with 72% at 3 years; 56% compared with 56% at 5 years; and 53% compared with 36% at 9 years; log-rank $p = 0.68$) [21]. There is also no difference in measures of long term graft function including FEV₁ and 6 min walk test, in incidence of acute rejection episodes or in quality of life [22]. CLAD represents an Achilles' heel to long term morbidity and mortality in lung transplantation and is a significant burden in both groups. It is possible that peri-operative events may establish a graft's steady march towards CLAD which EVLP-based interventions may provide an opportunity to ameliorate.

6. EVLP as a Therapeutic Platform

Steen first demonstrated that intrinsic elements of EVLP circuits can be manipulated with therapeutic value, including the use of perfusate with a high oncotic pressure to reverse or limit pulmonary oedema and positive pressure ventilation to reverse atelectasis [10]. Of the four commercialised EVLP devices available, the Organ Care System (OCS) is also portable, designed on the principle that moving the unit to the retrieval centre would limit cold ischaemic time and thus IRI [23]. The INSPIRE trial reported on outcomes from OCS-retrieved compared to SCS-retrieved standard-criteria lungs and found that incidence of grade III PGD within 72 h was less with an incidence of 17.7% in the OCS group (95% CI 11.8–25.1) and 29.7% in the standard group (95% CI 22.8–37.3, $p = 0.015$) although the mechanism underlying this difference, such as a potential reduction in IRI, was not understood and this did not equate to an early survival benefit. Other intrinsic elements of the set-up can also be manipulated to advantage. For example, most circuits apply positive airway pressure ventilation however the use of negative pressure ventilation has been associated with reductions in pro-inflammatory cytokines and lung injury, including the formation of oedema [11].

The window period provided by any EVLP circuit for assessment also offers a unique opportunity for the addition of therapeutics targeting both known and occult lung injuries. Higher doses can be employed without systemic side effects direct to target which also means and pharmacokinetic factors such as distribution to other compartments and hepatic

elimination are avoided [24]. Through the inbuilt infusion ports in EVLP circuits and endotracheal access, lungs may be subject to the known pharmacopeia of injectable or inhaled therapies as well as experimental interventions and a summary of potential candidates trialled so far can be found in Figure 2.

