A mock circulation loop to test extracorporeal CO\textsubscript{2} elimination setups

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Abstract

Background: Extracorporeal carbon dioxide removal (ECCO\textsubscript{2}R) is a promising yet limited researched therapy for hypercapnic respiratory failure in acute respiratory distress syndrome and exacerbated chronic obstructive pulmonary disease. Herein, we describe a new mock circuit that enables experimental ECCO\textsubscript{2}R research without animal models. In a second step, we use this model to investigate three experimental scenarios of ECCO\textsubscript{2}R: (I) the influence of hemoglobin concentration on CO\textsubscript{2} removal. (II) a potentially portable ECCO\textsubscript{2}R that uses air instead of oxygen, (III) a low-flow ECCO\textsubscript{2}R that achieves effective CO\textsubscript{2} clearance by recirculation and acidification of the limited blood volume of a small dual lumen cannula (such as a dialysis catheter).

Results: With the presented ECCO\textsubscript{2}R mock, CO\textsubscript{2} removal rates comparable to previous studies were obtained. The mock works with either fresh porcine blood or diluted expired human packed red blood cells. However, fresh porcine blood was preferred because of better handling and availability. In the second step of this work, hemoglobin concentration was identified as an important factor for CO\textsubscript{2} removal. In the second scenario, an air-driven ECCO\textsubscript{2}R setup showed only a slightly lower CO\textsubscript{2} wash-out than the same setup with pure oxygen as sweep gas. In the last scenario, the low-flow ECCO\textsubscript{2}R, the blood flow at the test membrane lung was successfully raised with a recirculation channel without the need to increase cannula flow. Low recirculation ratios resulted in increased efficiency, while high recirculation ratios caused slightly reduced CO\textsubscript{2} removal rates. Acidification of the CO\textsubscript{2} depleted blood in the recirculation channel caused an increase in CO\textsubscript{2} removal rate.

Conclusions: We demonstrate a simple and cost effective, yet powerful, “in-vitro” ECCO\textsubscript{2}R model that can be used as an alternative to animal experiments for many research scenarios. Moreover, in our approach parameters such as hemoglobin level can be modified more easily than in animal models.

Keywords: ECCO\textsubscript{2}R, Mock circulation, ECMO, ARDS, COPD

Background

Extracorporeal carbon dioxide elimination (ECCO\textsubscript{2}R) is a method to counteract hypercapnic respiratory failure, e.g., in severe acute respiratory distress syndrome (ARDS). Furthermore, ECCO\textsubscript{2}R may be used in the management of patients with acute exacerbations of chronic obstructive pulmonary disease (COPD) or patients waiting for lung
transplant [1, 2]. As mechanical ventilation in ARDS therapy can lead to ventilator-induced lung injury, ECCO₂R can be used to enable ultra-protective ventilation in patients without life-threatening hypoxemia [3–6].

Today, effective ECCO₂R therapy requires blood flow rates of more than 1 L/min and consequently cannulation with relatively large cannulas [1, 3]. Moreover, pure oxygen is frequently used as sweep gas in ECCO₂R therapy, in order to create a diffusion gradient for CO₂ to be cleared from the blood [4]. The invasiveness of catheters to generate a sufficient blood flow (BF), as well as mobility limitations due to heavy oxygen bottles, are burdens for potential mobile ECCO₂R devices [5]. Another limitation of current ECCO₂R devices is the exclusive permeability of the membrane lung (ML) for gaseous substances and hence the impermeability for dissolved bicarbonate [6]. Since the majority of carbon dioxide is transported as chemically bound HCO₃⁻, only approximately 10% of the total CO₂ can pass the ML, necessitating high extracorporeal blood flows to sufficiently deplete the blood of CO₂ [6].

So far, experimental evaluation of ECCO₂R setups heavily relies on animal models that are costly and require animal facilities. Considerable efforts to avoid animal experiments are made by both the scientific community and politics. As an alternative, MLs can be tested in mock circuits. Different mock designs have been described in the literature [7–10]. The aim of our study is to present a new mock circuit that reflects the anatomy of a mammal body connected to an ECCO₂R more closely and to validate that mock in three experimental scenarios.

