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Pre-clinical evaluation of an adult extracorporeal carbon dioxide removal system with active mixing for pediatric respiratory support

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Abstract

The objective of this work was to conduct pre-clinical feasibility studies to determine if a highly efficient, active-mixing, adult ECCO₂R system can safely be translated to the pediatric population. The Hemolung Respiratory Assist System (RAS) was tested *in-vitro* and *in-vivo* to evaluate its performance for pediatric veno-venous applications. The Hemolung RAS operates at blood flows of 350 – 550 mL/min and utilizes an integrated pump-gas exchange cartridge with a membrane surface area of 0.59 m² as the only component of the extracorporeal circuit. Both acute and 7-day chronic *in-vivo* tests were conducted in healthy juvenile sheep using a veno-venous cannulation

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strategy adapted to the *in-vivo* model. The Hemolung RAS was found to have gas exchange and pumping capabilities relevant to patients weighing 3 – 25 kg. Seven-day animal studies in juvenile sheep demonstrated that veno-venous extracorporeal support could be used safely and effectively with no significant adverse reactions related to device operation.

Keywords

extracorporeal carbon dioxide removal (ECCO₂R); gas exchange; Hemolung; *in vitro* characterization; pediatric extracorporeal membrane oxygenation (ECMO); pediatric respiratory assist device; pump-oxygenator

Introduction

Extracorporeal membrane oxygenation (ECMO) has been clinically utilized for four decades to provide respiratory support to children and neonates with cardiac and lung failure. For the pediatric population in need of extracorporeal pulmonary support, there is not yet an integrated system designed for long term support that has received regulatory review and approval. This has led to off-label use of existing components approved for short term cardiopulmonary bypass (CPB). These improvised devices are generally not optimized for chronic use and are often part of complex circuits, requiring specialized staff for device implementation and management (1). Their use has also been associated with mild to severe bleeding complications (2). Off-label use of medical devices is potentially dangerous, creating a great clinical need for the development of safe and effective pediatric devices.

Adult extracorporeal carbon dioxide removal (ECCO₂R) systems and pediatric ECMO share the common objectives of having a low blood flow rate and low priming volume while safely maintaining sufficient respiratory support. The oxygenation capabilities of ECCO₂R devices are overlooked in adult treatment due to the high blood flow rates necessary for sufficient O₂ delivery. With typical blood flow rates of 350 – 550 mL/min and a whole circuit priming volume of 270 mL, operating conditions of adult ECCO₂R devices are highly relevant to the pediatric population. The objective of this work was to conduct pre-clinical feasibility studies to determine if a highly efficient, active-mixing, adult ECCO₂R system can safely be translated to the pediatric population.

The Hemolung® Respiratory Assist System (RAS) is a highly simplified adult ECCO₂R system intended for partial respiratory support in adult patients with acute hypercapnic respiratory failure. The results of a clinical feasibility study of the Hemolung RAS in adults has been previously published (3). The Hemolung received post-market regulatory approval for use in the European Union, Canada, and Australia in early 2013. For this study, the Hemolung RAS was tested both *in-vitro* and *in-vivo*, in acute and chronic (7-day) studies in a juvenile sheep model, to evaluate its potential for pediatric applications. *In-vitro* testing focused on establishing the pumping performance of the existing system in a pediatric configuration as well as determining the gas exchange capabilities. *In-vivo* studies focused on verification of *in-vitro* testing in addition to evaluating the safety and performance of the Hemolung RAS in a pediatric configuration in longer-term studies. Healthy juvenile sheep were used for consistency between studies and to allow evaluation of treatment safety

independent of respiratory related injuries. The extracorporeal circuit was connected to all animals in for veno-venous support, as is clearly indicated in the absence of cardiac dysfunction (4). In all evaluations, we hypothesized that gas exchange capabilities of the Hemolung RAS in this model would be equivalent to those for the adult configuration performance at similar blood flows - minimum CO₂ removal of 50 mL/min at a venous partial pressure of CO₂ (PCO₂) equal to 45 mmHg. Target blood flow rates were set to a minimum of 280 mL/min, which approximates extracorporeal support conditions compatible with patients weighing 3 kg. We hypothesized that the Hemolung RAS could safely and reliably operate at this flow rate and gas exchange rate without failure or adverse reaction to treatment for 7 days.

Materials and Methods

Device description

The Hemolung RAS (ALung Technologies Inc., Pittsburgh, PA, USA) consists of a cylindrical cartridge containing a cylindrical bundle of hollow fiber membranes (HFMs) arranged lengthwise and potted annularly between the outside housing and a rotating, hollow, cylindrical core. A cross-sectional schematic of the Hemolung device is shown in Fig. 1. The rotating core centrifugally pumps blood from an upper central inlet channel through distribution channels in the upper end of the rotating core that run from the center of the core to the outer surface. From the distribution channels, the blood flows downward, across the bundle of hollow fibers in the space between the rotating cylinder and outer housing wall (see the expanded area in Fig. 1), exiting through an outlet port at the bottom of the outer housing.

