Tethered Liquid Perfluorocarbon Coating for 72 Hours Heparin-Free Extracorporeal Life Support

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Supplemental Methods:

TLP coating. Application of TLP to ECLS circuits and catheters was carried out in 2 phases. First, we sent entire ECLS circuits and components to Free Flow Medical Devices, LLC (Lancaster, PA, USA) for attachment of the perfluorinated silane tether layer. The tethered circuits were returned to our lab for conductance of experiments. Application of the liquid perfluorocarbon lubricant layer was carried out by us immediately before priming of the ECLS circuit with saline and start of cannulation. 150 mL of liquid lubricant, FluoroLube63 (FL63) was sterilized using a 0.2 µM filter and injected into the pre-membrane/venous face of the membrane lung. FL63 is a proprietary perfluorinated lubricant provided by Free Flow Medical Devices. Once the membrane was saturated, FL63 was drained from the oxygenator into the pump and tubing components and exposed to all blood-contacting surfaces. The excess FL63 was then withdrawn from the

circuit. The Avalon catheters with tethered layer applied were filled with FL63, then drained of lubricant immediately prior to cannulation.

Instrumentation. Female Yorkshire swine were anesthetized with 6 mg/kg Telazol® (tiletamine/zolazepam) delivered intramuscularly (IM). Atropine sulphate (0.05 mg/kg) was administered IM to prevent airway secretions. Endotracheal intubation was performed via direct laryngoscopy using a 7 mm endotracheal tube. A surgical plane of anesthesia was achieved using inhaled isoflurane (0.5 – 5% volume in oxygen). A 16 Fr Foley catheter was inserted trans-urethraly. Urine output was measured by the BARD® CRITICORE® Monitoring System (BARD Medical; Covington, Georgia, USA) to titrate fluid infusion during multi-day ICU care. The right jugular vein, left carotid artery and the left and right femoral arteries and veins were cannulated with 7-8.5 Fr sheath introducers (Arrow International Inc.; Reading, PA, USA) placed percutaneously with ultrasound guidance. Arterial sheaths were cannulated with IV lines for monitoring arterial blood pressure (ABP) and for collection of systemic blood samples. Venous sheaths were cannulated with IV lines for administration of fluids (normal saline) and anesthesia medications using an Alaris™ MedSystem III® Multi-Channel Infusion Pump (Alaris Medical Systems, Inc.; San Diego, CA, USA). Following venous line placement, animals were transitioned from inhaled anesthesia to total IV anesthesia (TIVA) for the remainder of the study. TIVA consisted of continuous infusion of fentanyl (1-4 mcg/kg/hr), ketamine (2-10 mg/kg/hr), midazolam (1-5 mg/kg/hr) and propofol (10-25 mcg/kg/min). Additionally, propofol boluses (1-3 mL) were delivered if evidence of spontaneous breathing, purposeful movement or discomfort were observed. Endotracheal intubation was converted to surgical tracheostomy using a 10.0 mm

tracheostomy tube. Animals were volume-control ventilated (Infinity V500, Dräger Medical; Lübeck, Germany) with room air, initial tidal volume (V_T) of 10 mL/kg and respiratory rate (RR) of 10-14 breaths/min. Positive end expiratory pressure (PEEP) was 5 cm H_2O. Animals were stabilized for 30 min prior to record of vital signs and collection of baseline samples. Urine output (UO), fluid balance, anesthesia rates and rectal core temperature were recorded hourly throughout the study.

Initiation of ECLS. Prior to cannulation, ECLS circuits were primed according to manufacturer's instruction using 1 L normal saline with 5000 U unfractionated heparin (UFH) added. Following initiation of ECLS, ventilator settings were incrementally adjusted as follows: V_T was reduced by 2 cc/kg to achieve a peak airway pressure below 35 cm H_2O; then, V_T was lowered to a minimum of 4 cc/kg. This progressive reduction of ventilator settings has been shown to reduce markers of VILI and pulmonary inflammation relative to standard lung-protective ventilation protocol of 6 mL/kg V_T in acute respiratory distress (ARDS) patients.[1] RR was incrementally reduced to a target of 4 breaths / min; and FiO_2 and ECLS settings were titrated to achieve a P_aO_2 > 60 mmHg and pH > 7.2.

