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Technique for xenogeneic cross-circulation to support human donor lungs ex vivo

W. Kelly Wu, MD,[a] Brandon A. Guenthart, MD,[b] John D. O'Neill, PhD,[c,d] Ahmed E. Hozain, MD,[e] Yuliya Tipograf, MD,[a] Rei Ukita, PhD,[a] John W. Stokes, MD,[a] Yatrik J. Patel, MD,[a] Meghan Pinezich, PhD,[f] Jennifer R. Talackine, MS,[a] Nancy L. Cardwell, BS,[a] Kenmond Fung, MS, CCP,[g] Gordana Vunjak-Novakovic, PhD,[f,h] and Matthew Bacchetta, MD, MBA[a,i]

From the [a]Department of Cardiac Surgery, Vanderbilt University Medical Center, Nashville, Tennessee; [b]Department of Cardiothoracic Surgery, Stanford University, Palo Alto, California; [c]Xylyx Bio, Inc., Brooklyn, New York; [d]Department of Cell Biology, State University of New York Downstate Medical Center, Brooklyn, New York; [e]Department of Surgery, State University of New York Downstate Medical Center, Brooklyn, New York; [f]Department of Biomedical Engineering, Columbia University, New York, New York; [g]Perfusion Services, New York − Presbyterian Hospital, New York, New York; [h]Department of Medicine, Columbia University Medical Center, New York, New York; and the [i]Department of Biomedical Engineering, Vanderbilt University, Nashville, Tennessee.

KEYWORDS:
ex vivo lung perfusion;
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xeno-support;
xenotransplantation

BACKGROUND: Xenogeneic cross-circulation (XC) is an experimental method for ex vivo organ support and recovery that could expand the pool of donor lungs suitable for transplantation. The objective of this study was to establish and validate a standardized, reproducible, and broadly applicable technique for performing xenogeneic XC to support and recover injured human donor lungs ex vivo.

METHODS: Human donor lungs (n = 9) declined for transplantation were procured, cannulated, and subjected to 24 hours of xenogeneic XC with anesthetized xeno-support swine (Yorkshire/Landrace) treated with standard immunosuppression (methylprednisolone, mycophenolate mofetil, tacrolimus) and complement-depleting cobra venom factor. Standard lung-protective perfusion and ventilation strategies, including periodic lung recruitment maneuvers, were used throughout xenogeneic XC. Every 6 hours, ex vivo donor lung function (gas exchange, compliance, airway pressures, pulmonary vascular dynamics, lung weight) was evaluated. At the experimental endpoint, comprehensive assessments of the lungs were performed by bronchoscopy, histology, and electron microscopy. Student's t-test and 1-way analysis of variance with Dunnett's post-hoc test was performed, and $p < 0.05$ was considered significant.

RESULTS: After 24 hours of xenogeneic XC, gas exchange (PaO2/FiO2) increased by 158% (endpoint: 364 ± 142 mm Hg; p = 0.06), and dynamic compliance increased by 127% (endpoint: 46 ± 20 ml/cmH_2O; p = 0.04). Airway pressures, pulmonary vascular pressures, and lung weight remained stable ($p > 0.05$) and within normal ranges. Over 24 hours of xenogeneic XC, gross and microscopic lung architecture were preserved: airway bronchoscopy and parenchymal histomorphology appeared normal, with intact blood−gas barrier.

CONCLUSIONS: Xenogeneic cross-circulation is a robust method for ex vivo support, evaluation, and improvement of injured human donor lungs declined for transplantation.

Abbreviations: CVF, cobra venom factor; EVLP, *ex vivo* lung perfusion; IJV, internal jugular vein; PA, pulmonary artery; PV, pulmonary vein; TPG, transpulmonary pressure gradient; XC, cross-circulation

Reprint requests: Matthew Bacchetta, MD, MBA, FACS, FAIMBE, Department of Cardiac Surgery and Biomedical Engineering, Vanderbilt University Medical Center, Medical Center East, 1215 21st Avenue South, Suite 5025, Nashville, TN 37232. Telephone: 615-322-248. Fax: 615-343-5993.

E-mail address: matthew.bacchetta@vumc.org

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Up to 80% of donor lungs are declined for transplantation,[1,2] often failing to meet criteria due to potentially reversible injuries such as aspiration pneumonitis, contusion, edema, or consolidation.[3,4] To improve the quality of donor lungs and increase their clinical utilization, extracorporeal perfusion techniques, including isolated ex vivo lung perfusion (EVLP) and cross-circulation (XC), have been developed.[5-8] Isolated EVLP, which re-circulates acellular or cellular perfusate in a closed or open circuit, is used in clinical transplantation to preserve and assess the viability of marginal quality lungs.[5,9,10] However, isolated EVLP remains limited in its duration of support and ability to recover organs with severe injuries,[11−13] due to a lack of *multisystem physiologic regulation* − including homeostatic balance, anabolic synthesis, metabolic clearance, and neurohumoral signaling.

Cross-circulation as a new approach for supporting whole lungs ex vivo, first developed and reported by our group,[7] exchanges whole blood between ex vivo donor lungs and an organism providing physiologic support. We previously showed that XC enables multiday maintenance[14] and functional recovery[7,15] of severely injured swine lungs. In preclinical studies, XC also maintained viability and enabled functional recovery of human donor lungs declined for transplantation by using swine to provide xenogeneic support.[15-17] During XC, physiologic regulation of the human donor lungs by the "xeno-support" swine provides a sustained homeostatic milieu for the ex vivo organ that significantly extends the time and opportunity for organ evaluation, therapeutic intervention, cellular regeneration, and functional recovery. The objective of this study was to establish and validate a standardized, reproducible technique for performing xenogeneic cross-circulation (Figure 1) to support human donor lungs ex vivo for 24 hours.

Materials and methods

Animals: Male Yorkshire/Landrace swine (*n* = 9) that were 3 to 8 months of age, with mean weight of 72.9 ± 24.8 kg (range, 54.6-123.0 kg) were used to provide xeno-support. Studies were approved by the Institutional Animal Care and Use Committees at Vanderbilt University Medical Center and Columbia University Medical Center. Animal care and procedures were conducted in accordance with the US National Research Council of the National Academies *Guide for the Care and Use of Laboratory Animals, Eighth Edition*.

