Perfusion

Ex vivo models for research in extracorporeal membrane oxygenation: a systematic review of the literature

Perfusion
2020, Vol. 35(1S) 38–49

DOI: 10.1177/0267659120907439
journals.sagepub.com/home/prf
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Abstract

With ongoing progress of components of extracorporeal membrane oxygenation including improvements of oxygenators, pumps, and coating materials, extracorporeal membrane oxygenation became increasingly accepted in the clinical practice. A suitable testing in an adequate setup is essential for the development of new technical aspects. Relevant tests can be conducted in ex vivo models specifically designed to test certain aspects. Different setups have been used in the past for specific research questions. We conducted a systematic literature review of ex vivo models of extracorporeal membrane oxygenation components. MEDLINE and Embase were searched between January 1996 and October 2017. The inclusion criteria were ex vivo models including features of extracorporeal membrane oxygenation technology. The exclusion criteria were clinical studies, abstracts, studies in which the model of extracorporeal membrane oxygenation has been reported previously, and studies not reporting on extracorporeal membrane oxygenation components. A total of 50 studies reporting on different ex vivo extracorporeal membrane oxygenation models have been identified from the literature search. Models have been grouped according to the specific research question they were designed to test for. The groups are focused on oxygenator performance, pump performance, hemostasis, and pharmacokinetics. Pre-clinical testing including use of ex vivo models is an important step in the development and improvement of extracorporeal membrane oxygenation components and materials. Furthermore, ex vivo models offer valuable insights for clinicians to better understand the consequences of choice of components, setup, and management of an extracorporeal membrane oxygenation circuit in any given condition. There is a need to standardize the reporting of pre-clinical studies in this area and to develop best practice in their design.

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Keywords
extracorporeal membrane oxygenation; ex vivo models; in vitro; extracorporeal life support

Introduction

Extracorporeal life support (ECLS) is a rapidly expanding field, with a growing number of clinical cases year by year.[1] In 2018, the Extracorporeal Life Support Organization (ELSO) registry reported more than 10,000 ECLS runs from 391 international centers, compared to 1,644 runs from 83 centers in 1990. These numbers represent neonatal, pediatric, and adult extracorporeal membrane oxygenation (ECMO) runs for cardiac and/or respiratory support from medical centers that are ELSO members. The actual numbers are expected to be significantly higher, as most centers do not report their data to the ELSO registry.[1,2]

Reasons for the growing interest and usage of ECLS include the many technical innovations that have been achieved in the last 25 years, resulting in easier handling of devices and presumably better patient outcome. The array of indications for ECLS has broadened, with the evolution of modern devices and the concomitant reduced complication rates. These include increasing numbers of patients who receive extracorporeal cardiopulmonary resuscitation (ECPR) and extracorporeal CO_2 removal ($ECCO_2R$).[3,4] However, growing numbers and improved survival rates cannot hide the fact that ECLS is still associated with complications leading to high morbidity and mortality in many patients. To further improve management, biocompatibility, and technical aspects of ECLS, ongoing research is needed.[5]

Clinical trials in intensive care within ECLS populations are difficult to perform due to the heterogeneity of the chronic and acute pathologies seen in such a disparate group. Animal studies are a valid alternative; however, such studies require large animals and well-equipped research institutes, and long-term experiments are expensive and difficult to perform. Therefore, ex vivo studies may be a feasible alternative to investigate some unsolved questions and problems in ECLS and help to improve the technology. The aim of this review is to give an overview on strengths and weaknesses of existing ex vivo models and to discuss future implications. A schematic model of an ex vivo circuit with different options is shown in Figure 1. The most relevant components are the membrane oxygenator, pump, and tubing of the system.