Blood Labs and Coagulation. For complete blood count and plasma samples for cytokine analysis, arterial whole blood was collected in ethylenediaminetetraacetic acid (EDTA) BD Vacutainer® tubes (1.8mg EDTA/mL blood) (Becton Dickinson; Franklin Lakes, NJ). To prepare plasma, dual centrifugation was performed (3,000 g, 10 min) and plasma was frozen until later analysis (-80 ºC). For thromboelastography, platelet aggregometry and platelet poor plasma (PPP) for coagulation testing, whole blood was collected in 3.2% sodium citrate BD Vacutainer® tubes (Becton Dickinson; Franklin Lakes, NJ). PPP was prepared by dual centrifugation (3,000 g, 10 min) and aliquots were

frozen until later analysis (-80 ºC). For assessment of syndecan-1, whole blood was collected in BD Vacutainer® serum tubes (Becton Dickinson; Franklin Lakes, NJ), incubated for 1.5 hours, centrifuged (3,000 g, 10 min) and frozen for later analysis.

Blood Gas Analysis. Systemic and pre-/post-membrane blood gases were analyzed using the i-STAT1 Blood Analyzer (Abott; Chicago, IL). Systemic blood gas samples were collected from the femoral artery. Pre-membrane blood samples were collected from the right femoral venous line which was positioned to withdraw blood before entry into the ECLS catheter. Post-membrane samples were collected from a port located within the ECLS connective tubing directly after blood passage out of the membrane lung. The membrane CO_2 removal was determined by the percent decrease in pCO_2 from a pre-membrane to post-membrane blood gas at each time point. O_2 transfer was calculated from pre- and post-membrane blood gases as previously reported.[2] Oxygenation index ([F_iO_2 x mean airway pressure] / P_aO_2) and P_aO_2 to F_iO_2 ratio (PFR) were calculated. Blood urea nitrogen (BUN), postassium (K+) and creatinine were measured using an i-STAT CHEM8+ cartridge (Abott; Chicago, IL).

Fixation of explanted circuits. At end of study, the ECLS circuit and catheter were immediately removed from the animal, rinsed with 1.5 L phosphate buffered saline (PBS) at 1 L/min flow, drained and filled with 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer for fixation. After 48 hours, the membrane was drained of fixative and disassembled to expose the polymethylpentene (PMP) gas exchange fibers, as we have previously described.[3] A PMP fiber layer was collected from the inlet face, center and outlet face of each ML. These segments were then further dissected into 4 equal 1 cm^2 quadrants. Samples from the Avalon catheter were collected from the inlet and outlet

connective tubing, the center of the catheter, and the catheter tip. Circuit tubing samples were collected from the pre-membrane and post-membrane lines immediately before and after the ML. All samples were dehydrated in graded ethanol (35%, 50%, 70%, 100%; 10 min each) and stored in a desiccator until imaging analysis.

Thrombus area Digital images of the membrane lung inlet, center and outlet PMP fiber layers were captured and used to determine the percent area of thrombus deposition on each layer, which was scored by 3 blinded reviewers using ImageJ Software (NIH; Bethesda, MD, USA) as previously described.[3]

Field Emission SEM imaging. The membrane, tubing and catheter segments were sputter-coated with gold-palladium at 10 nm thickness (Leica Microsystems Inc.; Wetzlar, Germany) and imaged using field emission SEM (FEI Teneo; Hillsboro, OR, USA). An accelerating voltage of 10 kV was applied with a spot size of 9.

Protein adhesion: Protein adsorption on circuit tubing and catheters was determined as previously reported.[4,5] Briefly, following rinse with 100 mL PBS, 2 cm segments were collected in duplicated from circuit connective tubing, catheter inlet/outlet tubing connections, catheter center and catheter tip. Additional tubing samples that had not been exposed to blood were cut from the circuit before use as controls. Segments were incubated in 1 wt% aqueous solution of sodium dodecylsulfate (SDS) buffer with agitation at 37 C for 1 h. Samples were centrifuged at 200 g for 10 min to sediment red blood cells, and total protein concentration in the supernatant was determined using the Pierce™ bicinchoninic acid (BCA) test kit (ThermoFisher Scientific; Waltham, MA). Total protein was divided by the blood-contacting surface area of the sample.