Human donor lungs: Donor lungs (*n* = 9) declined for transplantation by all clinical lung transplant programs and consented for research use were procured through local organ procurement organizations under protocols approved by the Institutional Review Board at Vanderbilt University Medical Center or Columbia University Medical Center. Procurements were performed by experienced surgeons using standard techniques,[18] in coordination with other teams deployed for organ procurement for clinical

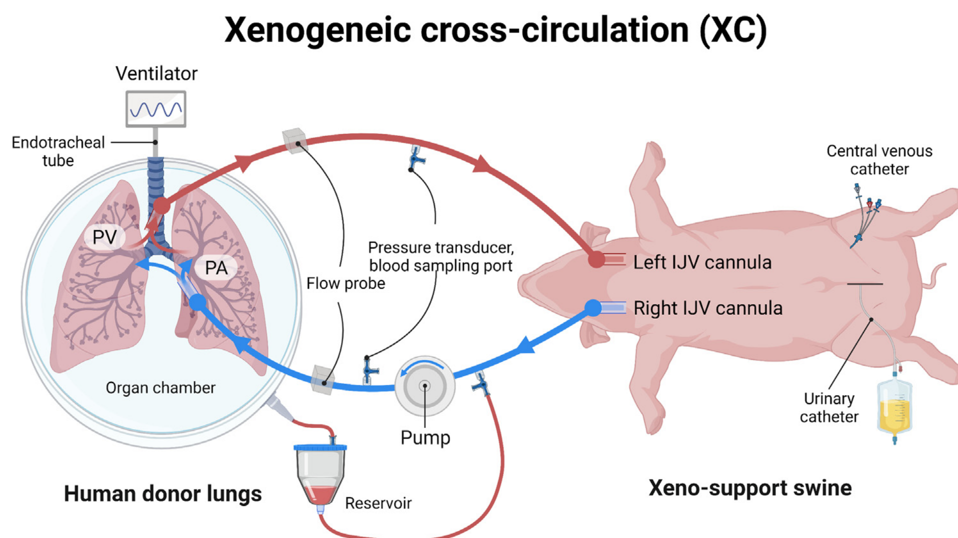

Figure 1 Xenogeneic cross-circulation setup. Key components include human donor lungs, xeno-support swine, and perfusion circuit. Swine blood drains from the right IJV cannula of the xeno-support swine, is propelled by a centrifugal pump through the PA to perfuse the human donor lungs, and returns from the PV to the xeno-support swine through the left IJV cannula. The trachea of the human donor lungs is connected to a ventilator through an endotracheal tube. Lungs are placed in an organ chamber, which is connected to a reservoir that returns lost blood to the circuit. Flow probes, pressure transducers, and blood sampling ports are integrated into the circuit. The xeno-support swine remains anesthetized, intubated, and mechanically ventilated throughout the procedure (swine ventilator not shown). A central venous catheter is placed in the femoral vein of the xeno-support swine, and a urinary catheter is placed in the bladder. A list of equipment and supplies, including suppliers and specifications, is in Supplementary Table 2, 3. IJV, internal jugular vein. PA, pulmonary artery. PV, pulmonary vein.

transplantation. Donor lungs with radiographic or bronchoscopic evidence of severe pneumonia, or with anticipated cold ischemia time exceeding 36 hours, were excluded from the study. Lungs meeting the study criteria were procured, placed in organ preservation solution (Perfadex, Vitrolife) at 4˚C, transported to our facility, and evaluated throughout 24 hours of xenogeneic XC.

Preparation of xeno-support swine: The preprocedure workflow is described in Figure 2A, and a detailed, step-by-step protocol is provided in the Supplementary Appendix. Briefly, swine were fasted for 4 hours prior to anesthetic induction, as previously described.[16,19] An auricular intravenous catheter and femoral central venous catheter were placed for drug administration, and an auricular arterial catheter was placed for hemodynamic monitoring. Intravenous immunosuppressive agents were administered: methylprednisolone (1 g, Pfizer), mycophenolate mofetil (500 mg, Genentech), tacrolimus (5 mg, Astellas Pharma), and cobra venom factor (1 mg, Sigma-Aldrich), as previously described (Figure 2B).[8] Importantly, transient hypersensitivity and hemodynamic instability associated with cobra venom factor administration were mitigated with antecedent administration of diphenhydramine (50 mg, West Ward Pharmaceuticals) and methylprednisolone. Intravenous antibiotics were also administered, as previously described.[8,19]

A urinary catheter was placed via open cystostomy for continuous bladder drainage during xenogeneic XC (Figure 3a). Bilateral internal jugular vein (IJV) access was obtained using ultrasound guidance or open surgical cutdown (Figure 3b). Intravenous heparin (30,000 IU, Fresenius Kabi) was administered, and cannulas (15-21 Fr, Bio-Medicus arterial, Medtronic) were placed in each IJV using Seldinger technique and serial dilation 3 minutes after heparin administration (Figure 3c). The right IJV cannula was inserted to a depth of approximately 18 cm until the tip was in the right atrium. The left IJV cannula was inserted to a depth of approximately 16 cm until the tip was in the innominate vein or superior vena cava. Cannulas were secured to the skin, and cervical incisions were closed (Figure 3d). A continuous rate infusion of heparin was initiated at 3,000 IU/h and titrated to maintain activated clotting time of 180 to 250 seconds throughout XC. Medications, suppliers and doses are listed in Supplementary Table 4 and 5.

Preparation of ex vivo *human lungs:* Lungs were procured and placed in cold organ preservation solution (Perfadex, Vitrolife) maintained at 4˚C on a slurry of ice. If the heart was procured for clinical transplantation, minimal pulmonary artery (PA) and left atrial cuff remained. To facilitate cannulation, reconstruction at the inflow and outflow was performed as needed using donor aorta (Figure 4a), donor pericardium (Figure 4b), a combination of donor aorta and pericardium (Figure 4c), or synthetic cuffs (Lung Cannula Set, XVIVO) (Figure 4d). The PA was cannulated with a 24 to 28 Fr cannula (tip at least 1 cm proximal to the bifurcation), and the pulmonary vein (PV) was cannulated with a 30 to 36 Fr cannula. Lungs were flushed with 1 liter of cold balanced crystalloid before clamping PA and PV cannulas. The trachea was intubated with a 9-mm endotracheal tube (tip at least 2 cm proximal to the carina). A list of cannulation components, including suppliers and specifications, is in Supplementary Table 3.