Methods
Composition of the dual-loop circuit

Standard experiment protocol

The presented in vitro ECCO₂R mock consisted of two circuits. The primary circuit was responsible for creating a hypoxic and hypercapnic (venous) environment, which represented the simulated vena cava, while the test ECCO₂R circuit washed out CO₂ and oxygenated the venous blood from the primary circuit.

The primary circuit consisted of a Getinge PLS (Maquet Cardiopulmonar GmbH, Getinge Group, Rastatt, Germany) circuit composed of a Rotaflow pump, Quadrox membrane lung, and BIOLine® coated PVC tubings. Two luer-lock connectors were installed into the circuit at 10 cm and 30 cm up-stream the centrifugal pump (Fig. 1). Sweep gas of the Quadrox PLS was provided via a gas blender with separate regulation of N₂, CO₂, and O₂ flows. Out of several flow rate combinations a sweep gas flow ($\dot{V}_{\text{sweep}}$) of 7.5 L/min N₂ and 0.55 L/min CO₂ was chosen to create a venous carbon dioxide partial pressure ($p_{\text{CO}_2}$) between 45 mmHg ± 5 mmHg and a venous oxygen saturation of 65% ± 5% (data not shown). Blood flow in the primary circuit was set to 5 L/min. For temperature control of 37 °C, a Maquet HU 35 (Maquet) was used accordingly.

Via the two luer-lock connectors, the test ECCO₂R circuit was connected to the main circuit. This junction between primary and test circuit could be throttled and simulated the cannulas in this model. The blood flow through these luer-lock connectors was called "cannula flow" in this work. The test circuit used 1/4 PVC tubings, a second Rotaflow centrifugal pump, and a pediatric Getinge Quadrox iD ML as the test ML. A $\dot{V}_{\text{sweep}}$ was applied to the pediatric Quadrox iD ML. At the sweep gas outlet of the ML, a mass flow sensor
(TSI41403) and a side-stream capnometer (Philips Intellivue) were installed. Blood flow in the test circuit could be adjusted by closing one of the luer-lock connectors partly or adjusting the rotations per minute on the Rotaflow pump.

As default, \( V_{\text{sweep}} \) rates of 0.3, 0.5, 0.7, 1, 2, 4, 6, and 7 L/min and blood flows of 0.6 L/min were used in the test circuit. One set of MLs was used per experiment. Each experiment was run for a maximum time of 2 h with one blood filling. A shift of up to two base excess points in 2 h was tolerated.

\( CO_2 \) removal rates \((N = 6)\) were calculated from the sweep gas flow rate \( V_{\text{sweep}} \) at the sweep outlet, the carbon dioxide partial pressure \( p_{\text{\text{CO}_2}} \) and the atmospheric pressure \( p_{\text{ges}} \) according to the following formula:

\[
V_{\text{CO}_2} = V_{\text{sweep}} \cdot \frac{p_{\text{\text{CO}_2}}}{p_{\text{ges}}}
\]
Choice of test fluid

The mock was filled with either 600 mL of fresh porcine blood or expired human packed red blood cells (PRBC). Porcine blood was added with 10,000 IE Heparin to avoid clotting, 1 g Meropenem to avoid bacterial growth during the measurement, and diluted with a solution of 0.9% saline to a hemoglobin concentration (Hb) of 7 g/dL. As an alternative, PRBC could also be used as test fluid after they were diluted with phosphate-buffered saline (1x) to a Hb of 7 g/dL and adjusted to a pH of 7.20 ± 0.30 with 1000 mmol/L NaHCO₃ solution. However, in this work, the default test fluid was undiluted porcine blood.

In order to evaluate the two blood fluids, CO₂ removal rates of fresh porcine blood and human expired PRBC were compared. Blood gas analyses were performed for both circuit fluids and compared. For fresh porcine blood, pH was in range of 7.20 ± 0.30, bicarbonate in the range from 20 to 25 mmol/L, and venous saturation of oxygen 65% ± 5%. In the shown PRBC measurement, the pH was 7.30 bicarbonate 21 mmol/L and venous saturation of oxygen 69%. Lactate was used to assess quality of the blood fluid. For fresh porcine blood, lactate values of up to 10 mmol/L before the measurement were tolerated. For expired PRBC, lactate levels of up to 20 mmol/L the beginning of the measurement series had to be tolerated.