The rotation of the cylindrical core also serves to generate secondary flow patterns adjacent to the fibers which enhance gas transfer efficiency by breaking down diffusional boundary layers. The thickness of the gap between the core and the bundle of hollow fiber membranes, as well as the radius and optimal rotational speed of the core were chosen to ideally generate Taylor vortices in the gap. The actual formation of Taylor vortices is exceedingly difficult to confirm for this design, however, gas exchange has been verified to increase by more than 60% due to rotation of the core as shown in Fig. 2. The shear stress generated on the outer surface of the core at maximum rotational speeds used for therapy (1400 RPM) is estimated to be approximately 100 dyne/cm², assuming laminar flow, and conservatively, up to 1000 dynes/cm² if laminar flow is not assumed. This range is well below the accepted level of 1,500 dyne/cm² for red cell lysis (5). In clinical feasibility study of the Hemolung device in adults, there were no reports of clinically significant hemolysis.

The hollow fiber membranes are microporous polypropylene coated with an ultrathin (1 micron) nonporous layer of siloxane to prevent wetting. The siloxane layer is further coated with covalently bound heparin to prevent thrombus formation. The total fiber surface area of the Hemolung is 0.59m², which is more than 90% of the total circuit surface area to which the blood is exposed. Sweep gas (pure O₂ or room air) is pulled through fibers under negative pressure with a vacuum pump to prevent air from crossing in to the blood in the event of a fiber break.

The core rotates freely on a fixed shaft with a bearing flushed by infused 0.9% NaCl sterile saline (30 mL/hr). The saline flush flows from inside the bearing space below the blood compartment across the seal and in to the blood compartment. The saline flush both prevents blood from leaking in to the rotor compartment, and helps to prevent stagnation of blood in the blood compartment space below the spinning core, adjacent to the seal. Magnets in the rotating core are coupled to driving-magnets on the Hemolung controller. Circuit blood flow is controlled by setting the core rotation rate on the controller interface. Sweep gas flow rate is also controlled directly on the controller interface. Gas pathway equipment necessary for measurement of the rate of CO₂ removal is integrated into the controller, and the CO₂ removal rate is displayed in real time on the user interface. In the pediatric configuration being investigated, the circuit inlet and outlet tubing (each 6ft [1.82m] long, ¼" (6.35mm) inner diameter) are connected to an 8–10 Fr one-piece femoral venous cannula (96830-008/96830-010; Medtronic, Minneapolis, MN, USA) and an 8 Fr one-piece arterial cannula (77008; Medtronic, Minneapolis, MN, USA), respectively. The Hemolung cartridge has a measured priming volume of 144 mL, and total circuit priming volume is 267 mL.

In-vitro testing

Pump performance testing—Pump performance evaluation of the Hemolung RAS in a pediatric configuration was conducted in a recirculating loop with carboxymethylcellulose (CMC) (Sigma-Aldrich, St. Louis, MO, USA) viscous blood analogue (3 – 4 cP). The loop consisted of the Hemolung cartridge and a reservoir. Flow rate was monitored with an online ultrasonic probe (Transonic Systems, Ithaca, NY, USA). Fluid pressure was measured differentially (PX771-025DI; Omega Engineering Inc., Stamford, CT, USA) at the Hemolung cartridge inlet and outlet with in-line sampling ports. Five pressure-flow measurements were recorded for various pump rotation rates by adjusting circuit resistance with an on-line Hoffman clamp.

Gas Exchange Testing—Gas exchange performance of the Hemolung in the pediatric configuration was conducted using parallel *in-vitro* circuits. A recirculating loop was used to balance blood gas tensions before flow was redirected to a single-pass circuit consisting of the Hemolung cartridge and a drainage reservoir. The recirculating balance loop consisted of the primary reservoir, a centrifugal pump (BPX-80; Medtronic, Minneapolis, MN, USA), and a commercial oxygenator (Affinity NT; Medtronic, Minneapolis, MN, USA) to control inlet O₂ and CO₂ gas tensions (with N₂/CO₂/O₂ sweep gas mixture). Following recirculation, flow was redirected to the single-pass circuit containing the Hemolung cartridge and drainage reservoir. Flow rates through the Hemolung cartridge were adjusted with a Hoffman clamp to match rates determined in pump performance testing for specific core rotation rates to simulate conditions of the pediatric configuration. Pure oxygen sweep gas was pulled through Hemolung fibers at 8.0 L/min. Testing followed AAMI standards for evaluation of gas exchange in blood oxygenators (6). Slaughterhouse bovine blood was collected the morning of testing and anticoagulated with sodium heparin (10,000 U/L). Total blood hemoglobin was adjusted to 12 ± 1 g/dL, and loop temperature was maintained at 37°C. Blood gas levels were adjusted to PCO₂ = 45 ± 5 mmHg, sO₂ = 65 ± 5% in the recirculating loop as verified by a RapidLab 248 blood gas analyzer (Siemens, Erlangen, Germany) and an in-line oxyhemoglobin saturation probe (Biotrend; Medtronic,

Minneapolis, MN, USA). Raw CO₂ removal rate (vCO₂) was recorded directly from the Hemolung RAS interface after adjusting to each rotation and blood flow rate. The exchange level was then normalized (vCO₂^{*}) to a PCO₂ = 45 mmHg to eliminate variability associated with small changes (< 5 mmHg) from the target PCO₂ (Equation 1).