Xenogeneic cross-circulation setup: The XC circuit is described in Figure 1. Before the procedure, circuit tubing and components were sterilized by ethylene oxide (Andersen). Pressure transducers (Edwards Lifesciences), flow probes (Sonotec), and blood sampling ports (Medline) were integrated into the circuit at donor lung inflow and outflow sites. A list of circuit equipment and supplies, including suppliers, model numbers, and specifications, is in Supplementary Table 2 and 3.

Initiation of xenogeneic cross-circulation: The *ex vivo* perfusion circuit was primed with 1 liter of warm balanced crystalloid solution, clamped, and connected to the xeno-support swine IJV cannulas using sterile wet-to-wet connection technique. Clamps were removed, and pump speed was slowly increased until flow was approximately 500 ml/min. Human donor lungs were placed in the *ex vivo* organ chamber (XVIVO). To initiate XC, the pump was stopped, and the circuit was clamped, cut, and connected to the PA and PV cannulas using sterile wet-to-wet connection

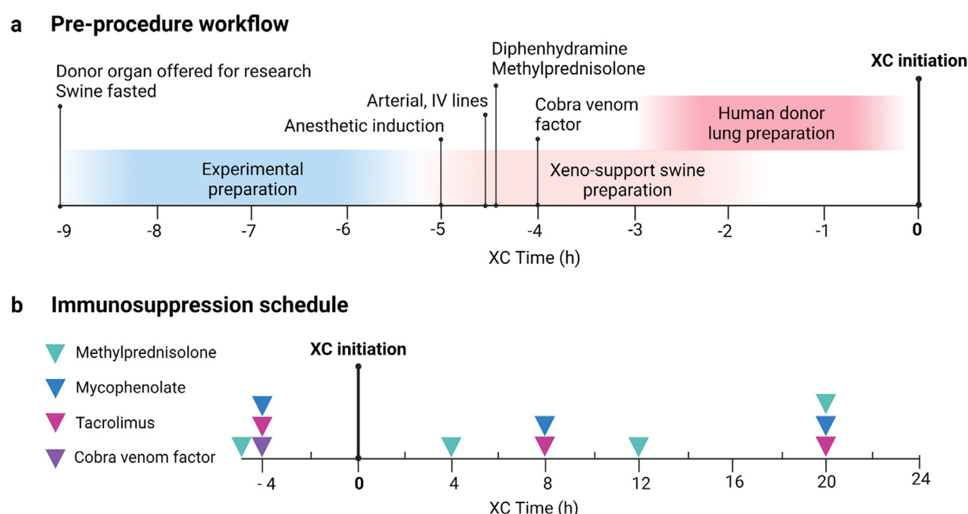

Figure 2 Xenogeneic cross-circulation timeline. (a), Pre-procedure workflow timeline before initiation of xenogeneic cross-circulation. Experimental preparation includes fasting of xeno-support swine, logistical coordination of donor organ procurement, and assembly of equipment, supplies, and medications. Xeno-support swine preparation includes anesthetic induction, intubation, placement of arterial and intravenous lines, administration of medications, bladder catheterization, and bilateral internal jugular vein cannulation. Donor lung preparation includes vascular reconstruction (if needed), pulmonary artery and pulmonary vein cannulation, tracheal intubation, and organ flush. (b), Schedule for immunosuppression. Dosing: methylprednisolone 1 g (initial dose) or 125 mg (remainder doses) IV, every 8 hours; mycophenolate 500 mg IV, every 12 hours; tacrolimus 500 mg IV, every 12 hours; cobra venom factor 1 mg IV, single dose. A list of medications, including suppliers and doses, is in Supplementary Table 4, 5. IV, intravenous. XC, cross-circulation.