Evaluation of three experimental ECCO₂R scenarios

Hemoglobin concentration and CO₂ removal

Three different hemoglobin levels (12, 7, 4 g/dL) were obtained by diluting fresh porcine blood with a solution of 0.9% saline and 24 mmol/L NaHCO₃. \( V_{\text{sweep}} \) and blood flow were set according to standard protocol. Blood gas analyses confirmed that bicarbonate was kept constantly at 24 mmol/L ± 3 mmol/L.

The air-driven ECCO₂R

Comparison of pure oxygen and air as sweep gases Pure oxygen or compressed air from gas cylinders were used to create the sweep gas flow. \( V_{\text{sweep}} \) and BF were set according to standard protocol.

The low-flow ECCO₂R

Addition of a recirculation channel to the setup A recirculation channel with variable throttle was introduced to the test ECCO₂R circuit according to Fig. 1. A part of the blood behind the test ML was brought back to the pump of the test circuit. Therefore, blood flow through the test ML was higher than the blood flow at the junction between test circuit and primary circuit (i.e., the simulated cannula flow). In this setup, \( V_{\text{sweep}} \) was kept constant at 5 L/min pure oxygen. The effect on CO₂ removal of a recirculation channel was compared to the regular circuit by closing the loop fully.

Acid-enhanced recirculation The acid-enhanced recirculation channel was built similar to the recirculation channel described before. An infusion of 0.9% NaCl adjusted to a pH of 1.0 with 25% hydrochloric acid was added via luer-lock to the recirculation channel.
channel (Fig. 1). Two different setups were compared. (A) A simulated cannula flow of 260 mL/min, a recirculation flow of 180 mL/min, and an acid infusion rate of 500 mL/h were chosen to evaluate the CO$_2$ removal rate of the test ML. (B) The second simulation used a simulated cannula flow of 290 mL/min and a recirculation flow of 170 mL/min and an infusion rate of 1200 mL/h. $V_{\text{sweep}}$ was kept constantly at 5 L/min for both setups.

**Statistics**
Statistical analysis was performed with GraphPad Prism 5.02 (GraphPad Software, Inc., La Jolla, CA). Parametric data was presented as mean ± SD. The Kolmogorov-Smirnov test was used to test for Gaussian distribution. Differences between groups were tested with unpaired $t$ test and ANOVA for Gaussian-distributed groups and with the Mann Whitney $U$ test and Kruskal-Wallis test for non-Gaussian-distributed groups. $P$ values $< 0.05$ (*) and $< 0.01$ (**) were considered significant, and $p$ values $< 0.001$ (***) were considered highly significant.

**Results**
Composition of the dual-loop circuit
Using the presented mock circuit model, continuous steady state measurements, with adjustable $p_{\text{CO}_2}$ and $O_2$ saturation values, were possible.

Choice of test fluid
The measurements with expired packed red blood cells showed CO$_2$ removal rates comparable to the results with fresh porcine blood diluted to the same hemoglobin concentration (Fig. 2). The non-linear progression of PRBC and porcine blood was similar.

![Fig. 2 Choice of test fluid. CO$_2$ removal rates ($V_{\text{CO}_2}$) were similar for fresh porcine blood and diluted packed red blood cells. This measurement was done with a blood flow of 0.6 L/min and $O_2$ sweep gas flows ($V_{\text{sweep}}$) of 0.3, 0.5, 0.7, 1, 2, 4, 6, and 7 L/min in the test circuit](image)
Evaluation of three experimental ECCO2R scenarios

Hemoglobin concentration and CO2 removal

A strong association of CO2 removal rate and hemoglobin concentration was found. The CO2 removal of the test ML was higher when a high Hb was used (Fig. 3). The differences in CO2 removal between the three Hb values were highly significant ($p < 0.001$). The higher the sweep gas flow rate, the greater was the difference of the three Hb values in CO2 removal.