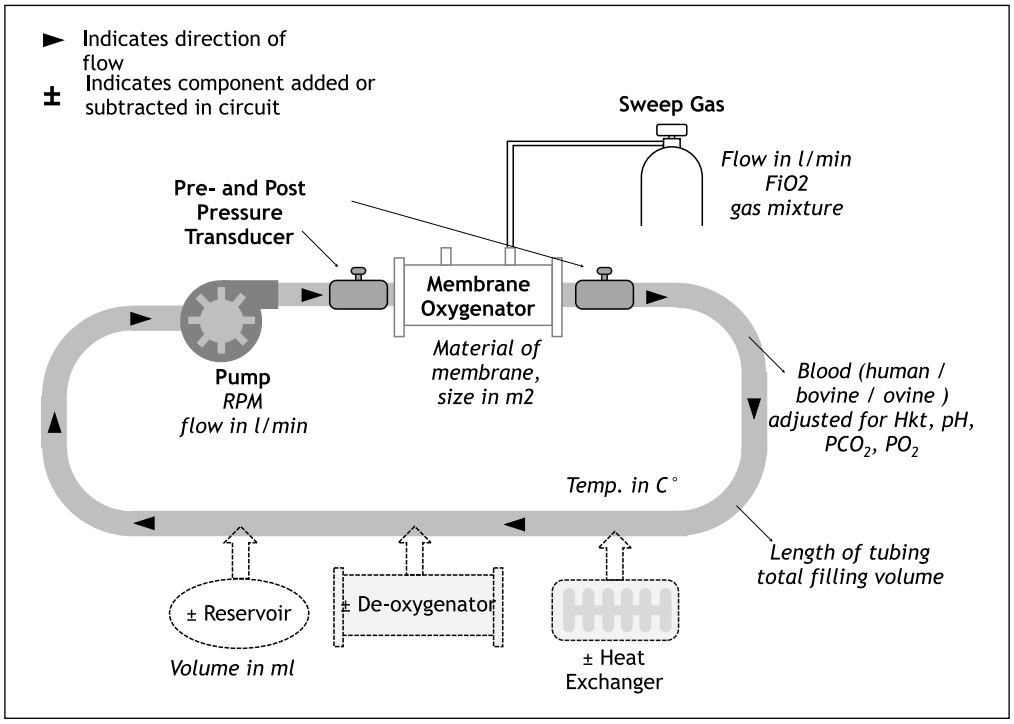

Figure 1. Scheme of an ex vivo circuit of extracorporeal membrane oxygenation showing all relevant components. A reservoir, second membrane oxygenator functioning as a deoxygenator, and a heat exchanger can be considered optional depending on the specific experimental question.
The variables given in italics are recommended to be reported on ex vivo models.

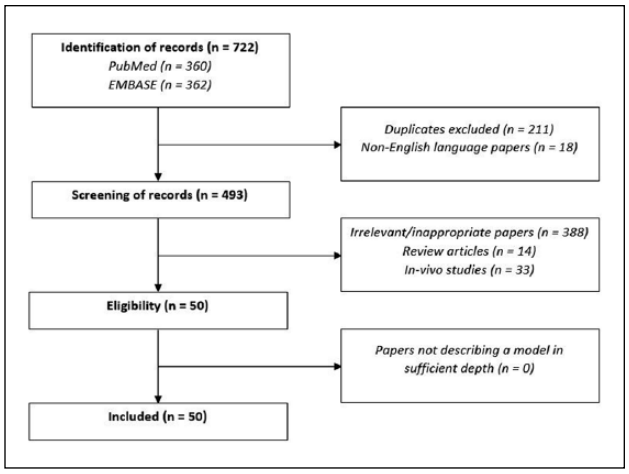

Figure 2. PRISMA flow diagram for inclusion and exclusion criteria.

Methods

A systematic review search strategy was designed in advance with the aim of retrieving all relevant studies on ex vivo ECMO models. A search of the MEDLINE (via PubMed) and Embase (via Ovid SP) indexed online databases from January 1996 to October 2017 was conducted.

Study selection occurred in two phases. Abstracts and citations were independently screened for relevance by experts in the field. Articles were excluded on the following basis: 1. if they did not report on an ex vivo model, 2. if they did not involve the use of ECMO or ECMO components, or 3. if the same model had been reported in a previous publication. The full text of all articles deemed relevant was retrieved. The search was limited to publications in English. The reference lists of screened studies were reviewed to identify publications not found by the original search strategy (Figure 2).

Oxygenator performance

The membrane oxygenator (MO) represents the central core of the ECMO circuit, as its role is to replace pulmonary alveolar function by transferring O_2 to the blood ($V'O_2MO$) and to remove CO_2 ($V'CO_2MO$).[6] Data related to MO performance therefore represent crucial information for its use in a clinical context. Ex vivo investigations conducted on mock circuits represent an acceptable alternative to evaluate MO performance. In the literature, several ex vivo studies have investigated different aspects of MO functioning among different models. In many cases, their results are applicable for clinical practice in ECMO management. The focus of ex vivo models on MO includes gas transfer capacity, flow dynamics (including pressure drop over the MO), and fluid loss.

Gas transfer

To evaluate MO gas exchange performance, mock circuits are designed to provide a continuous source of deoxygenated, CO_2-laden blood. They consist of two separate but integrated circuits: the first circuit is built on the MO under investigation, while the second circuit, including another MO, operates as an oxygen-consumption and carbon dioxide–generating device (deoxygenator) by supplying a gas mixture of nitrogen, oxygen, and CO_2 to the membrane. One or two blood reservoirs permit the integration of the separate circuit, acting as the blood return and mixing chamber.