$$vCO_2^* = vCO_2 \frac{45 \text{ mmHg}}{PCO_{2,\text{inlet}}} \quad [1]$$

Oxygen exchange (vO₂) was calculated based on blood side measurements from Equation 2, which accounts for both dissolved and hemoglobin-bound O₂ (7).

$$vO_2 = \left(\delta_{O_2}^{Hgb} \cdot Hgb \cdot [sO_{2,\text{out}} - sO_{2,\text{in}}] + \alpha_{O_2}^{37^\circ C} \cdot [PO_{2,\text{out}} - PO_{2,\text{in}}] \right) \cdot Q_b \quad [2]$$

where $\delta_{O_2}^{Hgb}$ = hemoglobin binding capacity to oxygen in volume gas per mass of hemoglobin (1.34 mL_{O₂}/gHgb)(8); Hgb = total blood hemoglobin concentration (g/dL); sO₂ = oxyhemoglobin saturation at inlet or outlet (~0–1); $\alpha_{O_2}^{37^\circ C}$ = oxygen solubility in blood (3.17 × 10⁻⁵ mL_{O₂}/mL_{blood}/mmHg); PO₂ = oxygen partial pressure at inlet or outlet (mmHg); and Q_b = blood flow rate (mL/min). Gas exchange data was collected at blood flows corresponding to rotation rates ranging from 500 – 2000 RPM to simulate a pediatric configuration. Measurements were also made at those rotation rates without adjusting loop resistance so that the O₂ rated flow rate (i.e. the maximum blood flow rate where 65% oxygenated blood can be fully saturated in a single pass through the device) could be determined.

In-vivo testing

Animal preparation and surgical procedure—Two acute (~6-hour) and twelve chronic (7-day) studies followed protocols approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Handling and care of animals was in accordance with the USDA Animal Welfare Act and the current guide for laboratory animal care and use prepared by the Institute of Laboratory Animal Resources at the National Academy of Sciences. Fourteen healthy juvenile sheep (19.0 – 32.5 kg) were induced with atropine (0.05 mg/kg) and Ketamine (22 mg/kg) prior to intubation with an endotracheal tube and placed on ventilation. Anesthesia was maintained during surgery with isoflurane (1–3.5%). In acute studies, a monitoring line was placed in the right carotid artery (for mean arterial pressure, MAP, and arterial blood gas analysis) and a Swan-Ganz catheter was floated to the pulmonary artery (PA) via the right external jugular (REJ), distal to the extracorporeal circuit (ECC) cannula insertion sites. Cardiac output (CO), central venous pressure (CVP), and PA blood gas analysis were achieved from the Swan-Ganz. In chronic studies, both venous and arterial monitoring lines were placed in the left neck vessels. Hemolung circuit cannula were inserted in the REJ using an open-technique after administering a heparin bolus (80 U/kg) with the drainage (pump inlet) cannula toward the

head and perfusion cannula (pump outlet) toward the superior vena cava (SVC). The REJ was ligated between cannula insertion points. The Hemolung circuit was pre-primed with heparinized saline, and extracorporeal flow was initiated immediately following connection to the animal. Anticoagulation was maintained with heparin between 1.5 – 2.3 times baseline activated partial thromboplastin time (aPTT). Baseline measurements included blood gas analysis, blood chemistry, complete blood count, aPTT, and plasma free hemoglobin (pfHb).

Acute in-vivo gas exchange—Ventilator settings were adjusted to match inlet blood gas tensions tested *in-vitro* ($\text{PCO}_2 = 45 \pm 5$ mmHg; $\text{sO}_2 = 65 \pm 5\%$). Gas exchange measurements were taken with a constant sweep flow of 8 L/min at various blood flows, with the maximum flow limited by the total blood flow through the REJ. Exchange data was also collected at the maximum blood flow with varying sweep gas flows (2 – 8 L/min). After verification of blood gas levels, arterial, PA, Hemolung cartridge inlet (venous), and Hemolung cartridge outlet blood samples were drawn for gas analysis. CO, CVP, MAP, cartridge core rotation rate, and CO₂ removal rates were also recorded.

Chronic (7-day) in-vivo testing—After initiation of ECC flow, the insertion site was closed and cannula were secured to the neck. The animal was extubated and awoken in the OR before transfer to the ICU where it was cared for in stanchions. Antibiotic (Kefzol; 1 g/8hrs) was given for the full 7-day study and analgesic (Banamine; 2.2 mg/kg/6–8hrs; IV) for up to 72 hours in the ICU. Blood samples for venous gases, arterial gases, pump outlet gases, complete blood count, serum chemistry, coagulation parameters, and pfHb were taken at predetermined intervals. All Hemolung data (CO₂ removal, core RPM, Q_g , Q_b) was logged every 2 minutes by the controller software. Venous blood samples were drawn from the left external jugular via the venous maintenance catheter, and assumed to be hematologically equivalent to Hemolung inlet flow from the REJ. Venous PCO₂ was measured every 8 hours and used for CO₂ removal normalization according to Equation 1. Pump outlet blood gases were measured daily to monitor oxygenation. Arterial blood samples for gas analysis were taken every 2 hours for the first 24 hours of treatment after which only as necessary to monitor animal well-being (approximately every 24 hours).