Histology. Tissue samples from lungs, kidney, liver, jejunum, left ventricle and aorta were fixed in neutral-buffered 10% formalin for at least 48 hrs, trimmed, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin (H&E). Histological images were recorded using a 10x objective (Zeiss Axioskop; Oberkochen, Germany) and were evaluated by a pathologist blinded to study conditions to assess signs of toxicity, end-organ damage and diffuse alveolar damage in the lungs.[6]

References:

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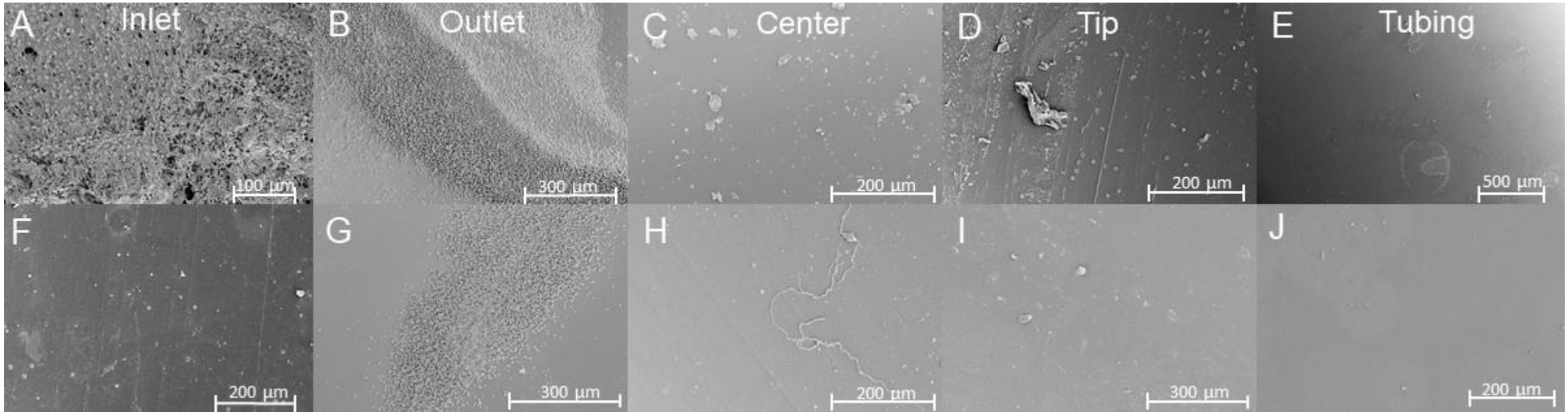
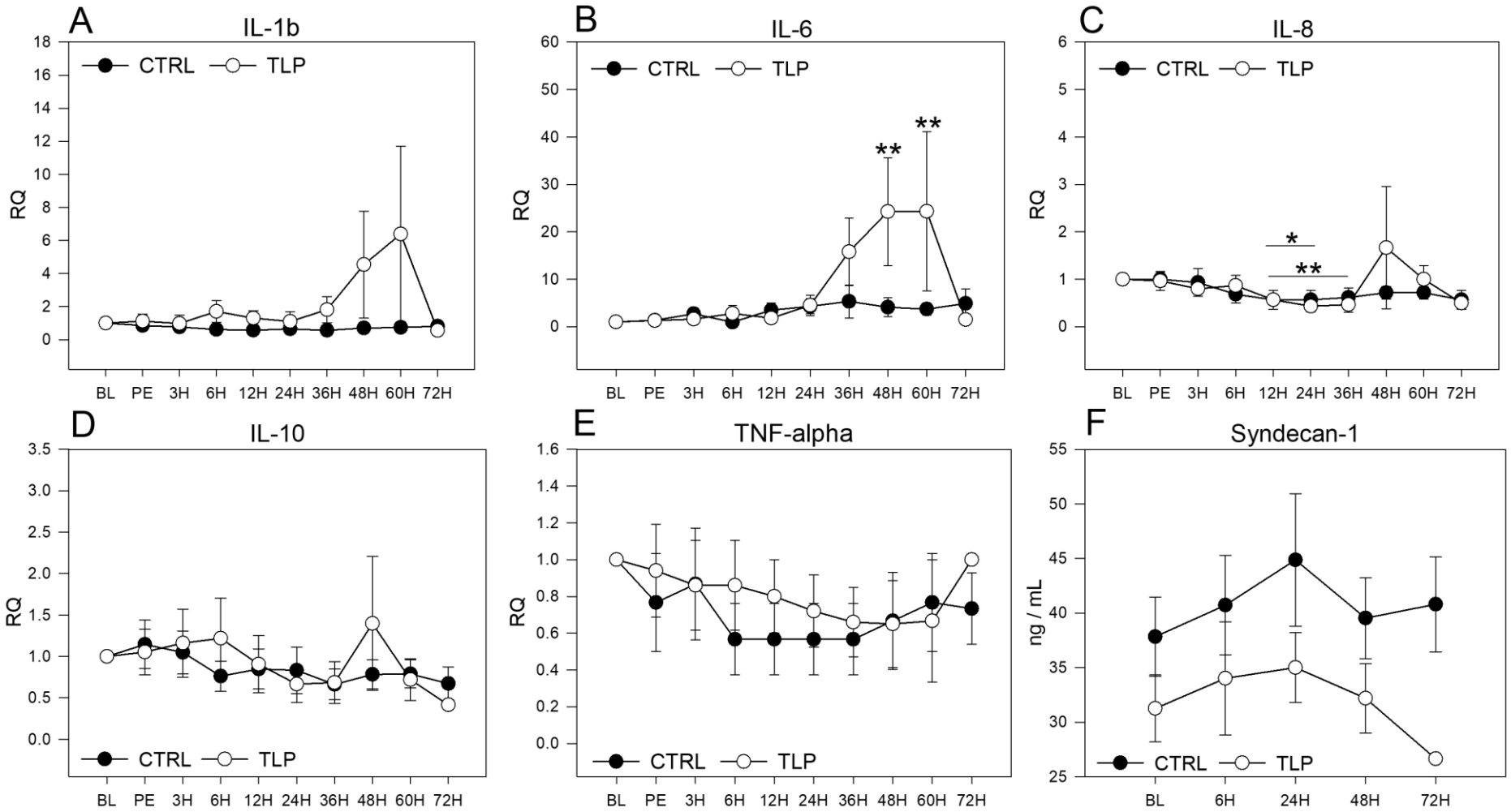
Supplemental Table 1. ECLS Circuit Variables