Four different ex vivo models focusing on gas transfer capacity (Table 1) were identified. According to the Association of American Medical Instrumentation (AAMI) standards,[16,17] the primed blood was deoxygenated and then adjusted to obtain a standard venous blood saturation of $65 \pm 5\%$, with CO_2 partial pressure (p_vCO_2) of 45 ± 5 mm Hg and base excess (BE) of 0 ± 5 mEq/L. Hemoglobin (Hg) level was maintained at 9 g/dL in early studies and then increased to 12 g/dL according to the change in required standards. Temperature was maintained at 37°C with a heat exchanger integrated into the deoxygenator or both the deoxygenator and the tested MO.

MO inlet and outlet O_2 and CO_2 were measured using a mass spectrometer, respectively. Blood Hg level (g/dL), Hg saturation (SO_2, %), O_2 (p_aO_2, mm Hg), CO_2 (p_aCO_2, mm Hg) partial pressures, and bicarbonate blood concentration (HCO_3^-) were measured with a gas analyzer from blood samples taken at the inlet (pre-MO) and the outlet (post-MO) of MO sampling ports.

Blood oxygen content (C_aO_2, MO/dL) and MO oxygen transfer rate ($V'O_2MO$, MO/minutes) were calculated from blood gas analysis results, at a given blood flow (BF, L/minute) as follows

$$C_aO_2 = \frac{\left(Hg \times SO_2 \times 1.34\right)}{100 + p_aO_2 \times 0.003}$$

$$V'O_2ML = BF \times \left(C_{post}O_2 - C_{pre}O_2\right) \times 10$$

Blood total CO_2 content (C_aCO_2, mmol/L) and MO CO_2 removal ($V'CO_2MO$, MO/minutes) were calculated in two different ways. Latest studies used data obtained from MO blood sample ports as it follows

$$C_aCO_2 = HCO_3^- + 0.03 \times p_aCO_2$$

$$V'CO_2MO = 22.4 \times \left(C_{pre}CO_2 - C_{post}CO_2\right) \times BF$$

Van Meurs et al.[7] calculated the transfer from the change in CO_2 concentration ($[CO_2]_e$, ratio) between the inlet and outlet gas port concentration ($\Delta[CO_2]_e$), as follows

Table 1. Studies with ex vivo models focused on membrane oxygenator's gas transfer.

	Author	Circuit model	Membrane oxygenator	Endpoint
1	Van Meurs et al.[7]	Two roller pumps, one oxygenator, one consumption membrane, two reservoirs, one heat exchanger, and one gas blender	Hollow fiber: SciMed 0.8 M^2 and 0.6 M^2 (SciMed Life Systems, Inc., Minneapolis, MI, USA)	CO_2 transfer, O_2 transfer, and pressure drop
2	Niimi et al.[8]	Two centrifugal pumps, one oxygenator, one deoxygenator, one reservoir, one heat exchanger, and one gas blender	Hollow fiber: Mera HP Excelungprime, HPO-20H-C (Senko Medical Instrument Mfg. CO., Ltd, Tokyo, Japan)	Gas transfer and hemolysis
3	Kawahito et al.[9]	One centrifugal pump, one roller pump, one oxygenator, one deoxygenator, one venous reservoir, one arterial reservoir, one heat exchanger, and one gas blender.	Hollow fiber: pre-production model PPM-03 and PPM-04 (Fuji Systems Inc., Tokyo, Japan)	Gas transfer and biocompatibility (hemolysis and pressure drop)
4	Karimova et al.[19]	Two roller pumps, one oxygenator, one deoxygenator, one blood reservoir, one heat exchanger, and one gas blender	Hollow fiber: Hilite® 800 LT (Medos Medizintechnik AG, Stolberg, Germany)	Durability of ECMO circuit in terms of oxygenator function, sterility, and release of plasticizers
5	Lawson and Holt[63]	Two centrifugal pumps, one oxygenator, and one guardian canister	Hollow fiber: Jostra Quadrox D Oxygenator (Maquet Cardiopulmonary AG, Hirrlingen, Germany)	Insensible water loss
6	Camacho et al.[13]	One roller pump, one oxygenator, one reservoir, and one heat exchanger	Hollow fiber: 0.8 M or 0.4 M (Avecor Cardiovascular Inc., Plymouth, MN, USA)	Water loss at various ECFRs, gas flow rates (sweep), and from two sizes of membrane oxygenators
7	Alexander et al.[32]	One roller pump, one oxygenator, one reservoir, and one heat exchanger	Hollow fiber: Minimax Plus oxygenator (Medtronic, Dublin, Ireland)	Insensible water loss for a given sweep rate and relative sodium increase
8	Gill and O'Shaughnessy[35]	One centrifugal pump, one oxygenator, one reservoir, one hemofilter, and one heat exchanger	Hollow fiber: Hilite® 2400LT oxygenator (Medos Medizintechnik AG, Stolberg, Germany)	Water loss over a 24-hour period at various gas flow rates
9	Li Li et al.[34]	One centrifugal pump, one oxygenator, one gas blender, and one heat changer	Hollow fiber: Jostra Quadrox D oxygenator (Maquet Cardiopulmonary AG)	Fluid loss at different sweep gas flow rates and different temperatures