Study termination and necropsy—At the end of each study, a final round of blood samples were drawn. A heparin bolus was administered (100 U/kg) prior to euthanasia. The Hemolung circuit was disconnected and flushed with heparinized saline and inspected for thrombus deposition. A complete necropsy was conducted with ECC cannula in place. Indwelling vessels were opened and inspected for gross damage or thrombus related to cannula placement.

Statistical Analysis—Measurements associated with hematology and organ function on Day 7 of chronic *in-vivo* studies were compared to baseline levels using a two-tailed Student's t-test in Microsoft Excel. Equality of variance (i.e. hetero- or homoscedasticity) was determined with and f-test.

Results

In-Vitro Testing

The pump performance curves for the Hemolung cartridge are shown in Fig. 3A. The pump curves represent the ability of the Hemolung to generate a pressure head against an afterload for a given pump speed and circuit flow. Test conditions and the resulting pump curves roughly simulate veno-venous attachment since net head across inflow and outflow cannula during *in-vitro* testing was negligible. The Hemolung RAS achieved fluid flow rates of 0.3 L/min at 1000 RPM with a pressure head of approximately 100 mmHg and with both cannula (i.e. 8 or 10 Fr inflow and 8 Fr outflow). Maximum blood flow was 0.82 L/min at 2000 RPM against a pressure head of 320 mmHg.

In-vitro gas exchange rates in blood with and without pediatric cannulation are shown in Fig. 3B together with necessary O₂ delivery to fully replenish metabolic consumption versus body weight in kilograms (W_{kg}) for pediatric applications (125 mL O₂/BSA, where $BSA[m^2] = [4 * W_{kg} + 7]/[90 + W_{kg}]$ (9,10)). The plots are overlaid such that W_{kg} aligns with relevant circuit blood flow rates (125 – 150 mL/min/kg) (11). Both normalized CO₂ removal and O₂ delivery increased approximately linearly versus blood flow with and without circuit cannula. The maximum transfer rates with pediatric cannulation were 108 mL CO₂/min and 56 mL O₂/min at 0.83 L/min of blood flow and a pump speed of 2000 RPM. Without cannula, the highest transfer rates were 154 mL CO₂/min ($Q_b = 2.0$ L/min; 1825 RPM), and 125 mL O₂/min ($Q_b = 2.3$ L/min; 2000 RPM). The CO₂ removal measurement at 2.3 L/min was omitted since a steady state reading could not be reached at that flow due to the sampling time restrictions associated with the single-pass test loop. Blood at the device outlet remained 100% saturated at the controller's maximum motor speed (2000 RPM; $Q_b = 2.3$ L/min) with inlet sO₂ = 63%, indicating the O₂ rated flow exceeds 2.3 L/min.

Acute In-Vivo

The Hemolung RAS was successfully connected to two animals (S008–13, S009–13) for acute *in-vivo* testing. Maximum blood flow rates available to the Hemolung circuit (i.e. the blood flow rate delivered to the right jugular) were 0.33 L/min at 1000 RPM and 0.47 L/min at 1500 RPM in each animal. Maximum normalized CO₂ removal and O₂ transfer rates were 65.0 ± 8.9 mL CO₂/min and 22.2 ± 2.8 mL O₂/min at $Q_b = 0.47$ L/min as shown in Fig. 4A. Both CO₂ and O₂ transfer rates varied linearly with blood flow at constant sweep gas flow of 8.0 L/min. Circuit blood flow rates in each of the two studies did not match exactly due to the variation in blood vessel diameter for each animal, however gas transfer repeatability between cartridges can be inferred from the overlapping datasets.

Exchange rates with varying blood flow and fixed sweep gas flow are shown in Fig. 4B as a fraction of gas transfer at maximum sweep gas flow (8.0 L/min). Oxygen transfer was generally insensitive to increasing sweep gas flow as anticipated, achieving 88% of the O₂ delivery at 2.0 L/min compared to 8.0 L/min. CO₂ removal increased significantly between sweep gas flows of 2.0 and 4.0 L/min (54%), then appeared to plateau as flow approached 8.0 L/min (< 10% increase from $Q_g = 4.0$ to 8.0 L/min). The diminishing return in gas

exchange for increasing sweep gas flow is reflective of the high blood flow to sweep gas flow ratio ($Q_b:Q_g$) and indicates that further increasing sweep gas flow would not significantly affect CO_2 removal.