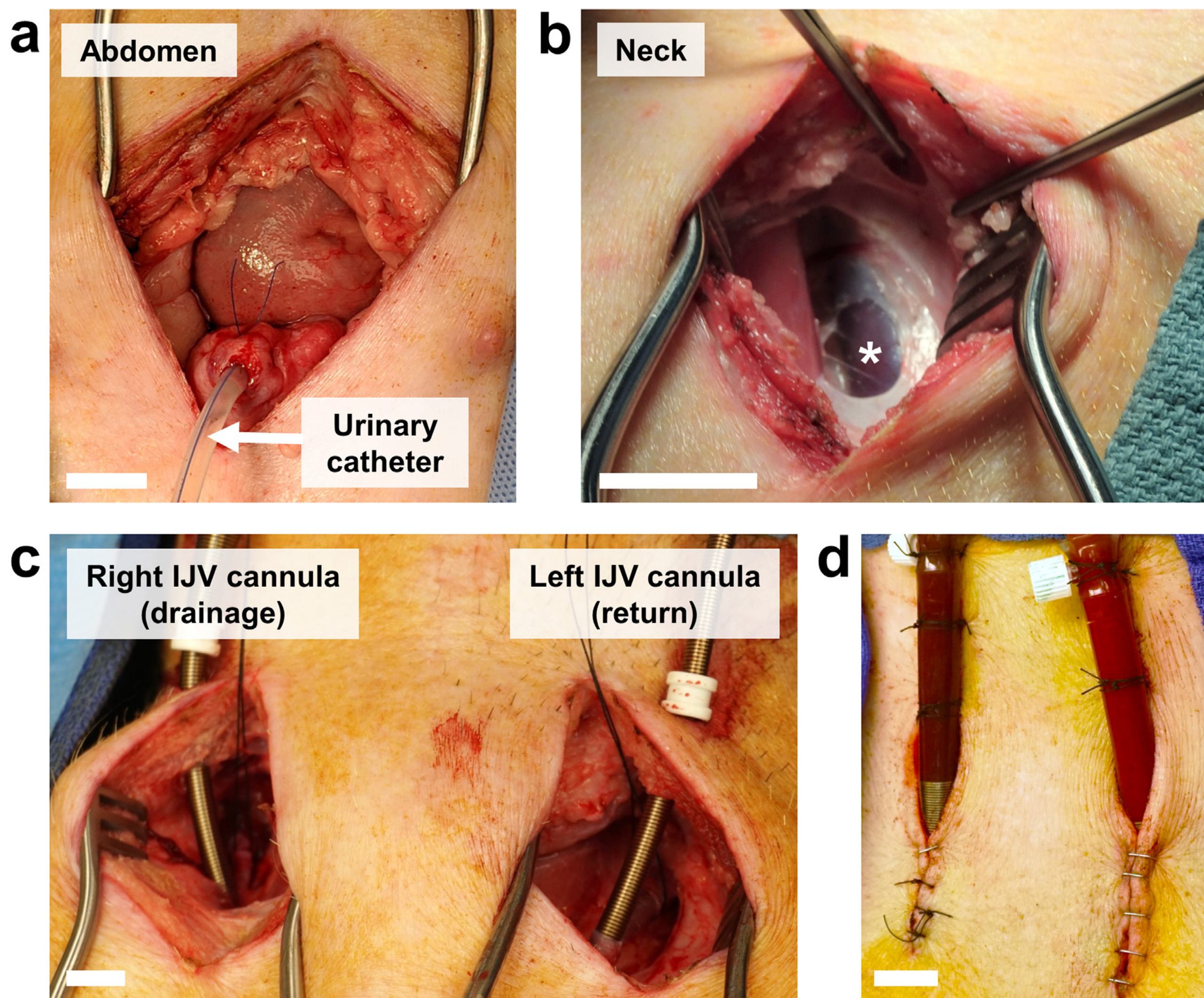

Figure 3 Xeno-support swine preparation. (a) Bladder catheterization via open cystostomy. (b) Exposure of an internal jugular vein (*) via cervical incision. (c) Placement of drainage and return cannulas in the internal jugular veins for xenogeneic cross-circulation. (d) Right and left IJV cannulas secured to skin with closure of cervical incisions. The color difference between right IJV drainage cannula (*left*, dark red due to deoxygenated blood) and left IJV return cannula (*right*, bright red due to oxygenated blood) is visible confirmation that *ex vivo* donor lungs are performing gas exchange. Scale bar: 2 cm.

technique. Clamps were removed, the pump was restarted, and swine blood perfused through the ex vivo lungs − marking the initiation of xenogeneic XC between human donor lungs and xeno-support swine. Pump speed was initially adjusted to maintain flow at 5% of estimated donor cardiac output. Blood loss into the organ chamber was collected in a hardshell cardiotomy reservoir (Maquet) and returned to the circuit as needed. Hemodynamic instability of xeno-support swine was medically supported with fluid administration and vasoactive infusions (Supplementary Appendix). Experimental baseline (0 hour) was designated as the timepoint immediately after initiation of XC (i.e., reperfusion). After reperfusion, baseline data and samples were collected. A list of troubleshooting tips is in Supplementary Table 1.

Maintenance of xenogeneic cross-circulation: A standard lung-protective strategy for perfusion and ventilation of *ex vivo* human donor lungs was used throughout xenogeneic XC (Table 1). Over the first hour, pump speed was gradually increased to 10% to 20% of donor cardiac output. Lung surface temperature was monitored using an infrared camera (T530, FLIR), and ventilation of *ex vivo* human donor lungs was initiated after average lung surface temperature was 30°C, as previously described (Supplementary Fig. 1).[20,21] The ventilator (Servo-i, Maquet) was set to volume-controlled, continuous mandatory ventilation mode, with initial tidal volume of 2 to 3 ml/kg, respiratory rate of 8 to 10 breaths per minute, fraction of inspired oxygen (FiO_2) of 21%, and positive end-expiratory pressure (PEEP) of 5 cmH_2O. After *ex vivo* human donor lungs achieved normothermia, tidal volume was increased to 4 − 6 ml/kg. Temperature of *ex vivo* lungs was regulated by management of xeno-support swine, including use of heating pads, forced air warming devices, and timely closure of surgical sites. Lung recruitment maneuvers were performed by increasing PEEP to 10 cmH_2O and maintaining inspiratory holds for 10 seconds every 4 hours (Supplementary Figure 2).

Functional evaluation of ex vivo *human lungs:* Respiratory function was evaluated every 6 hours throughout 24 hours of xenogeneic XC. Blood samples were collected from the PA and PV, blood gas analysis was performed using a point-of-care analyzer (epoc, Heska), and gas exchange parameters (PaO_2/FiO_2, change in pCO_2) were calculated. Peak inspiratory pressure (PIP) and plateau pressure ($P_{plateau}$) were recorded to determine dynamic

Table 1 Perfusion and Ventilation Strategy for Initiation of Xenogeneic Cross-Circulation

	TIME AFTER REPERFUSION (min)						
SUPPORT PARAMETER	0	10	20	30	40	50	60+
Flow rate[a] (ml/min)	200	250	300	350	400	500	600
% of donor cardiac output	5	6	8	9	10	13	15
% of maintenance flow	33	42	50	58	67	83	100
Lung surface temperature (°C)	10	25	30	35	37	37	37
Tidal volume (ml/kg donor)	0	0	2	3	3	3	4[b]

[a]Based on estimated donor cardiac output of 4 L/min, and target flow rate of 15% of donor cardiac output.
[b]Tidal volume thereafter may be gradually increased to 6 to 8 ml/kg, pending stability of ventilatory parameters.

compliance (C_{dyn} = TV/(PIP − PEEP)) of *ex vivo* human donor lungs. Vascular flows and pressures at the PA and PV were continuously monitored and recorded (ADInstruments). Lung weight was obtained using a top-loading balance (W-4830, WeighMax). Airways were assessed with a 3.8 mm flexible bronchoscope (aScope 4, Ambu).