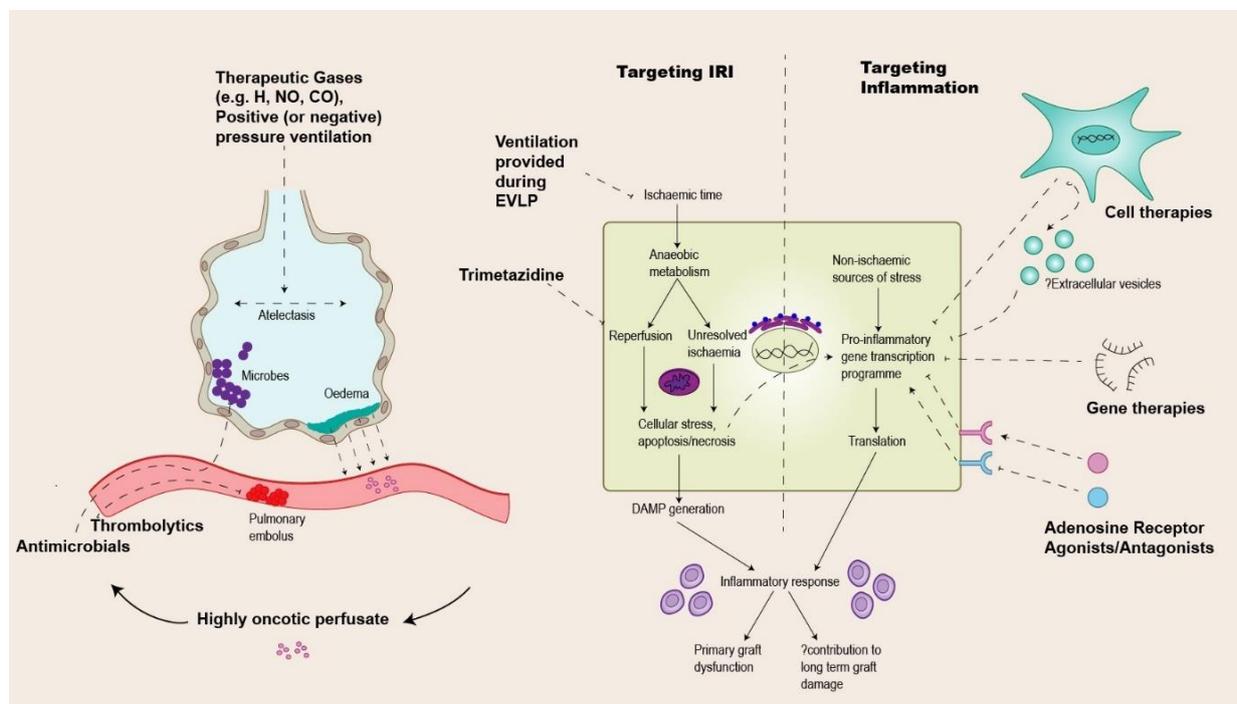


Figure 2. Summary of areas targeted by the potential therapeutics trialled for use in EVLP. Ventilation provided during EVLP is included as a means to combat ischaemic time and damage in the case of the portable EVLP device commercially available.

Donor lung infection is common either due to circumstances preceding death or through perimortem inoculation via ventilation or aspiration and poses a significant risk in highly immunosuppressed recipients, particularly during induction therapy. In one study, 18 human lungs initially assessed as unsuitable for transplantation were infused with high doses of the broad-spectrum antibiotic combination meropenem and amphotericin B during EVLP [25]. Bronchoalveolar lavages were taken before and after treatment and microbial concentrations analysed. Of the 13 that had positive bacterial cultures, antibiotic therapy was associated with a significant decrease in bacterial load. Six lungs underwent reassessment and were successfully transplanted with all recipients surviving to discharge. Nakajima et al. (2016), demonstrated use of high-dose ciprofloxacin, vancomycin and meropenem combination was associated with reduced bacterial counts as well as improved oxygenation status, pulmonary compliance and pulmonary vascular resistance compared to the controls with no antibiotic intervention [26].

EVLP also offers the opportunity to directly treat virally infected donor lungs, for example where hepatitis C positive donor lungs are available for serologically negative recipients [27]. Pre-clinical studies by the Toronto group included performing an 'intense lung wash' on Hepatitis C positive lungs where the perfusion protocol was prolonged to nine hours and included an exchange of the perfusion solution and circuit [28]. Comparing with standard protocol EVLP with hepatitis C positive lungs, the wash out achieved a reduction in HCV RNA viral loads. They achieved even better clearance of HCV RNA by applying photodynamic therapy (PDT), which involves the administration of the photosensitising drug methylene blue and its activation by red light irradiation, which is then thought to exert its effect on HCV via reactive oxygen species generation. In the first clinical study in this area, the group used a 6 h circuit exchange protocol intending to wash out HCV in one group and an alternative light therapy using UVC irradiation of circuit

perfusate in another [29]. In the wash out group, although all patients became viraemic after transplantation, EVLP using this protocol did seem to delay HCV RNA detection time. In the UVC treatment group, 2/11 patients did not become viraemic at all in the post-transplant period including at 6 month follow up. Treatment is of course possible after transplantation by direct acting antivirals in hepatitis C however this work does provide a model for limiting transmission of other viruses using EVLP.

Pulmonary embolism is also a common pathology in donor lungs. Machuca et al. (2013) first described the use of EVLP in treating pulmonary embolism leading to successful transplantation [30]. In this case, study, although large thrombi were extracted by retrograde perfusate flush during procurement, pulmonary arterial pressure and pulmonary vascular resistance on EVLP remained high leading to a diagnosis of suspected residual thrombus. Thrombolysis with alteplase led to improvements in pulmonary arterial pressure and conversion to successful lung transplantation.

A variety of other therapeutic directions explored in animal models have shown promise, including pre-existing medications. Trimetazidine, an anti-anginal medication, limits ischaemia by promoting glucose metabolism over fatty acid metabolism in ischaemic tissue leading to increased ATP production and additionally inhibits mitochondrial permeability transition pore opening, responsible for damage during reperfusion [31]. In a porcine EVLP model, trimetazidine was associated with a less inflammatory expression profile post-EVLP, with reduced myeloperoxidase levels in lavage samples and improved gas exchange post-transplantation compared to controls.

The adenosine receptor family may also provide valuable therapeutic targets. Among their other actions, individual adenosine receptors are known to stimulate either pro-inflammatory or anti-inflammatory signalling pathways, therefore immunomodulation might be achieved with use of both agonists and antagonists [24]. For example, an antagonist to the pro-inflammatory A_{2B} receptor trialled in a porcine EVLP model was associated with a statistically significant improvement in lung compliance, a decrease in IL-12 and reduced in neutrophil infiltration [32]. Conversely, use of an agonist to the anti-inflammatory A_{2A} receptor in a porcine model was associated with statistically significant reduction in oedema, reduction in interferon- γ (IFN- γ) and increased oxygenation [33].