Chronic In-Vivo

All 12 animals were successfully connected to the Hemolung RAS. Seven days of treatment was achieved in seven animals, with five studies ending prematurely. None of the five early terminations were a result of the Hemolung RAS, but were due to complications associated with central venous catheters or monitoring lines. A summary of all animal outcomes is listed in Table 1. The first animal (S021-11) died unexpectedly after 19 hours of treatment due to cranial hematoma and ischemic brain injury. The cannulation in the first animal differed from subsequent animals in that the drainage and perfusion cannula were placed in independent jugulars (i.e. left and right). We believe this arrangement restricted blood flow away from the head, resulting in cranial edema and heightened intracranial pressure, which was the primary cause of the ischemic injury that ultimately lead to study termination. The cannulation strategy was subsequently modified such that both drainage and perfusion cannula were placed in the right external jugular. In two cases (S022-11 and S006-13), the animal was electively euthanized after 5 and 86 hours of treatment due to cannula dislodgement (drainage and perfusion, respectively) from the REJ. Two studies were terminated due to respiratory related issues. Animal S023-11 died suddenly approximately 19 hours after initiation of therapy due to pulmonary embolism. A necropsy conducted immediately following the event revealed that the venous monitoring line had migrated 3 – 4 cm above the SVC-right atrial junction and was surrounded by thrombus. The pump outflow cannula, which was closest to the thrombus was clean and properly positioned inside the jugular lumen. During surgery, prior to inserting the Hemolung perfusion cannula, a lack of backflow observed in the REJ suggests the thrombus may have developed prior to cannulation. Animal S010-13 experienced respiratory distress shortly following surgery and ventilator extubation, indicated by arterial hypercapnia and hypoxemia. Oxygen was provided by mask intermittently for ~2 hours until blood gases recovered, at which time the animal was alert and stable. At hour 84, the animal became agitated with shortness of breath prior to collapsing, at which time the animal was euthanized per IACUC protocol requirements. The Hemolung cartridge, circuitry, and indwelling vessel were clear of thrombus or blockage. Upon opening the right chest a large consolidated clot (6 – 8 cm axial plane diameter) was found within the chest, superior to the heart and lungs (dorsal to the bronchial region) extending 8 – 10 cm up the trachea. A small perforation (2 – 3 mm) was found in the LEJ immediately distal to the tip of the indwelling maintenance line. Margins of the perforation were rough, and advanced clotting in the surrounding tissue was observed. The tip of the maintenance line was cut to a point for ease of insertion prior to placement during surgery, possibly causing the puncture during placement. Gradual aggregation of a consolidated clot in the chest due to repeated agitation from animal movement in the days following surgery culminating in respiratory distress would be consistent with our observations.

The daily averaged blood flow rates are shown in Fig. 5A. Circuit flow was maintained at or above target flows (280–300 mL/min) for the duration of most studies. In two animals

(S005–13 and S007–13), the jugular became fully exsanguinated at target flows resulting in suction of the vessel onto the drainage cannula, so pump speeds were reduced to achieve 240–260 mL/min (Day 1 averages), where no flow limitations were observed. In these animals, daily average blood flow decreased by 2 – 4%. Cranial edema was observed post-operatively (within 3 hours) in three animals (S021–11, S041–12, and S010–13), possibly an adverse reaction to surgery or a result of increased flow resistance in the cranial venous return due to maintenance line and Hemolung circuitry in the jugulars. The Hemolung cartridge pump speed was increased to raise circuit flows in the latter two animals, increasing fluid drainage from the head. Edema waned over several hours in these animals, at which time pump speeds were reduced.

Normalized CO₂ removal rates were averaged daily and are shown in Fig. 5B. CO₂ removal rate was anticipated to be similar to the levels achieved in adult sheep with the Hemolung RAS (a minimum of 50 mL/min at a venous PCO₂ = 45 mmHg) (12). Daily venous PCO₂ averages are listed in Table 2 together with the overall average acid/bases status of venous blood. The overall average reduction in blood PCO₂ due to circuit flow (inlet minus outlet) was 24.5 ± 1.9 mmHg, as shown in Fig. 6. Normalized CO₂ removal rates greater than 50 mL/min were maintained for the full duration of all but one study (S005–13), in which low CO₂ removal rates correspond with reduced blood flow rates. Blood drawn from the Hemolung circuit perfusion line remained at 100% oxygen saturation for the duration of all studies, indicating maximal oxygenation of circuit blood flow. Arterial blood gases and acid/base status of each animal was monitored, the daily averages of which are provided in Table 3. Arterial pH and oxygenation levels remained within normal physiologic ranges throughout testing. Arterial pCO₂ and HCO₃⁻ each remained at the lower end of normal ranges for the majority of testing, likely due to the CO₂ removed by the external circuit in addition to that eliminated by the healthy lungs. Base excess was consistently below the typical reference range, which is not surprising given the lower levels of CO₂ and HCO₃⁻, with the exception of a spike on Day 3 that was consistent in most animals. This observation is common in chronic sheep studies, and is generally related to diet as animals acclimate and resume more normal eating habits, which can slightly alkalize circulating blood.

The hematology data from collected blood samples are summarized in Table 4. Necropsy and cartridge inspection conducted at the conclusion of each 7-day study revealed minor thrombi concentrated at the fiber knitting in the devices as shown in Fig. 7A, but no significant thrombus formation was found in the cartridge or extracorporeal circuit in any of the animals. Some coagulation was observed in one cartridge (S005–13) near the top and bottom of the fiber potting (Fig. 7B). The accumulation was potentially due to flow stasis in those areas, which was likely related to the below-target average blood flow rates. Fibers in proximity of the cartridge outlet were clear of aggregation (Fig. 7C). No evidence of thrombus formation or vessel injury was found after REJ cannula removal in any animal.