Histologic evaluation of ex vivo *human lungs:* Wedge biopsies of lung parenchyma were obtained according to a predetermined, randomized sampling map, as previously described,[7] after 24 hours of xenogeneic XC. Tissue samples were prepared and imaged by light and transmission electron microscopy, as previously described.[16,19]

Termination of xenogeneic cross-circulation: After endpoint data and samples were collected, donor lungs were inflated, and the trachea was clamped. EVLP and ventilation were stopped, and all cannulas were clamped. Lungs were disconnected from the xenogeneic XC circuit, flushed with cold organ preservation solution (Perfadex, Vitrolife) until the effluent was clear, and stored in an isolation bag on ice. Xeno-support swine were disconnected from the circuit and euthanized according to institutional protocol.

Statistical analysis: No data were excluded from analysis. Student's *t*-test and 1-way analysis of variance with Dunnett's *post-hoc* test were performed using statistical analysis software (Prism 8.2.1, GraphPad), and $p < 0.05$ was considered statistically significant. Results are reported as mean values ± standard deviation, unless otherwise specified. Mean changes in parameters reported in Table 2 were determined by calculating the percent change for each experiment, then calculating the mean ± standard deviation of percent changes across all experiments.

Results

Xenogeneic cross-circulation supported and improved the respiratory function of *ex vivo* human donor lungs (Table 2), with no mortality of xeno-support swine. After 24 hours of xenogeneic XC, human donor lungs exhibited a mean increase in oxygenation of 158% ($p = 0.06$), with mean endpoint PaO_2/FiO_2 above 350 mm Hg (Figure 5a). Removal of CO_2, assessed by the change (Δ) in pCO_2 between the pulmonary artery and vein, steadily improved (Figure 5b). Dynamic compliance of donor lungs increased by 127% (p = 0.04) and was maintained above 30 ml/cmH_2O (Figure 5c). Peak inspiratory pressure (PIP) remained below 20 cmH_2O, and plateau pressure ($P_{plateau}$) was stable (Figure 5d). Transpulmonary pressure gradient, the average difference in pressure between pulmonary artery and vein, was maintained at 12 ± 6 mm Hg (Figure 5e), and mean flow was 409 ± 283 ml/min. Lung weight did not significantly change ($p = 0.28$) (Figure 5f).

After 24 hours of xenogeneic XC, gross appearance of *ex vivo* human donor lungs was normal (Figure 6a), with normal airways on bronchoscopy (Figure 6b). Microscopically, xenogeneic XC preserved donor lung parenchyma (Figure 6c), including alveolar structure (Figure 6d), pulmonary vascular histomorphology (Figure 6e), and small airway architecture (Figure 6f). Transmission electron microscopy confirmed preservation of the alveolar

Table 2 Function of Ex Vivo Donor Lungs ($n = 9$) After 24 Hours of Xenogeneic Cross-circulation

Functional parameter	Baseline	Endpoint	Mean change[a]	*P*-value
Gas exchange				
PaO_2/FiO_2 (mm Hg)	181 ± 89	364 ± 142	+ 158 ± 182%	0.06
Δ pCO_2 (mm Hg)	10 ± 9	25 ± 15	+ 363 ± 390%	0.20
Ventilation				
Dynamic compliance (ml/cmH_2O)	30 ± 19	46 ± 20	+ 127 ± 174%	0.04
Peak inspiratory pressure (cmH_2O)	17 ± 6	15 ± 4	− 3 ± 34%	0.62
Plateau pressure[b] (cmH_2O)	10 ± 1	14 ± 4	+ 23 ± 30%	0.71
Perfusion				
Transpulmonary pressure gradient (mm Hg)	10 ± 5	14 ± 7	+ 51 ± 66%	0.52
Organ integrity				
Lung weight (kg)	1.3 ± 0.4	1.2 ± 0.5	− 5 ± 16%	0.28

[a]Reported as the percent change average across all experiments.
[b]Data represent $n = 4$ donor lungs.