Variable	Group	Post-ECLS	3 hr	6 hr	12 hrs	24 hrs	48 hrs	72 hrs
Flow (L / min)	CTRL	1±0	1±0	1±0	1±0	1±0	1±0	1±0
	TLP	1±0	1±0	1±0	1±0	1.1±0.1	1.1±0.1	1.0
Sweep gas (L / min)	CTRL	5±0	5±0	5±0	5±0	5±0	5±0†	5±0
	TLP	5±0	5±0	6±1	7±2	10±4	14±4	10
Pump RPM	CTRL	2780±40	2660±20	2630±20	2620±40	2650±40	2700±40	3300±430
	TLP	2840±40	2760±40	2750±40	2730±40	2840±150	3000±170	2750
P ven (mmHg)	CTRL	-77±6	-67±1	-64±2	-62±2	-63±2	-64±2	-63±1
	TLP	-71±2	-67±2	-66±2	-65±2	-73±9	-79±9	-64
∆p	CTRL	23±1	21±1	20±1	19±1	21±1	23±1	142±85
	TLP	31±1	28±1	26±2	27±1	38±7	34±2	27

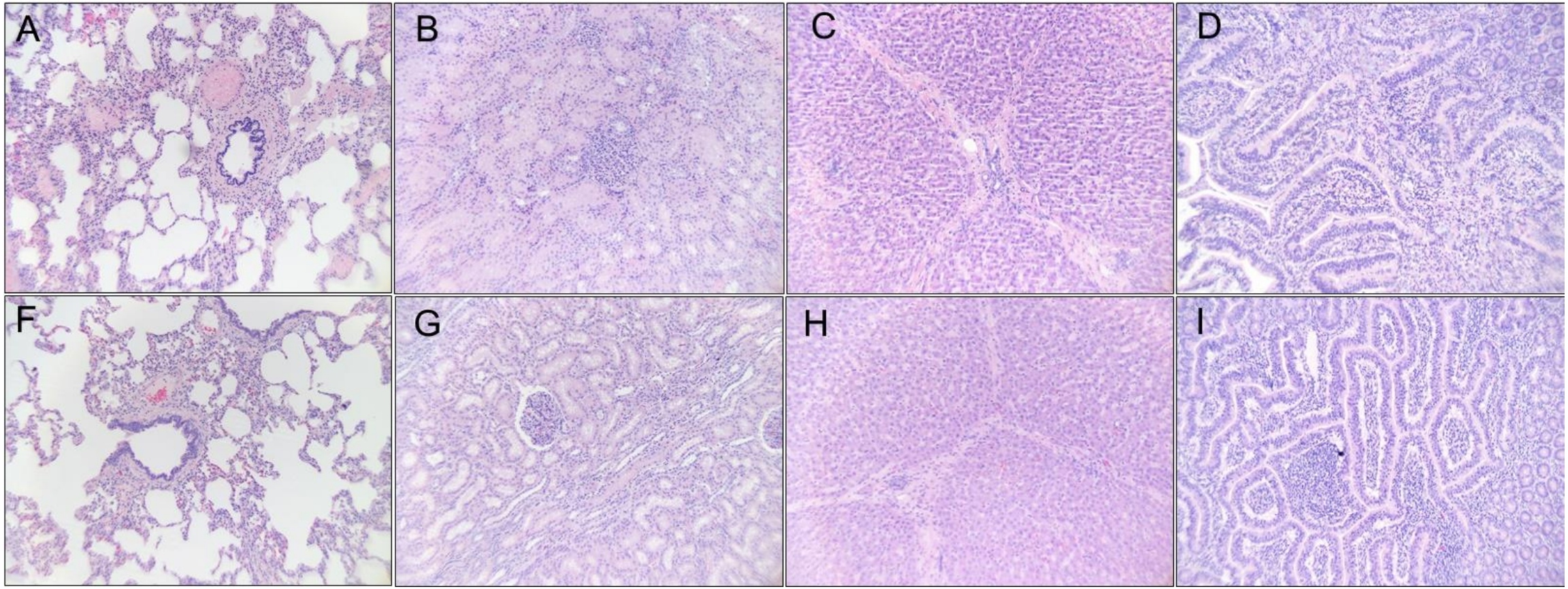
Mean ± standard deviation in control animals (CTRL; n=5) receiving extracorporeal life support (ECLS) with heparin-coated circuits and continuous heparin infusion and tethered liquid perfluorocarbon (TLP; n=5) group receiving ECLS using TLP-coated circuits and no systemic anticoagulation for 72 hours. RPM = pump revolutions per minute, Pven = membrane inlet pressure/negative pressure, ∆p = membrane pressure gradient/pressure drop. *Indicates significant change from baseline. †Indicates significant difference between control (CTRL) and tethered liquid perfluorocarbon (TLP) Groups. Significance $p<0.05$.



Supplemental Figure 1. Field emission scanning electron microscopy images collected from segments of 19 Fr dual-lumen catheters for veno-venous extracorporeal life support following 72 hours in vivo circulation in swine (n=5/group). Control catheters (CTRL; top row, panels A-E) were used with continuous heparin infusion and are compared to tethered liquid perfluorocarbon (TLP; bottom row, panels G-L) coated catheters used without systemic anticoagulation. Samples were collected from the catheter inlet connection (A, F), outlet connection (B, G), center of the catheter (C,H) and catheter distal tip (D, I). Greatest deposition in both groups was observed on the inlet and outlet connections. There was no apparent difference between groups in sample appearance.