Discussion

Conventional pediatric ECMO systems are complex, associated with risk of hemorrhaging, and often require off-label use of circuit components due to inadequate research attention specifically to pediatric extracorporeal support (1). These issues can be addressed with a

simple-to-use, highly efficient, pediatric respiratory assist system that integrates pump and oxygenator components to minimize complexity. The Hemolung RAS is an adult ECCO₂R system with a circuit priming volume and fiber surface area comparable to existing pediatric ECMO systems (Table 5) that operates at flows similar to renal dialysis (350–550 mL/min) (13). In these studies, we evaluated the performance and safety of the Hemolung in a pediatric configuration in *in-vitro* gas exchange and pump testing, and at low blood flows (~280 ml/min) in acute and seven-day chronic *in-vivo* studies in juvenile sheep.

Extrapolated *in-vitro* gas exchange trends for the pediatric cannulation case do not predict sans-cannula performance well for either CO₂ or O₂ and vice versa. This effect is due to the enhancement in transfer efficiency attributed to the ActiveMix cartridge design. The circuit flow resistance contributed by the small diameter pediatric cannulae requires greater pump speeds to achieve similar blood flow rates with cannula versus without. At the higher rotation rate the effectiveness of ActiveMix increases, resulting in more efficient gas transfer. The required pump speed to achieve 0.83 L/min with cannula was double that of the sans-cannula case (2000 versus 1000 RPM), resulting in 30–40% higher exchange rates due to the ActiveMix effect. While the true O₂ rated flow could not be determined due to equipment limitations, we found it to be at least 2.3 L/min, which approximately matches or exceeds all pediatric oxygenators commercially available and in development (11). Compared to metabolic consumption, the Hemolung RAS can deliver O₂ sufficient for full metabolic support for patients 3 – 25 kg, or up to ~10 kg with the specific cannulation tested at a pump rotation of 2000 RPM. Full respiratory function is not necessarily required or desired, so partial support could be provided for a broader patient population.

The strong dependence of gas transfer on blood flow for low blood flow ranges (<500 mL/min) was anticipated for both O₂ and CO₂ in acute *in-vivo* gas exchange testing. O₂ transfer with extracorporeal gas exchangers is limited primarily by the rate of hemoglobin exposure (i.e. blood flow rate) given the large partial pressure differential across the HFM wall (~600 – 700 mmHg). In contrast, CO₂ removal is typically dependent on sweep gas flow and is less sensitive to blood flow as a result of the low partial pressure differential across the HFM wall (~0 – 50 mmHg). Given that sweep gas flow is much greater than blood flow under typical operating conditions, however, accumulating CO₂ in the sweep gas is quickly dispelled from the fibers, and a high blood to sweep gas PCO₂ gradient is maintained for increasing blood flow.

Complications associated with the chronic *in-vivo* studies that resulted in premature termination were not directly related to Hemolung RAS circuit or operation. The cause of cranial edema in some animals was unclear (i.e. reaction to surgery or inadequate drainage from the head) however the condition was transient and when addressed did not cause further complications. In cases of respiratory related deaths, no evidence was found to indicate that injuries were a consequence or exacerbated by connection to the Hemolung. In both animals, pre and post-mortem observations are consistent with issues that would derive from the venous monitoring line.

Circuit flows relevant to patients of approximately 3 kg (~280 ml/min) were maintained for the duration of 7-day *in-vivo* studies with the exception of two animals, in which

physiologic limitations (i.e. low jugular blood flow) prevented reaching target flows. Low initial circuit flow rates may also explain the small, gradual decline in blood flow (2 – 4 %/ day) in these two studies, and the minor instances of thrombus found within the cartridge fiber bundle used for S005–13 (Fig. 7B and 7C). Thrombus in oxygenators is commonly found in areas of flow stasis, which would be consistent with our observations given that the fiber bundle in proximity to the outlet port was mostly clear of cell aggregation or thrombus. These observations may indicate a minimum operable blood flow for the Hemolung RAS in a pediatric configuration of 280 mL/min, which is similar or lower than recommended minima for commercially available pediatric oxygenators.

Normalized CO₂ removal was maintained at or above the adult target minimum rate of 50 mL/min for all 7-day studies with the exception of the animal with declining blood flows (S005–13). Since the sweep gas flow was much greater than the blood flow under chronic operating conditions, CO₂ removal was sensitive to changing blood flows. This is verified by comparison of *in-vitro* to S005–13 *in-vivo* gas exchange data, where normalized CO₂ removal varied with blood flow by 0.11 mL CO₂/mL_{blood} and 0.10 mL CO₂/mL_{blood}, respectively.