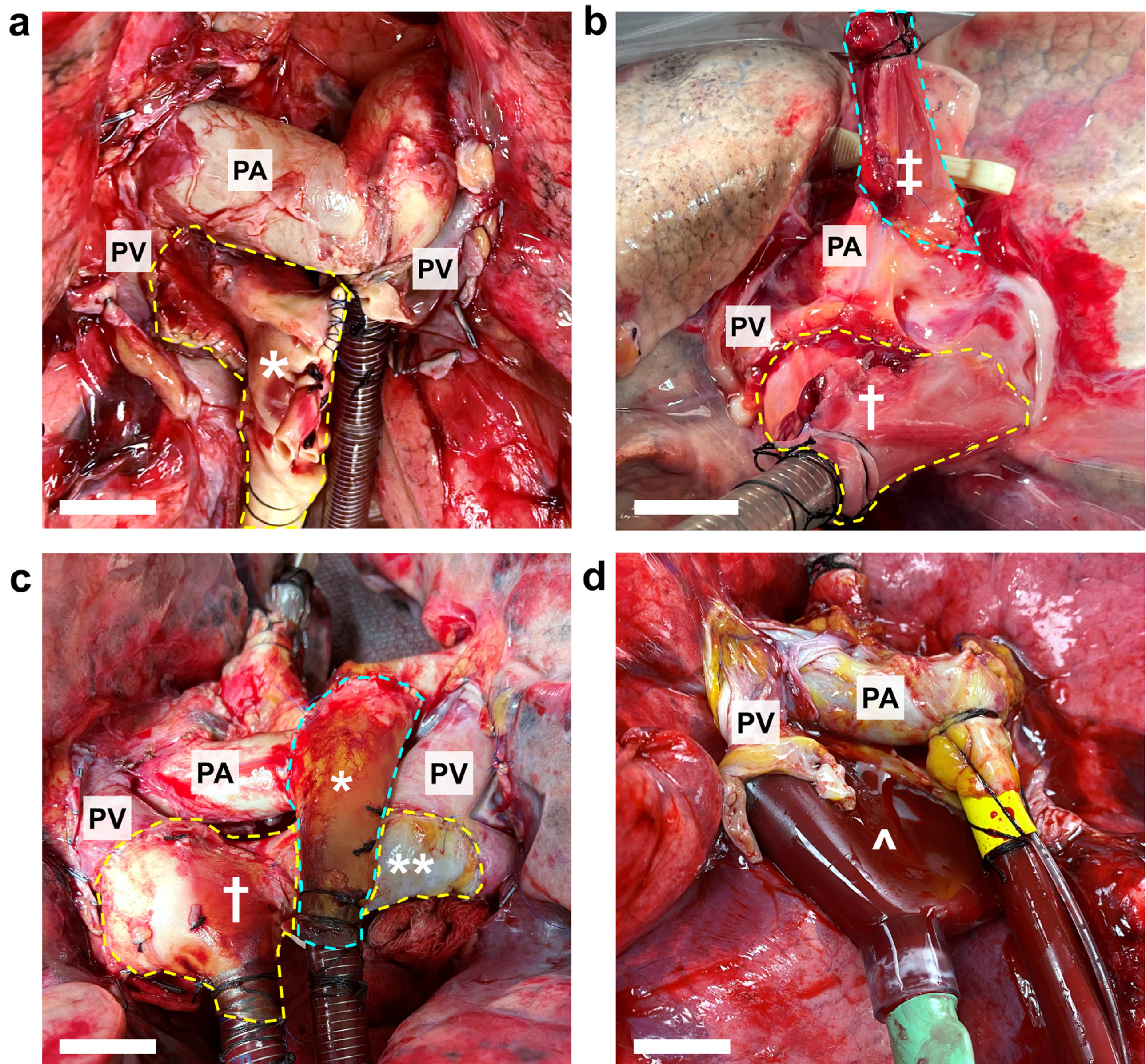

Figure 4 Reconstruction and cannulation of *ex vivo* human donor lungs. (a) Donor aortic arch (*) used to interpose PV and reconstruct the left atrial cuff. (b) Donor pericardium used to create tubular (‡) and funnel-shaped (†) interposition grafts for the PA and PV, respectively. (c) Donor aorta (*) used to reconstruct the main PA. Donor aorta (**) and donor pericardium (†) used to reconstruct the left atrial cuff. (d) Funnel-shaped left atrial cannula (^) anastomosed to the left atrial cuff. Blue outline: PA reconstruction. Yellow outline: PV reconstruction. PA, pulmonary artery. PV, pulmonary vein. Scale bar: 2 cm.

−capillary barrier (Figure 6g) and integrity of type II pneumocytes (Figure 6h).

Discussion

The technique for xenogeneic cross-circulation is an experimental method to support and improve the function of *ex vivo* donor lungs declined for transplantation. By providing *multisystem physiologic regulation*, XC enhances the durability of *ex vivo* organ support and recovery beyond the capabilities demonstrated by isolated organ perfusion systems.[7,8,14−16] In this study, xenogeneic XC was performed for 24 hours, and donor lungs declined for clinical transplantation showed improvements meeting functional criteria for transplantation. Key technical considerations and envisioned applications of xenogeneic XC are discussed below.

Xeno-support swine: In prior XC studies, several swine breeds that are commonly used in research were successfully used as allo-support swine, including Yorkshire,[15] Yorkshire x Landrace (crossbreed),[19] and NIH miniature swine.[7] In this study, Yorkshire and Yorkshire x Landrace swine were used as xeno-support swine with no apparent differences, suggesting that experimental xenogeneic XC is not limited to use of a single or specific breed. As availability of genetically-modified swine for therapeutic uses