Supplemental Figure 2. Mean ± standard deviation of systemic cytokine levels expressed as the relative quotient (RQ) from blood samples collected throughout 72 hours of extracorporeal life support (ECLS). Control animals (CTRL; n=5) received ECLS with heparin-coated circuits and continuous heparin infusion versus tethered liquid perfluorocarbon (TLP) animals (n=5) that received ECLS using TLP-coated circuits and no systemic anticoagulation. (A) Interleukin-1β (IL-1b), (B) interleukin-6 (IL-6), (C) interleukin-8 (IL-8), (D) interleukin-10, (E) tumor necrosis factor α, and (F) endothelial damage marker sydnecan-1 were measured at baseline (BL), post-initiation of ECLS (PE) and at 3, 6, 12, 24, 36, 48, 60 and 72 hours post-ECLS. *Indicates significant change from baseline in CTRL. **Indicates significant change from baseline in TLP. †Indicates significant difference between groups. All tests were two-sided with significance $p<0.05$.



Supplemental Figure 3. Representative images from control animals (CTRL) receiving ECLS with heparin-coated circuits and continuous heparin infusion and tethered liquid perfluorcarbon (TLP) animals receiving TLP-coated ECLS without systemic anticoagulation for 72 hours circulation. CTRL images (top row, panels A-D) were comparable to TLP images (bottom row, panels F-I) in this subset of animals where tissue from the lung (A, F), kidney (B, G), liver (C, H) and jejunum (D, I) were unremarkable with no signs of toxic injury, organ damage or thrombosis/hemorrhage.