Some changes in hematological parameters were observed on Day 7 versus baseline, but none were manifested clinically. WBC and fibrinogen levels increased with significance however both remained well within normal ranges for sheep. HCT, Hgb, ALT, and Creatinine were all below physiologic norms on Day 7, however none deviated significantly from baseline concentrations, which were also out of normal ranges. No concerning levels of pfHb were observed in any animal during 7-day studies. The results of these tests are consistent with the outcomes reported in the human clinical testing of the Hemolung (3). Meaningful comparisons to existing technology could not be made since to our knowledge this is the first reported pediatric pre-clinical study to evaluate a respiratory assist device in chronic *in-vivo* studies. Existing devices used for pediatric ECMO are reconfigured CPB systems used off-label, so laboratory data is unavailable for this application. Several pediatric devices intended for a similar application as that investigated here are described in the literature (11,14–18), however no *in-vivo* testing > 24 hours has been reported.

The use of juvenile sheep as a representative model for neonatal and pediatric patients with lower body weights is a potential criticism of these studies. The fraction of cardiac output through the Hemolung circuit in the chosen animal model does not necessarily match that of intended patients, though patients 3 – 25 kg may benefit from the absolute flows tested here. The physiological impact on lower weight patients may therefore differ in ways undetectable here. Restrictions on age and weight of sheep due to necessary weaning periods prevent use of smaller animals, however other groups have used similar ovine models for neonatal feasibility studies including those partnering with the United States FDA (19). We therefore feel that the model chosen can appropriately represent the dedicated patient population for the discussed application.

Conclusions

The objectives of this study were to characterize performance of the Hemolung RAS in a pediatric configuration and to conduct feasibility studies to determine the efficacy of direct translation to the pediatric population. The Hemolung RAS was found to have gas exchange and pumping capabilities relevant to patients weighing 3 – 25 kg. Seven-day animal studies in juvenile sheep demonstrated that veno-venous extracorporeal support could be provided safely at low-flows relevant to patients of approximately 3 kg with no significant adverse reactions related to device operation. These study outcomes suggest the potential exists for use of the Hemolung RAS in a veno-venous pediatric configuration to safely provide respiratory support utilizing a significantly less complex system than traditional pediatric ECMO.

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Fig. 1.

Tear-away schematic of the Hemolung RAS gas exchanger cartridge. Identified is the blood pathway from the inlet to the fiber bundle, the rotating cylindrical core containing the centrifugal pump, and the annular fiber bundle. The rotating cylindrical core generates secondary flow pathways that enhance gas exchange efficiency, shown in the scaled view of the bundle at right.

Fig. 2. CO₂ removal rate as a function of the rotational speed of the core with blood flow (Q_b) maintained at a constant rate using an external pump. The rate of CO₂ removal increases by over 60% at a core rotation of 1400 RPM due to the secondary flow patterns created by the motion of the core surface.

Fig. 3.
 (A) Hemolung in vitro pump curves in a viscous (3–4 cP) blood analogue solution. (B) *In-vitro* gas exchange in blood with and without simulated pediatric cannulation (10/8 Fr inflow/outflow) versus blood flow rate plotted with necessary O₂ delivery to replenish metabolic consumption versus body weight (W_{kg}).

Fig. 4.

Acute *in-vivo* gas exchange versus blood flow rate and sweep gas flow rate. (A) Measured exchange rates are shown for each animal (S008-13 and S009-13) independently. Q_g was maintained at 8.0L/min. (B) Gas exchange rates were normalized to gas exchange at maximum Q_g (8.0L/min). $vCO_2/vCO_{2, 8.0L/min}$ and $vO_2/vO_{2, 8.0L/min}$ values were averaged across both animals. Error bars represent one standard deviation.

Fig. 5.

Seven-day *in-vivo* Hemolung performance data. (A) Daily blood flow rates. Tests ended prior to 24hrs are omitted. (B) Daily average normalized CO₂ removal rates (mL/min).

Fig. 6.

Daily average PCO₂ at the inlet and outlet of the Hemolung device. Inlet samples were collected from the venous sampling line in the left neck, which is representative of blood in the external circuit. Outlet samples were collected directly from the circuit tubing. Data from all chronic in vivo animals was averaged for each 24 hour period. Error bars represent one standard deviation.

Fig. 7.

(A) Typical cartridge following seven-day *in-vivo* study. Some minor thrombus formation in the fiber wefts, indicated with an arrow. (B) Cartridge used in S005–13 after seven days of treatment showing some coagulation in areas of possible flow stasis. (C) Outlet of Hemolung cartridge (S005–13) and above fibers clear of thrombus.

Table 1 Summary of outcomes in twelve chronic *in-vivo* studies.

Test	Animal	Weight	Study Length	Study Outcome
1	S021-11	27.2 kg	19 h	Cranial hematoma with ischemic brain injury
2	S022-11	22.9 kg	5 h	Drainage cannula dislodged
3	S020-11	22.2 kg	168 h	Elected termination
4	S024-11	28.0 kg	168 h	Elected termination
5	S023-11	26.0 kg	19 h	Pulmonary embolism
6	S025-11	26.0 kg	168 h	Elected termination
7	S041-12	30.2 kg	167 h	Elected termination
8	S042-12	30.2 kg	166 h	Elected termination
9	S005-13	26.8 kg	169 h	Elected termination
10	S006-13	26.7 kg	86 h	Dislodged cannula and elective euthanasia
11	S007-13	19.0 kg	168 h	Elected termination
12	S010-13	19.5 kg	84 h	Respiratory related collapse and elective euthanasia