The air-driven ECCO2R

Comparison of pure oxygen and air as sweep gases Performance of CO2 removal ($\dot{V}_{CO2}$) showed a plateau at $\dot{V}_{Sweep} \geq 6$ L/min and above. Below that plateau, CO2 removal rate increased with rising $\dot{V}_{Sweep}$ rates, although the measured $p_a CO2$ at the sweep gas outlet of the ML was decreasing with rising sweep gas flow rates (Table 1).

The CO2 removal using air was, in most cases, 15–20% less efficient ($p < 0.05$) compared to pure oxygen at the same $\dot{V}_{Sweep}$.

The low-flow ECCO2R

Impact of a recirculation channel on CO2 removal The opening of the channel for recirculation caused an increase of the blood flow through the ML of the test circuit. However, the dynamics in fluid flow between the primary and test circuits as a whole was changed. The pump speed of the test circuit had to be increased to maintain a simulated cannula flow, similar to that before onset of recirculation.

The addition of a recirculation channel in some settings achieved an increased CO2 removal rate while in other settings decreased CO2 removal rates are observed (Table 2). The reduced CO2 removal rates were mainly observed for very high recirculation

Fig. 3 Hemoglobin concentration and CO2 removal. Hemoglobin concentrations were set to 12, 7, and 4 g/dL. A blood flow of 0.6 L/min and O2 sweep gas flows ($\dot{V}_{sweep}$) of 0.3, 0.5, 0.7, 1, 2, 4, 6, and 7 L/min were used in the test circuit. CO2 removal rate was higher for high hemoglobin concentrations ($p < 0.001$)
ratios \( \text{recirculation ratio} = \frac{\text{recirculation flow}}{\text{membrane flow}} \times 100\% \) relative to the simulated cannula flow, such as 88%.

**Impact of HCl infusion on CO₂ removal**
The addition of an HCl infusion at 500 mL/h (1200 mL/h) to the recirculation channel with a simulated cannula flow of 260 mL/min (290 mL/min) and a recirculation flow of 180 mL/min (170 mL/min) led to a significant increase in CO₂ removal rate (Fig. 4). The effect was more pronounced for an acid infusion rate of 1200 mL/h than for 500 mL/h.

**Table 1** Impact of pure oxygen and air as sweep gases on CO₂ removal

| \( V_{\text{Sweep}} \) [L/min] | Sweep gas | \( p_c \text{CO}_2 \) [kPa] | \( V_{\text{CO}_2} \) [mL/min] | \( p \) |
|-----------------|----------|-----------------|-----------------|---|
| 7               | \( \text{O}_2 \) | 1.41            | 98.92 ± 2.28    | \( p < 0.05 \) |
| 7               | Air      | 1.26            | 89.68 ± 4.06    |               |
| 6               | \( \text{O}_2 \) | 1.56            | 94.03 ± 0.97    | \( p < 0.05 \) |
| 6               | Air      | 1.35            | 81.37 ± 1.07    |               |
| 4               | \( \text{O}_2 \) | 2.12            | 85.05 ± 1.04    | \( p < 0.05 \) |
| 4               | Air      | 1.77            | 72.00 ± 1.16    |               |
| 2               | \( \text{O}_2 \) | 3.09            | 62.51 ± 0.98    | \( p < 0.05 \) |
| 2               | Air      | 2.74            | 54.67 ± 0.64    |               |
| 1               | \( \text{O}_2 \) | 4.05            | 40.91 ± 1.41    | \( p < 0.05 \) |
| 1               | Air      | 3.58            | 36.14 ± 0.57    |               |
| 0.7             | \( \text{O}_2 \) | 4.35            | 30.14 ± 0.62    | ns |
| 0.7             | Air      | 4.27            | 29.61 ± 0.55    |               |
| 0.5             | \( \text{O}_2 \) | 4.58            | 23.29 ± 0.32    | \( p < 0.05 \) |
| 0.5             | Air      | 4.12            | 20.11 ± 0.4     |               |
| 0.3             | \( \text{O}_2 \) | 4.7             | 13.99 ± 0.25    | \( p < 0.05 \) |
| 0.3             | Air      | 4.3             | 13.13 ± 0.11    |               |