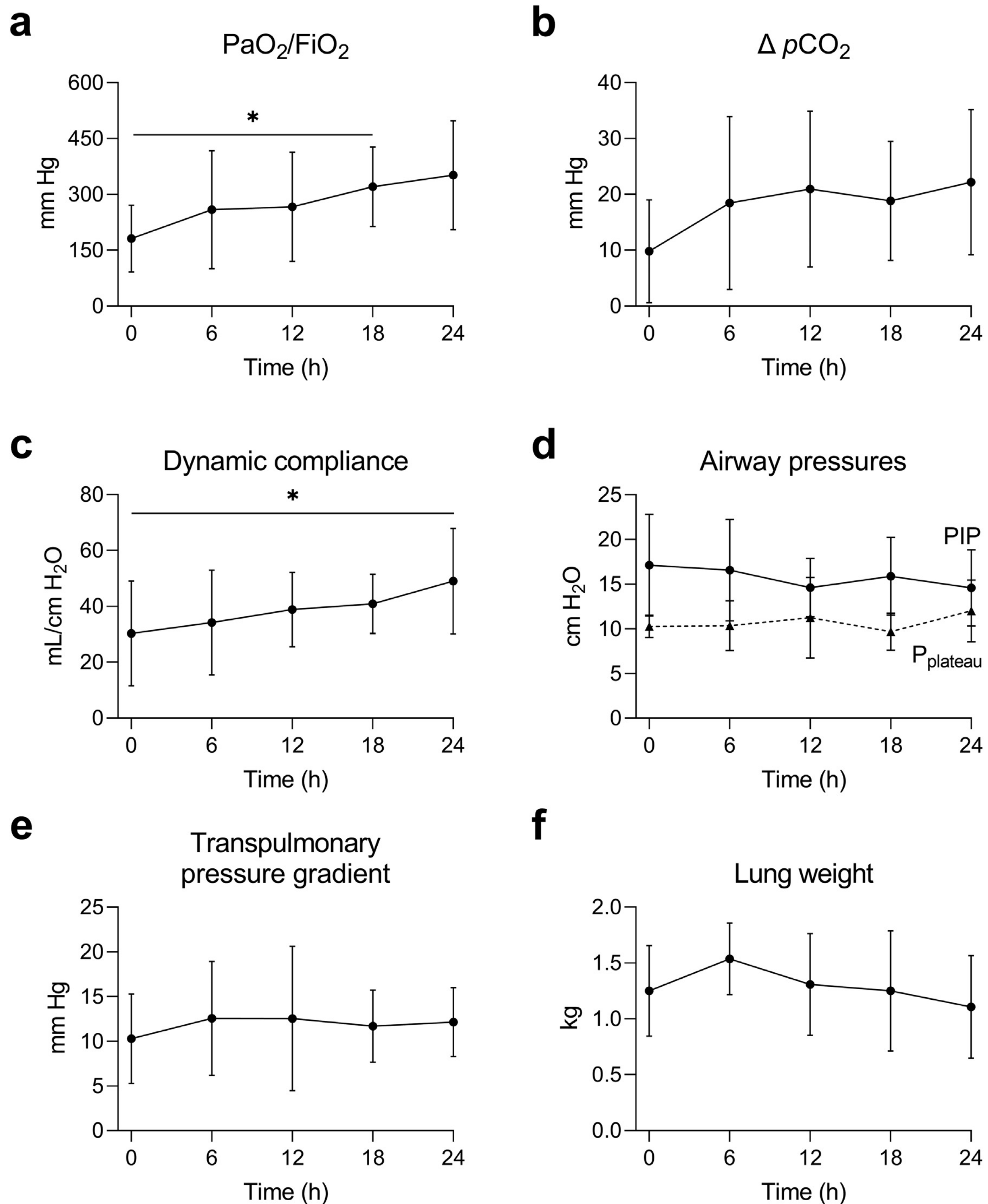

Figure 5 Human donor lung function over 24 hours of xenogeneic cross-circulation support. (a), PaO_2/FiO_2. (b), Change (Δ) in pCO_2. (c), Dynamic compliance. (d), Airway pressures: peak inspiratory pressure (PIP) and plateau pressure ($P_{plateau}$). (e), Transpulmonary pressure gradient (P_{PA}-P_{PV}). (f), Lung weight. All values represent mean ± standard deviation. $* p < 0.05$.

increases,[22] future studies may investigate the potential benefits of using humanized swine for xeno-support during xenogeneic XC.

Immunosuppression: Cross-circulation of whole blood between a living swine and human donor organ is feasible using a standard immunosuppression regimen with cobra venom factor, a complement C3 analogue used for complement depletion in vertebrate animals.[23,24] Future studies will investigate the effects of other immunosuppression on immune response, and perform deeper analyses of immunological interactions to optimize immunosuppression regimens.

Logistics: As soon as declined donor lungs are offered for XC, we commence preparation of xeno-support swine[16] so that the 4 hours required for swine fasting overlaps with the time required for donor organ procurement and transportation, effectively minimizing cold ischemia time. Meanwhile, we prepare the procedure room in coordination with the organ procurement team in a process analogous to the protocols and procedures in clinical transplantation.

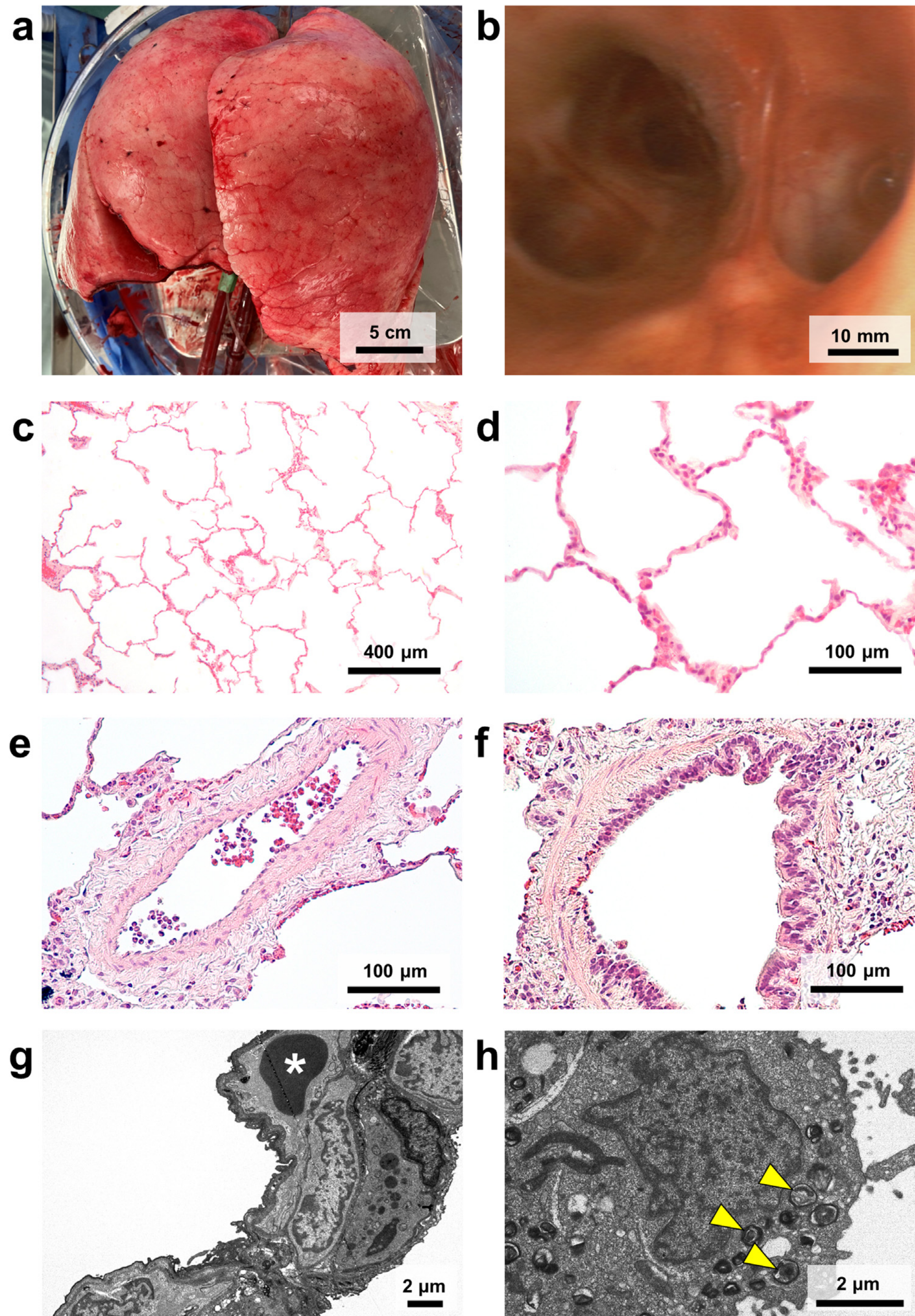

Figure 6 Human donor lungs after 24 hours of xenogeneic cross-circulation support. (a), Gross appearance. (b), Representative bronchoscopy. (c-f), Representative hematoxylin and eosin staining of lung parenchyma at low (c) and high (d) magnification, arterioles (e), and small airways (f). (g), (h), Representative transmission electron microscopy demonstrating intact alveolar-capillary barrier with red blood cell (*) (g) and type II pneumocyte with lamellar bodies (arrows) (h).