ECMO: extracorporeal membrane oxygenation; ECFRs: extracorporeal fluid flow rates.

$$V'CO_2ML = \Delta\left(CO_2\right)_e \times GF$$

In three studies[7–9], the mock circuit was used to compare oxygenator performance in terms of biocompatibility and gas transfer capacity. In these studies, the oxygenator performance was evaluated in different conditions of surface area and membrane coating.[7–9,18] In addition, Karimova designed the mock circuit and assessed the gas transfer capacity, sterility, and release of plasticizers of a wet pre-primed circuit after a 2-week period of storage.[19]

Fluid loss

A series of different ex vivo studies investigated the insensible water loss from the MO (Table 2). It has been theorized that water transport of MO depends mainly on the combination of two different mechanisms: 1. bulk convection, due to a hydrostatic pressure gradient, and 2. activated diffusion, driven by a concentration gradient.[12,13] Furthermore, water loss is inversely proportional to the membrane thickness, and it is influenced by the micro-structure of the membrane material as well as the size of the pores, if any, in the membrane.[32] Moreover, the method of micro-pore manufacturing could also influence the amount of water leakage.[33]

Almost all the literature found is based on ex vivo tests, using circuit setup to simulate clinical conditions. Table 1 shows the ex vivo circuit's characteristics focusing on fluid loss.

Since fluid and electrolyte balance are a primary concern and particularly challenging for neonatal and

Table 2. Summary of studies with ex vivo models focused on pharmacokinetics.

	Hasni[20] and Lemaitre[21]	Leven[22]	Shekar[23]	Shekar[24]	Sinnah[25]	Watt[26]	Harthan[64]	Wagner[28]	Mehta[29]	Bhatt-Mehta[30]	Preston[31]
First priming	HWB	NaCl	Plasma-Lyte + albumin	Plasma-Lyte + albumin	Plasma-Lyte	RBC, FFP, Plasma-Lyte A, heparin, $NaHCO_3$, and Ca-gluconate		Crystalloid + albumin	CO_2, Plasma-Lyte A/RLa, and albumin	CO_2	Plasma-Lyte
Final medium	HWB	HWB	HWB	HWB	Hg + albumin	RBC and FFP/as above ± albumin	RBC/alb/Bic	RBC and plasma	HWB, Plasma-Lyte, and A/RLa	RBC and FFP	Bovine whole blood
Anticoagulation			Heparin 5,000 IU	Heparin 5,000 IU	Heparin 5,000 IU			Heparin 100 IU		Heparin	Heparin 4 IU/mL
ACT (s)			220-250	220-250	220-250						
Tubing	PVC	PVC		PVC	PVC	PVC Sorin Smart Tubing	PVC	PVC	PVC and ST	PVC and ST	PVC and ST
Coating tubing	Bioline	Bioline	Bioline	Bioline	Bioline	Phosphorylcholine	Carmeda				Uncoated
Hollow-fiber oxygenator	Quadrox PMP 1.8 m^2 PU 0.4 m^2 PC housing Bioline coating	Quadrox PMP 1.8 m^2 PU 0.4 m^2 PC housing Bioline coating	Quadrox D PMP 1.8 m^2 PE 0.6 m^2 Bioline coating	Quadrox PMP 1.8 m^2 PE 0.6 m^2 Bioline coating	Quadrox PMP 1.8 m^2 PE 0.6 m^2 Bioline coating	Yes/Yes/- Quadrox iD/Adult PMP 1.8 m^2 PE 0.6 m^2 Quadrox iD/Ped PMP 0.8 m^2 Bioline coating	Quadrox D PMP 1.8 m^2 PE 0.6 m^2 Bioline coating	Quadrox D PMP 1.8 m^2 PE 0.6 m^2 Bioline coating	Medtronic Silicone 1.5 m^2	Medtronic Silicone 0.6 m^2	Quadrox D PMP 1.8 m^2 PE 0.6 m^2 Baby RX PP 0.5 m^2 X-coated Stainless heater PC housing

RBC: red blood cells; Hg: hemoglobin; PVC: polyvinyl chloride; PMP: polymethylpentene; ACT: Activated clotting time; HWB: human whole blood; FFP: fresh frozen plasma; ST: Super tygon; PU: polyurethane; PE: polyethylene; PP: polypropylene

pediatric population,[15] almost all tests were run at flow rates that could approximate clinical parameters seen in infants ECMO; only Li Li et al.[34] performed a study to assess the adult ECMO fluid loss. Nearly, all tests were run over at least 24 hours to obtain practical results for a clinical fluid balance assessment. All test circuits employed were primed with normal saline or colloid solution, and it may have affected the membrane water loss because of the lower solution viscosity. Meanwhile, the use of transfused whole animal blood would have carried intrinsic limitations, based on the red blood cells relatively short half-life in the in vitro setting and physical size differences in between species.[32] Initial studies have mainly used roller pumps, while more recent tests employed centrifugal pumps. Temperature was controlled with heat exchangers connected to the ECMO circuit or directly integrated to the MO.