**Table 2** Effect of a recirculation channel on CO₂ elimination

| Simulated cannula flow [mL/min] | Recirculation channel flow [mL/min] | Membrane flow [mL/min] | Recirculation ratio (%) | \( V_{\text{CO}_2} \) [mL/min] | \( p \) |
|-------------------------------|------------------------------------|------------------------|-------------------------|-----------------|---|
| 910                           | Closed                             | 910                    |                          | 66.67 ± 0.69    | \( p < 0.05 \) |
| 900                           | 500                                | 1400                   | 35.7                    | 69.63 ± 0.73    |               |
| 500                           | Closed                             | 500                    |                          | 58.31 ± 0.61    | ns |
| 500                           | 280                                | 780                    | 35.9                    | 57.97 ± 0.4     |               |
| 300                           | Closed                             | 300                    |                          | 48.66 ± 0.46    | \( p < 0.05 \) |
| 300                           | 240                                | 540                    | 44.4                    | 49.93 ± 0.43    |               |
| 200                           | Closed                             | 200                    |                          | 42.25 ± 0.71    | ns |
| 200                           | 140                                | 340                    | 41.2                    | 42.32 ± 0.99    |               |
| 100                           | Closed                             | 100                    |                          | 28.76 ± 1.22    | \( p < 0.05 \) |
| 100                           | 80                                 | 180                    | 44.4                    | 30.93 ± 0.62    |               |
| 330                           | Closed                             | 330                    |                          | 55.38 ± 0.88    | ns |
| 330                           | 1140                               | 1470                   | 77.6                    | 54.45 ± 1.26    |               |
| 180                           | Closed                             | 180                    |                          | 45.16 ± 0.96    | \( p < 0.05 \) |
| 180                           | 1310                               | 1490                   | 87.9                    | 41.24 ± 0.43    |               |
Discussion
Composition of the dual-loop circuit
The ECCO₂R mock described in this work shows a reasonable alternative method to gain information about ECCO₂R and simulate clinical settings. While animal-based ECMO or ECCO₂R test setups remain gold standard, for certain scientific issues, the animal model might not add additional benefit despite being a lot more complex and costlier. The test circuit in this model was inspired by the Homburg lung, the most commonly used ECCO₂R platform in our center, which is based on the Rotaflow platform and a pediatric Quadrox iD oxygenator [11].

One advantage of our mock setup in comparison to animal-based ECCO₂R experiments is the ability to modify blood gas parameters and the hemoglobin level more easily.

Other mock circulation models have been described in the literature that differ however from our approach. Schumer et al. created a closed loop ECMO mock with a single pump, a deoxygenation ML, and the tested oxygenation ML in series [10]. Barret et al. demonstrated a low blood flow single loop ECCO₂R model and used a Hemolung test ML. The impact of the sweep gas flow (air) on CO₂ removal and the relationship between $V_{\text{sweep}}$ and dead space was investigated [7]. Moreover, Sun et al. tested four commercial MLs in a closed loop circuit with one centrifugal pump [12]. The CO₂ removal rates for the tested Maquet Quadrox pediatric iD MLs were in the same range as our results using the same ML for a Hb of 15 g/dL (Table 3).

In contrast to those approaches, our model consists of two interconnected circuits where each circuit has its own centrifugal pump. This allows to create individual blood flows in the primary circuit and the test circuit. Consequently, the physiological blood flow of a mammal body (primary circuit, BF = 5 L/min) connected to an ECCO₂R

| $V_{\text{sweep}}$ | This study (BF 0.6 L/min) | Sun et al. (BF 0.5 L/min) [12] |
|-----------------|----------------------------|---------------------------------|
| 1 L/min         | 41 mL/min                  | 39 mL/min                       |
| 2 L/min         | 63 mL/min                  | 61 mL/min                       |
| 4 L/min         | 85 mL/min                  | 92 mL/min                       |

Table 3: Comparison of CO₂ removal rates of the pediatric oxygenator Quadrox iD for different blood flow (BF) and sweep gas flows ($V_{\text{sweep}}$).
device (test circuit) can be demonstrated more precisely. Similar to our approach, de Villiers Hugo et al. have created a dual mock to investigate a low-flow system with a modified dialysis unit [8].