Table 2

Daily average venous blood gases and acid/base status

	Normal	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
pH	7.35 – 7.45	7.36 (0.02)	7.39 (0.02)	7.41 (0.02)	7.40 (0.03)	7.39 (0.02)	7.38 (0.02)	7.38 (0.03)
B.E., mEq/L	-2 – +2	-4.7 (1.5)	-2.0 (1.6)	0.1 (2.0)	-0.1 (2.1)	-0.5 (1.7)	-0.6 (2.3)	-1.3 (1.5)
pCO ₂ , mmHg	35 – 45	36.3 (2.8)	37.5 (2.6)	39.1 (3.8)	39.2 (3.8)	39.9 (3.4)	40.6 (3.4)	39.4 (3.8)
HCO ₃ ⁻ , mEq/L	22 – 26	19.8 (1.3)	22.3 (1.4)	24.2 (2.3)	24.2 (2.1)	23.8 (1.8)	23.7 (2.0)	23.1 (1.7)
sO ₂ , %	94 – 100	49.1 (4.8)	47.2 (7.0)	48.4 (8.0)	43.9 (9.6)	45.4 (7.1)	47.5 (6.7)	46.9 (8.5)

Data given as mean (±sd).

Table 3

Daily average arterial blood gases and acid/base status

	Normal	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
pH	7.35 – 7.45	7.39 (0.03)	7.40 (0.01)	7.41 (0.01)	7.39 (0.01)	7.40 (0.02)	7.41 (0.01)	7.38 (0.01)
B.E., mEq/L	-2 – +2	-3.7 (2.1)	-2.1 (1.4)	0.5 (0.9)	-2.4 (1.8)	-2.0 (0.7)	-0.7 (1.2)	-2.5 (1.2)
pCO ₂ , mmHg	35 – 45	34.5 (2.9)	35.5 (2.4)	38.8 (1.7)	38.3 (2.4)	36.7 (2.0)	37.4 (2.1)	37.2 (2.1)
HCO ₃ ⁻ , mEq/L	22 – 26	20.4 (2.0)	21.9 (1.5)	24.6 (0.9)	22.7 (1.4)	22.1 (0.7)	23.3 (1.2)	21.7 (1.3)
sO ₂ , %	94 – 100	97.4 (1.6)	97.6 (1.4)	95.4 (3.0)	95.9 (1.6)	96.3 (2.9)	97.2 (2.2)	97.3 (1.5)

Data given as mean (±sd).

Table 4 Hematology during 7-day chronic *in-vivo* studies.

	Normal	Baseline	Days 1–6	Day 7
Platelets, k/ μ L	250–750	389 (281)	267 (200)	540 (257)
Fibrinogen, k/ μ L	100–500	106 (68)	140 (61)	194 (56) ^a
HCT, %	27–45	24.2 (6.7)	20.4 (4.0)	21.1 (3.8)
PfHb, mg/dL	0–40	12.8 (5.9)	11.1 (4.4)	11.0 (4.0)
BUN, mg/dL	8–20	13.8 (3.8)	11.8 (4.8)	12.0 (2.4)
Creatinine, mg/dL	1.2–1.9	0.6 (0.1)	0.6 (0.2)	0.5 (0.1)

Data are given as mean ($\pm$ sd).^aDay 7 data compared to baseline data with Student's t-test and differ with statistical significance for $p < 0.05$ .Values above or below the normal range for sheep are listed in **bold**.

HCT = hematocrit, PfHb = plasma-free hemoglobin concentration, BUN = urea nitrogen.

Table 5

Total circuit priming volume and gas exchange surface area of Hemolung RAS and clinically available pediatric ECMO systems.

	Gas Exchanger <i>mL</i>	Pump + Tubing ^a <i>mL</i>	Total Volume ^b <i>mL</i>	Membrane Surface Area <i>m</i> ²
ALung Hemolung RAS	114	58–116 ^c	172–230	0.59
Medos hilite® 800 LT	57	72–164	129–221	0.32
Medos hilite® 2400 LT	98	72–164	170–262	0.65
Maquet QUADROX-i Neonatal	38	72–164	110–202	0.38
Maquet QUADROX-i Pediatric	81	72–164	153–245	0.80
Sorin Lilliput 2	105	72–164	177–269	0.64
Medtronic Minimax® Plus	149	72–164	221–313	0.80

^a Circuit Pump estimates account for range of example centrifugal pumps with lower (Levitronix Pedimag: 14 mL) and higher (Medtronic BP-50: 48 mL) priming volumes. Tubing volume assumes ¼" (6.35 mm) inner diameter with total length of 6–12 ft (1.82–3.66 m) (13)

^b Excludes priming volume of cannula

^c Gas exchanger includes pump and oxygenator components

Medos hilite® 800 LT and 2400 LT technical data found at www.medos-ag.com

Maquet QUADROX-i Neonatal and Pediatric technical data found at www.maquet.com

Sorin Lilliput 2 technical data found at www.sorin.com

Medtronic Minimax® Plus data found at www.medtronic.com