Insensible water loss evaluation was based on a replacement method in all tests. It assumes that in the experimental noncompliant systems, closed to the external environment, all fluid loss occurred via evaporation across the membrane surface area. The amount of fluid lost is quantified based on the assumption that the same volume of liquid automatically drawn back into the circuit from a fluid-filled burette or be manually replaced, refilling the circuit reservoir to its original level. Only one study employed a second method to measure the fluid loss. It measured the fluid condensing at 0°C directly from the exit gas port of the MO.[13] This collection method showed lower values than the replacement one. The difference may be due to inability to completely condense all water vapor, and the authors were unable to measure the relative humidity of the gas exiting from the condensation jars.

These models show a strong correlation between sweep gas flow and fluid loss; Gill and O'Shaughnessy demonstrated that humidifying gases reduces the fluid loss significantly, suggesting a possible strategy to be adopted for the clinical practice.[35] The results of these studies demonstrate the importance of the insensible water loss from an ECMO oxygenator that should be taken into account by clinicians in managing fluid and electrolyte balance of patients.

Pump performance and flow characteristics

Pump performance is critical to the design of ECLS systems, particularly in mitigating complications. The foremost of these complications is hemolysis, which is maximal within the pump due to high shear stress. Blood trauma secondary to high shear stress is also responsible for the development of acquired coagulopathies, such as acquired von Willebrand syndrome, placing patients supported with ECLS at a higher risk of bleeding.[36] Pumps must be reliable, minimizing the chance of catastrophic failure. Recently, evidence has emerged suggesting that the nature of flow produced by a pump may impact organ perfusion and ultimately patient outcome.[37–39] Pulsatile flow has been associated with improved cerebral and coronary perfusion, a reduction in inotropic support, and a decrease in the rate of gastrointestinal bleeding in studies on cardiopulmonary bypass (CPB) and left ventricular assist devices (VADs).[37–39] The pivotal importance of the pump and the improvements in outcome witnessed with progressive enhancements mean that researchers and clinicians must have robust experimental models with which to test future designs. Ex vivo models represent the entry point for translational research in pump technology; here, we consider several factors in detail.

When developing a novel pump design, ex vivo modeling provides a reliable, rapid, and cost-effective method of assessing performance characteristics and reliability. Several groups have described models of varying complexities with this aim. Casas et al.[40] used a simple setup to test the safety and performance of a novel ECMO/CPB device. This system was designed with reuseable components that would also prove cost-efficient if used as disposables. The circuit consisted of a magnetically coupled centrifugal pump driving a straight-blade impeller and a control console incorporating pressure, flow, and temperature sensor arrays. A water–glycerine solution was used during mock circulation, which aimed to assess the reliability of the pump design under a range of pressures, flow rates, and pump speeds. The loop was simple in nature, consisting solely of the pump and associated equipment (control console, power supply), a thermometer, and flow/pressure sensors.

More complex designs often seek to account for systemic variables such as vascular resistance and compliance. Pantalos et al.[41,42] have published several papers describing iterative revisions of their pediatric cardiopulmonary assist system (pCAS), which integrates a pump mechanism and a hollow-fiber oxygenator in a single housing. Seeking to recapitulate an in vivo setting more comprehensively, they have reported using a mock circulation comprising two pneumatic VADs, which simulate the right and left ventricles. Connected to each ventricle are upstream bladder bulbs, which represent the atria. Varied tubing and cannula configurations are then used to approximate vascular anatomy as desired.

There is an increasing interest in the effects of pulsatility on tissue perfusion during ECLS, correspondingly several groups have established mock circulatory loops designed to trial pumps capable of generating pulsatility. Evenson et al.[43] developed an ex vivo model of neonatal ECLS to investigate the effects of pulsatility on flow, pressure, and hemodynamic energy. As the model was

limited by a failure to vary circuit factors which influence hemodynamic performance, a modification to the experimental setup, which includes electrocardiogram triggering based simulation of multiple atrial and ventricular arrhythmias, was made.[44,45] Trittenwein et al.[46] have described an ex vivo pulsatile ECMO circuit, which incorporates a double-chamber pneumatically driven VAD as a pump. In this model, a mock neonatal circulation was constructed using a series of stiff and elastic components simulating arterial and venous capacitance.

Ex vivo ECMO simulations are relevant investigatory tools in pump design and evaluation. They range from simple resistance circuits to advanced patient simulators, with investigators often building complexity as the testing process advances.[47] They can be designed to incorporate an array of sensors, including high-speed photography or ultrasound imaging.[48] Future mock circulatory loops will use computer-controlled components to facilitate simulation of a wide range of cardiovascular states.