Unlike most of the described mocks, our construction allows to measure continuously [1, 10, 13]. Using N$_2$ and CO$_2$ as sweep gases on the primary circuit, a constant venous milieu with a $p_{\text{CO}_2}$ of 45 ± 5 mmHg can be established throughout the whole measurement series.

**Limitations of the dual-loop model**

However, our study has some limitations. As most experiments were carried out with porcine rather than human blood, the data cannot be directly compared to clinical patients. However, we did evaluate diluted expired human packed red blood cells, although in one exemplary scenario, and found no relevant difference in CO$_2$ elimination compared to fresh porcine blood. However, like in most mock circuits, the absence of endothelium and its interaction with the blood limits generalization.

Moreover, the two preclinical scenarios, air-driven and low-flow ECCO$_2$R, were measured with undiluted porcine blood samples, as obtained by the butcher, in order to keep already complex setups as simple as possible. Porcine Hb values of 13.5 ± 1.5 g/dL are rather high compared to typical clinical scenarios of patients with hypercapnic respiratory failure. However, a scenario with diluted porcine blood samples was added to consider the association of different hemoglobin concentrations and CO$_2$ removal. Not for clinical reasons but to analyze a broad range, the lowest Hb level was set to a rather low 4 g/dL.

**Choice of test fluid**

We conducted this experiment to demonstrate that both fresh porcine blood and diluted expired packed red blood cells qualify for mock circuit fluid with rather similar properties. While we used both fluids in our initial tests with mock circuits, at this point, we prefer fresh porcine blood. Availability of diluted packed red blood cells is limited, as clinical operations only rarely generate expired PRBC. Those few PRBC that are older than 6 weeks have to be discarded and can be redirected to research. Although expiration of PRBC does happen, timing is not predictable, and quality and storage time of those expired packed red blood cells is very inhomogeneous. Moreover, we observed that deoxygenating the system to a sufficient venous environment in the primary circuit was easier when using fresh pig blood. The impact of storage time on biochemical processes of PRBC has been described in literature before.

Prolonged storage of PRBC leads to a left shift of the oxygen dissociation curve and the affinity of oxygen for hemoglobin in the PRBC increases [14, 15]. Moreover, the increase of affinity results in a decrease of 2,3-bisphosphoglycerate concentration in PRBC during storage longer than 2 weeks [16]. Furthermore, a lower pH, due to lactate production by erythrocytes, induces the depletion of 2,3-bisphosphoglycerate and increases the affinity of hemoglobin for O$_2$ [15]. As a result, the ability to deoxygenate in the primary circuit is better for fresh porcine blood than for old PRCBs.

In addition, fresh porcine blood samples did not require pH adjustment, as there was very little variance in the samples and the amount of lactate was much lower.
Moreover, it was paramount to avoid relevant pH drifts during experiments, and fresh porcine blood was also more stable in this regard.

**Evaluation of three experimental ECCO₂R scenarios**

**Hemoglobin concentration and CO₂ removal**

The hemoglobin level in the blood has a strong impact on the ability of the test ML to remove CO₂, as hemoglobin is one of the carriers of CO₂ in the blood. About 13% of the total CO₂ amount in the blood is bounded on Hb to carbaminohemoglobin [16, 17]. A lower hemoglobin concentration in the blood reduces the ability to unload CO₂ from carbaminohemoglobin in exchange for O₂ at the test ML according to human lungs [18, 19]. Moreover, as described by the Haldane effect, the upload of oxygen to hemoglobin in the test ML induces a release of protons (H⁺) from the hemoglobin molecules [20, 21]. In the blood, there is a reversible equilibrium between bicarbonate and carbon dioxide catalyzed by the enzyme carbonic anhydrase that is stored in erythrocytes [15, 20, 22]. Reducing hemoglobin level, the buffer capacity to provide protons declines as well as the amount of the enzyme carbonic anhydrase to catalyze the reaction. Therefore, less CO₂ can possibly be eliminated at the test ML.