Cell therapies are an area of particular interest in EVLP research. Whole cells or their miRNA-containing extracellular vesicle (EV) derivatives can be administered to EVLP circuits and are thought to be entrapped in the lung parenchyma where they can exert juxtacrine and paracrine immunomodulatory effects on surrounding tissue [24]. Mesenchymal stem cells (MSCs) can be derived from the umbilical cord or bone marrow and have low immunogenicity given their very low major histocompatibility complex (MHC) class I expression levels, absence of MHC class II markers and lack of co-stimulatory molecules involved in T cell activation [34]. The intravascular administration of MSCs in a porcine EVLP model demonstrated both their parenchymal retention and a demonstrable reduction in IL-8 concentrations in perfusates [35]. MSC-derived EVs administered in a murine EVLP-autotransplantation model aimed at simulating ischaemia-reperfusion showed significant reductions in oedema, neutrophil infiltration, myeloperoxidase levels and pro-inflammatory cytokines in the treatment group [36]. The administration of both MSCs and MSC-derived EVs to human lungs has shown improvements in alveolar fluid clearance, with EVs also improving lung function [37,38]. Multipotent adult progenitor cells (MAPCs), an alternative cell therapy with shared characteristics with MSCs have also been preliminarily explored for use in EVLP, with one porcine EVLP model demonstrating reduced neutrophil infiltration and pro-inflammatory cytokines TNF- α , IL-1 β and IFN- γ in lavage samples in the treatment group compared to controls [39].

Intrabronchial gas therapy administration may be another important intervention possible during EVLP. For example, hydrogen gas is a known antioxidant and has been trialled in a porcine EVLP model where it was associated with statistically significantly lower pulmonary vascular resistance and peak airway pressure and reduced expression of IL-1 β , IL-6, IL-8 and TNF- α [40]. When similarly treated porcine lungs were transplanted

in a follow-up study, the hydrogen-treated group had superior arterial blood gas results post-transplant and expressed higher concentrations of the anti-inflammatory cytokine IL-10 [41].

Gene therapy is in its early stages in normothermic machine perfusion research but has theoretical potential. Genes may be either introduced, for example through a viral vector, or their expression inhibited using RNA interference (RNAi) technologies. Viral vectors are associated with inflammation when used systemically and EVLP represents an opportunity to avoid this response [24]. Machuca et al. (2017) delivered genes encoding IL-10 via the adenovirus vector intrabronchially in a porcine EVLP model, noting superior gas exchange, less histological evidence of inflammation and reduced IFN- γ production in the treatment group [42]. Any number of genes in the genome might be manipulated using these technologies and this is potentially an area of significant future development.

7. The Future Potential for EVLP

EVLP offers logistical advantages through the extension of preservation time without extending cold ischaemic time, meaning operations might be performed in normal working hours and transplantation surgeries can be performed in series rather than parallel, the latter of which is only possible in select centres. Notably, one retrospective study noted there was no difference found in early outcomes comparing grafts with a preservation time >12 h facilitated by EVLP with a group receiving grafts with a preservation time of <12 h [43].

Beyond transplantation, several innovation areas for EVLP have been suggested. First, EVLP provides an excellent and translatable platform for thoracic oncology drug trials [44]. EVLP could be coupled with autotransplantation for the administration of intolerably toxic chemotherapies [44] and antimicrobials [45] in the setting of otherwise incurable malignancies or multi-resistant lung infections. Finally, an area of great future potential lies in bioengineering of lungs, involving decellularization, seeding of the remaining tissue scaffold with multipotent progenitor cells of recipient origin and subsequent recellularization to produce a chimeric organ aimed at circumventing the issue of rejection. EVLP has already been shown to be an effective tool for decellularization and recellularization [46] and may provide a platform for assessment and safe use of bioengineered lungs in the future when this field progresses.

8. Conclusions

The potential for EVLP in clinical medicine and research is vast. In human lung transplantation it has been shown as a safe and effective tool for detailed lung assessment which can lift our lung utilisation rates from their current despairingly low levels. EVLP also provides a platform for the introduction of myriad therapies which may provide solutions to many of the issues encountered by patients who undergo lung transplantation and the clinicians who care for them.

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