Hemostasis and coagulation

In the clinical practice, the management of coagulation is crucial given the well-described problems of bleeding and thrombosis on ECMO.[49,50] Ex vivo experiments allow a standardized, controlled, and repeatable model to be developed and the impact of individual circuit components to be assessed without confounding factors from differing host responses. However, the data derived from ex vivo models regarding biocompatibility and hemostasis are necessarily limited and may not predict the real-life problems faced with patients at the bedside. One limitation is that studies are generally restricted to a short period of time (6 hours) and provide data around the initial impact of the circuit on certain aspects of hemostasis. The lack of interaction with a live biological system is a further limitation. However, the advantages of the mock loops are that specific components of the coagulation pathways may be explored, and this can help direct further research into biocompatible membranes and to provide a focus for subsequent clinical studies. Thus, we will focus on some of the models which have been developed and the resulting data.

Hemolysis is a common problem in extracorporeal support, and it is thought that factors from both patient and circuits contributed to this problem.[51] Meyer et al.[52,53] evaluated the hemolytic potential of neonatal ECMO circuits at simulated low flow rate using four models: centrifugal and roller pumps each with two different oxygenators (silicone and polymethylpentene hollow fiber). The experiments were conducted for 6 hours using a mock loop primed with porcine whole blood anticoagulated with heparin. Blood flow was maintained at a constant 300 mL/minute. Gas flow was with a mixture of O_2 and air to maintain pH, PO_2, and PCO_2 within a normal range. Circuit blood was maintained at 38°C. A collapsible plastic bag was used as a venous reservoir, and the outflow tubing was constricted using a smaller arterial catheter to create a resistance with a pressure gradient of 230 mm Hg. These experiments showed that the extracorporeal systems developed plasma-free Hg at a similar rate compared with static blood control with no difference in the mean normalized index of hemolysis between centrifugal and roller pumps using silicone or polymethylpentene hollow-fiber oxygenators.

The use of a standardized "compact" loop[54] instead allowed to study the hemolysis with a lower priming volume and fewer circuit elements which may impact the results. These compact loops are very cheap and easy to establish. In one study, five compact loops were prepared with a specific flow and pressure relevant to each clinical condition (CPB, right and left VADs). This study showed that lower rotational speed and total pressure changes were associated with less hemolysis.

Bleilevens et al.[55] evaluated the biocompatibility of the iD-pediatric membrane oxygenator (Bioline coating, Getinge, Rastatt, Germany) using two different priming solutions for 12 hours. This experiment was designed to understand the impact of the priming solution on markers of biocompatibility. Approximately, 120 mL of porcine blood were used to set this experiment. In the first test, the mock loop was tested without modification of the sweep gas (0.6 L/minute blood flow and 3 L/minute sweep gas flow at 0.21 FiO_2, according to manufacturer's recommendation). In the second test, room air was replaced with a CO_2-enhanced gas (5% CO_2, 21% O_2, and 74% N_2) in combination with a nutrient solution to provide buffering and nutrition for the blood. When the unbuffered nutrient poor loop was compared with the buffered nutrient rich loop, the unbuffered loop had a significantly higher pH and hemolysis demonstrated by a rise in plasma-free Hg. Hence when interpreting in vitro tests of circuits, it is necessary to also look at the circulating blood solution.

Meyer et al.[52] evaluated the generation of platelet-derived microparticles in the different mock loops using the flow cytometry. Microparticles are small cell-derived membrane vesicles, between 0.1 and 1 µm, which are pro-inflammatory. These experiments showed that platelet-derived microparticles progressively increased over time in all four systems and the static control. Centrifugal pumps were associated with higher microparticle generation than the roller pump, regardless of which membrane oxygenator was used.

Other experiments performed by Van Poucke et al.[56] tested platelet function during long-lasting extracorporeal membrane circulation with moderate hypothermia (simulating the heart–lung machine environment used

to manage complex cases of cardiac surgery). Mock loops with a centrifugal pump, a membrane oxygenator with integrated heat exchanger, and a venous reservoir were set up. All components were coated with phosphorylcholine. Three healthy human volunteers provided whole venous blood which was then added to the circuits primed with saline and plasma expander. The mock circuits were run for a period of 32 hours, maintaining at the temperature of 32°C for almost 24 hours. Results from these tests showed that hypothermia in association with ECMO negatively affects platelet function and that spontaneous recovery of platelet function does not start immediately after rewarming.