**The air-driven ECCO₂R**

In this scenario, various sweep gas flows are tested to find an economical $\dot{V}_{\text{sweep}}$ range. While a decrease in oxygen flow by 1 or 2 L/min alone might not seem significant in terms of macroeconomics, it is nevertheless an important step to a more efficient ECCO₂R design.

As shown in Table 2, for a blood flow of 600 mL/min, a plateau is reached, applying $\dot{V}_{\text{sweep}} \geq 6$ L/min. Therefore, an efficient mobile ECCO₂R with limited sweep gas supply should stay at the lower sweep gas flow rates of this plateau. Similar results have been shown by Barret et al. for a blood flow of 400 mL/min and a plateau for $\dot{V}_{\text{sweep}} \geq 4$ L/min [7].

We propose that the plateau is caused by a decrease of the final $p_g$CO₂ in the gas phase at the outflow of the test ML, when high $\dot{V}_{\text{sweep}}$ rates are applied. We assume, that using low sweep flow rates, an equilibrium between CO₂ in gas and in the blood is established early in the hollow fiber of the ML, and the $p_g$CO₂ determined is similar to the $p_a$CO₂ in the blood. Increasing $\dot{V}_{\text{sweep}}$, the equilibrium is established subsequently, or the period of contact is too short to reach equilibrium. Therefore, the measured $p_g$CO₂ of high flow rates is lower than for low flow rates. According to Fick’s principle, a high difference between $p_a$CO₂ and $p_g$CO₂ induces a higher diffusion flux, hence a higher CO₂ elimination. However, high sweep flow rates result in a final $p_g$CO₂ in the gas phase that is practically zero. A further increase cannot decrease the $p_g$CO₂ in the gas phase below zero and therefore has minimal effect on the diffusion gradient and the CO₂ removal rate.

The second aspect of this experiment was the idea to use ambient air as a sweep gas alternative to pure oxygen. This would eliminate the above discussed problem of sweep gas economy altogether. However, as shown in Table 2, CO₂ removal rate of ambient air is significantly lower than that of pure oxygen. We assume, that this stands in close connection to the Haldane effect as well [21]. Ambient air leads to lower oxygen
concentrations in the blood compared to pure oxygen. By uploading less oxygen to hemoglobin, less CO₂ dissociates from the molecule in exchange [17]. Moreover, a lower pO₂ in the blood causes a smaller release of protons from hemoglobin and therefore less CO₂ formation and dissociation [18]. Despite this reduction on CO₂ removal, we still like the idea of a portable ECCO₂R with ambient air. A turbine setup, such as used by many CPAP devices, could utilize the infinite supply of ambient air and is most likely less heavy than a gas cylinder. This makes a patient on such a hypothetic ECCO₂R device independent from pure oxygen and gas cylinders and might promote the mobility of the patient.

The low-flow ECCO₂R
A major problem of low-flow cannulas, such as dialysis catheters, is their inability to create a sufficient BF across the ML, leading to an increased risk of ML clotting. A previous study has shown that there is a correlation of areas with low flow speed and thrombus formation in membrane oxygenators [23]. One solution for this problem is to increase flow speed in the membrane lungs by using smaller oxygenators, with smaller cross sections. However, in our opinion it is preferable to use commonly available oxygenators with higher surface area. Therefore, another approach is the use of a recirculation channel, a trick to recirculate the limited blood volume from the cannula more than once before sending it back into the patient. However, only flow volume is increased by such a recirculation channel, not the CO₂ content, because the recirculated blood was already CO₂ depleted during the first passage through the ML. To further increase CO₂ removal from the mixed blood, HCO₃⁻ can be converted into membrane diffusible CO₂ with the addition of acid.