Upon receipt of the donor organ, the xeno-support swine is anesthetized, intubated, given immunosuppressive induction therapy, and cannulated while the human donor lung is prepared for xenogeneic XC.

Cold ischemia time: In this study, xenogeneic XC significantly improved human donor lungs with a mean total cold ischemia time of 16.0 ± 6.1 hours (range, 9.8-27.5 hours; n = 8), whereas isolated EVLP (low-flow acellular and high-flow cellular) has shown limited ability to recover donor lungs with cold ischemia time greater than 8 hours.[25] Notably, the reparative potential of lungs with extremely long cold ischemia times may be limited, as 1 lung that had total cold ischemia time of 37.8 hours, including clinical EVLP time, developed progressive pulmonary edema, increased airway pressures, and poor compliance, resulting in termination after 12 hours of xenogeneic XC.

Lung cannulation: Since heart donation and prior clinical EVLP can variably affect the pulmonary artery and left atrial cuff, we describe multiple reconstruction techniques to facilitate cannulation in a variety of scenarios.[14,26]

Correct placement of the PV cannula is particularly important to enable continuous unobstructed outflow and prevent excess pressure at the PV (e.g., due to kinking or obstruction of the cannula, or collapse of pulmonary veins or reconstructed conduits), which can lead to hydrostatic pulmonary edema and impaired lung compliance. Direct pressure transduction by a catheter inserted through the synthetic cuff or reconstructed vessel may offer more accurate readings than downstream pressure sensors.[5]

Lung reperfusion: During initial reperfusion, circuit flow and xeno-support swine fluid management were monitored to prevent rapid reperfusion and volume overload, which can exacerbate reperfusion injury.[27-29] We also used lung surface thermography (Supplementary Figure 1) as previously described[7,15] to identify regional perfusion abnormalities and monitor organ temperature during re-warming.[20,21]

Maintenance of xenogeneic XC: Our lung perfusion and ventilation strategy with xenogeneic XC is similar to protocols used in clinical EVLP,[9,30] and has enabled functional recovery of *ex vivo* lungs across a variety of injuries.[7,15,16] Future studies may investigate development of advanced EVLP and ventilation strategies tailored to donor lungs with specific injury etiologies.

Lung interventions and evaluation: Xenogeneic XC is compatible with investigational diagnostics (e.g., transpleural imaging[31]) and therapeutics (e.g., surfactant replacement[12,15]) that can improve *ex vivo* organ recovery. In this study, lungs were systematically maintained on xenogeneic XC for a predetermined duration (24 hours). In the clinical setting, lungs would be maintained on xenogeneic XC only for the time necessary to recover. At the conclusion of xenogeneic XC, lungs can be procured from the circuit, flushed, and placed on ice, similar to clinical EVLP.

Study limitations: The marked variability in type and pattern of injury, reason for being declined, and overall quality of the human lungs available for this study led to notable variability in improvement and recovery of *ex vivo* donor lungs, and is an inherent limitation of the study design. Although limited characterization of the immunologic response (such as infiltration of swine immune cells and the cytokine milieu) during xenogeneic XC was previously reported by our group,[16] additional immunologic and immunomodulatory studies are needed to inform future translational efforts. Importantly, the risk for zoonotic infection during xenogeneic XC was not evaluated and remains to be fully investigated.

Envisioned applications: Beyond *ex vivo* donor organ support and recovery, the technique for xenogeneic XC has broad applicability in transplantation research and can be used to investigate: immunomodulatory strategies, therapeutic interventions, ischemia–reperfusion injury, and whole organ bioengineering.[30] Clinically, xenogeneic XC could be performed at centralized facilities to recover donor lungs that do not meet conventional isolated EVLP criteria, or fail to recover on isolated EVLP. In contrast to xenotransplantation, xenogeneic XC could mitigate risk of chronic rejection by: (1) only transiently exposing human donor lungs to xenoantigens, and (2) enabling allotransplantation of human organ into human recipient. Informed by previous studies that identified trace residual swine cells in human lungs after xenogeneic XC,[16] future studies will quantify and evaluate the clinical significance of swine residues. Importantly, donor lungs recovered by xenogeneic XC (including risk of infectious transmission) remain to be evaluated after transplantation. Overall, by integrating advantages of EVLP and xeno-support, our technique for xenogeneic XC provides *multisystem physiologic regulation* that effectively improves ex vivo donor lung support and recovery, and could facilitate expansion of the donor organ pool in the future.

Disclosure statement

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B.A.G. is a consultant for Xylyx Bio, Inc., which has licensing and commercial interests in cross-circulation technologies. J.D.O. is an employee and officer of Xylyx Bio, Inc. J.D.O., G.V-N., and M.B. own stock in Xylyx Bio, Inc. B.A.G., J.D.O., K.F., G.V-N., and M.B. are co-inventors on a patent application (WO2018013849A1) for cross-circulation as a platform for extracorporeal organ recovery, regeneration, and maintenance. W.K.W., R.U., J.W.S., and M.B. are coinventors on a patent application for an alternate configuration of cross-circulation for the same purpose.

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Author contributions

W.K.W., B.A.G., J.D.O., A.E.H., G.V-N., and M.B. conceptualized and designed the study. W.K.W., B.A.G., J.D. O., A.E.H., Y.T., J.W.S., M.B., and Y.J.P. performed the cross-circulation procedures and data acquisition. R.U., M. P., J.T., N.L.C., K.F. performed data acquisition. W.K.W., J.T., and N.L.C. performed histologic processing and examination. W.K.W., B.A.G., and J.D.O. analyzed the data. W.

K.W .performed the statistical analyses. W.K.W., B.A.G., and J.D.O. primarily wrote the manuscript. All coauthors edited and approved the final manuscript.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.healun.2022.11.002.

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