Pharmacokinetics

Plastic components of the ECMO circuit may adsorb and thus reduce the free fraction of several drugs. Each drug tested has its pharmacokinetic profile of individual medication largely dependent on its context and test setting. The protein/albumin content of the circulating fluid affects sequestration of drugs that bind to albumin, as does the lipophilicity of the drug. For neonatal and pediatric patients, tubing/coating of the tubings accounts for the largest artificial surface area exposed to the circulating ECMO blood. For adults, it may be the polyvinyl chloride tubing or the hollow-fiber membrane surface inside an oxygenator. Coating materials not only promote biocompatibility by reducing activation platelets and the immune system but also protect the patient from phthalates (tubing plasticizers) to diffuse into the patient's blood.[57] However, the primary aim of tubing coating would be to mimic the milieu interieur in an ECMO setting in all possible aspects, such as temperature, Hg concentration/hematocrit, electrolytes, protein content, and oxygenation content/Hg saturation.

Today, products may be certified for use up to a month, which is possible due to the development of the coating materials. Coatings may be heparin dependent (Bioline, Carmeda, and Rheoparin®; Medos, Xenios, Heilbronn, Germany) that covalently bind heparin to the surface or non-heparin dependent. Albumin was one of the first molecules to be used for pre-coating with the purpose of providing a base layer of protein that would delay or mitigate the biological response to the heavily hydrophobic surfaces. Adsorbed albumin is able to increase the hydrophilicity of the surface, and concurrently provide a competitive protein that the fibrinogen must displace. In ECMO tubing, albumin is covalently linked to surfaces (e.g. Safeline®, Maquet Cardiopulmonary AG, Hirrlingen, Germany) to ensure its retention and to prevent displacement. In addition to heparin and albumin, there are several other surfaces that have been developed with the aim of increasing hydrophilicity or surface charge in order to mimic endothelial cells that are lining mammalian blood vessels. These include phosphorylcholine, polymethoxyethylacrylate (SMARTx®, Sorin, LivaNova, Belgium), X-coating® (Terumo, Ann Arbor, MI, USA), polyethylene oxide, and triblock surface-modifying additives.

During the early stage of ECMO development, the oxygenator membrane was a known site for plasma leakage and foaming due to the use of silicone or the later polypropylene for respiratory membrane. Today, the fluid-tight polymethylpentene membrane is the most common material for oxygenator; however, polypropylene hollow-fiber oxygenators still exist on the market. The ex vivo mock setup has been essential in the development of artificial/polymer surface area suitable for clinical setting and for addressing issues concerning the composition of the flow medium.

A total of twelve studies were identified, in which eight ex vivo ECMO models were used to assess drug pharmacokinetics (PKs). Eleven studies (91.7%) used human blood, and one study used bovine blood. All studies were prospective and observational; none of these studies were randomized or directly compared two different ECMO models on drug PKs. One ex vivo study evaluated results together with data obtained in one ovine in vivo series and antibiotic concentrations obtained in one critically ill patient.[58] The details of the identified studies are presented in Table 2.

A total of 31 drugs were assessed in these models: 13 were antibiotics (amoxicillin, ampicillin, cefazolin, cefepime, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, linezolid, meropenem, oxacillin, piperacillin, vancomycin), four were antifungals (caspofungin, fluconazole, micafungin, voriconazole), nine were sedatives/anticonvulsants (dexmedetomidine, fentanyl, fosphenytoin, lorazepam, midazolam, morphine, phenobarbital, propofol, thiopental), two were inotropes (dopamine, epinephrine), and also cyclosporine, heparin, and hydrocortisone have been tested. Plasma levels of tested drugs differed according to their protein binding capacity, stability, and lipophilicity as shown by Shekar et al.[59]

Miscellaneous

Ex vivo models for the simulation of ECMO circuits provide valuable means to identify strategies for optimizing clinical interventions into complex clinical scenarios.[10,11,14,23,60–62]

One example is the different air handling capacities of a circuit depending on the isolated circuit components. Gill et al. have managed to identify that centrifugal pumps manage not only to generate a significantly greater amount of gaseous micro-emboli than roller pumps but also to propel them through a low-resistance oxygenator to a greater extent than a roller pump. In their study, they used a blood-primed circuit, in which

1 mL of air was infused pre-pump over 10 in via a syringe pump and air detection cuvettes were placed pre-pump, post-pump, and post-oxygenator.[61] Their ex vivo model managed to determine that the air handling capability of the Quadrox D oxygenator (Maquet Cardiopulmonary AG) was dependent upon the type of pump (centrifugal or roller pump), and whether the application of an air-eliminating filter can prevent circuit air introduction from intravenous infusions.[61]

Some "real-time" monitoring technologies, which have proven to be very valuable in CPB-supported patients, have been transferred into ECMO circuits. To use such sensors in ECMO, the evaluation of long-term usage is advisable.