Impact of a recirculation channel on CO₂ elimination When first evaluating recirculation channels, an obviously reduced CO₂ removal compared to an otherwise identical setup without a recirculation channel was noticeable. Pump rotation speed was kept constant when opening the loop inducing a lower simulated cannula flow (data not shown).

However, for the comparisons shown in this paper, we decided that the simulated cannula flow, which defines the amount of CO₂-rich blood that goes into the test setup, should be held constant. With matched simulated cannula flows, the overall trend for lower CO₂ removal rates with recirculation channels is gone, albeit at the expense of higher pump rotation speeds.

Small and large recirculation ratios vary CO₂ removal efficiency because of a different CO₂ content in the mixed blood and a different total membrane flow. Increasing the recirculation ratio decreases the carbon dioxide partial pressure in the mixed blood (p<sub>m</sub>CO₂) hence the transmembrane gradient, but on the other hand, increases flow speed in the ML, two factors with inverse influence on CO₂ removal rate. With ECMO flow rates, rather than ECCO₂R flow rates in this work, Madhani et al. also found an increased CO₂ transfer rate for blood recirculation [24]. However, in his setup, a cannula flow of 3.5 L/min and a recirculation flow of 6.5 L/min were used. This recirculation ratio of 65% is above our efficiency increasing ratios but below those recirculation ratios, where our setup showed a decrease in efficiency. We suggest that there is an
optimal recirculation ratio, somewhere between 50 and 75%. The evaluation of this optimum should be subject of future research.

**Impact of acidification of the recirculated blood** Achieving increased CO\(_2\) removal by local acidification has been described in previous studies [2, 5, 6, 9, 25–27]. However, this is the first study to combine an acidification step with a recirculation channel. The acidification can be used to overcompensate slight disadvantages in the CO\(_2\) washout of recirculation channels with high recirculation ratio. The acid transforms bicarbonate (effectively chemically dissolved CO\(_2\)) into physically dissolved CO\(_2\), hence restoring the \(p_m\)CO\(_2\) level in the recirculated blood and increasing CO\(_2\) removal in the ML. In our study, the strong inorganic acid HCl was used, as Cl\(^-\) has little metabolic effects. However, like previous studies, we also identified hemolysis caused by both the acid and mechanical blood trauma in the bends of the recirculation channel as major problem that needs to be solved before this approach can be used in clinical studies [25].

**Conclusion**

Our ECCO\(_2\)R mock represents a novel method to generate data with animal free experiments as it simulates a mammal body connected to an ECCO\(_2\)R. As a practical consequence of this work, we suggest that a gas cylinder free and mobile ECCO\(_2\)R could use air as sweep gas with only moderate reduction in efficiency. Moreover, we conclude that recirculation channels and acid enhancement are promising tools to improve ECCO\(_2\)R therapy that should be topic of future research.

All in all, our model is an improvement of so far established mock circuits, and more ECCO\(_2\)R setups will be tested in the future.

**Abbreviations**

ECCO\(_2\)R: Extra corporal carbon dioxide removal; CO\(_2\): Carbon dioxide; Hb: Hemoglobin; ML: Membrane lung; \(V_{\text{sweep}}\): Gas flow rate; \(p_m\)CO\(_2\): Carbon dioxide partial pressure in gas phase; \(p_v\)CO\(_2\): Carbon dioxide partial pressure in venous blood; \(p_m\)CO\(_2\): Carbon dioxide partial pressure in mixed blood; \(P_{\text{atm}}\): Atmospheric pressure; BF: Blood flow; ARDS: Acute respiratory distress syndrome; COPD: Chronic obstructive pulmonary disease; PVC: Polyvinyl chloride; PRBC: Packed red blood cells; \(\dot{V}_{\text{CO}2}\): CO\(_2\) removal rate; HCL: Hydrochloric acid

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**Authors’ contributions**

LSS and AMJ performed the experiments and wrote the manuscript. NS, FS, RWM, JS, QTD, and RB assisted in the development of the study and helped create the manuscript. PML and AJO designed, supervised the experiments, and revised the manuscripts. LSS and AMJ contributed equally to the manuscript. The authors read and approved the final manuscript.

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**Availability of data and materials**

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**Competing interests**

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