For such evaluation, a circuit consisting of a silicone membrane oxygenator and a stainless steel heat exchanger was constructed by Schreur et al.,[23] and a standard venous reservoir bag was used to represent the patient. This circuit was primed with fresh porcine blood and conditioned with the addition of CO_2 to simulate typical venous blood under ECMO conditions. This model enabled them to investigate the difference between different O_2 saturation measurement tools.[23]

Another question which has been investigated by ex vivo research is how to determine the recirculation fraction which inevitably occurs when putting a patient on veno-venous (VV) ECMO. Being aware of the existing phenomena, ex vivo models have been applied to evaluate methods which not only confirm its relation to cannula position, design, and patient hemodynamics but also allowed measurement of the recirculation fractions. Linton et al.[60] developed an in vitro method for measuring cardiac output and shunt fraction during VV ECMO by injecting lithium chloride downstream the oxygenator in an ex vivo circuit attached to an ex vivo patient circuit.

Ex vivo experiments might also be a useful addition to in vivo experiments, because of the strength or advantage that simulated circuits provide conditions that allow control of one variable at a time, which is difficult to achieve in animal or clinical studies. Shekar et al.[10] combined simulated circuits and ovine models of ECLS, allowing an incremental and systemic approach for characterizing the effects of ECLS on host physiology. The use of simulated circuits together with healthy and critically ill ovine models receiving ECMO can help to gain insight into the contributions of circuit factors relative to the influence of critical illness as well on changes in the pharmacokinetics of drugs.

A relevant question in supporting patients on peripheral veno-arterial (VA) ECMO concerns the adequacy of cerebral oxygenation when competition flow is experienced between the variable oxygenated blood ejected by the native ventricle and the artificially oxygenated blood being injected into the arterial circulation. To investigate this phenomenon, Xie et al. used a blood-based mock circulatory loop simulating respiratory failure in a central and peripheral cannulation VA ECMO setup. Cerebral circulation was mimicked by an additional loop, in which flow was recorded at increasing cardiac outputs and the researchers managed to simulate the relationship between native cardiac output and cerebral perfusion in a setting with respiratory failure.[11]

It is clear that a pre-clinical evaluation tool, the ex vivo model, offers valuable insights to the clinicians as to better understand the consequences of choice of components, setup, and management of an ECMO circuit in any given condition. Besides being an important tool for translational science, ex vivo models can also help clinicians in training programs to gain understanding and improve technical skills.[62] Technically related to ECMO devices are also ex vivo organ care systems used for perfusion of donor organs.[27]

Limitations of ex vivo models

As discussed above, not a single ex vivo ECMO model can be suitable to address all specific research questions. Therefore, the model always has to be adjusted and specified in regard to the element of interest. For example, while a blood reservoir is useful in a model to test a certain pump or technical aspect, it will be a relevant confounding factor in models aiming to examine coagulation and biocompatibility. While ex vivo ECMO models have specific limitations in accordance with the addressed question, there are also general limitations. A main limitation has to be considered is the fluid/blood used for the model. While the disadvantages of blood replacement fluids or ovine/bovine blood lie in the differences in rheology and coagulation also fresh human whole blood has limitations. First transfused blood is in general anticoagulated with, for example, citrate, and metabolism of anticoagulants is not given as in an in vivo model. Furthermore, the blood volume of an ex vivo model is not comparable to a patient, and therefore constant recirculation of single blood components through pump head and oxygenator occurs more often in shorter time, causing elevated blood trauma which will influence hemostasis, pharmacokinetics, results of membrane performance, and flow characteristics. The blood volume and ongoing hemolysis in ex vivo models limit also the reasonable time tests can be run for.

Summary

Ex vivo models bear a great potential to investigate different aspects of ECMO therapy. A number of clinically relevant lessons have been learnt; for example, it is appreciated that hemolysis is associated with shear stress, pressure changes, rotational speed, and pump

type (centrifugal or roller). Ex vivo testing has also provided data about the impact of circuits on platelets and systemic inflammation.

There is not a single model that would be ideal to study all of these aspects; therefore, the model has to be specifically designed for the relevant aspect that is to be studied. A schematic model is given in Figure 1, indicating relevant parts and variables that routinely should be reported. This review reports on all previously established models and shows that there is significant heterogeneity in both design and reporting. Understandably, this has created significant difficult in interpreting and comparing research results produced with different settings, and comparison of results gets more relevant with the expanding availability of different equipment that should be comparable.

Although models cannot all be the same, we advocate that there is a need to standardize the reporting of pre-clinical studies in ECMO and ECLS research. This could be achieved by the introduction of a minimum data set for pre-clinical ECMO studies.

Acknowledgements

The authors thank Mr Eric Lee-Jan Wu, Critical Care Research Group, Prince Charles Hospital, Brisbane, QLD, Australia, for his help in designing Figure 1.

Author Contributions

All listed authors read and approved the final manuscript for submission. Furthermore, all of the authors contributed to the conception and the design of the review and its revision prior to submission.

Availability of Data and Material

The data sets analyzed during this study are available from the corresponding author on reasonable request.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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