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Alkaline Liquid Ventilation of the Membrane Lung for Extracorporeal Carbon Dioxide Removal ($ECCO_2R$): In Vitro Study

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Abstract: Extracorporeal carbon dioxide removal ($ECCO_2R$) is a promising strategy to manage acute respiratory failure. We hypothesized that $ECCO_2R$ could be enhanced by ventilating the membrane lung with a sodium hydroxide (NaOH) solution with high CO_2 absorbing capacity. A computed mathematical model was implemented to assess NaOH–CO_2 interactions. Subsequently, we compared NaOH infusion, named "alkaline liquid ventilation", to conventional oxygen sweeping flows. We built an extracorporeal circuit with two polypropylene membrane lungs, one to remove CO_2 and the other to maintain a constant PCO_2 (60 ± 2 mmHg). The circuit was primed with swine blood. Blood flow was 500 mL × min^{-1}. After testing the safety and feasibility of increasing concentrations of aqueous NaOH (up to 100 mmol × L^{-1}), the CO_2 removal capacity of sweeping oxygen was compared to that of 100 mmol × L^{-1} NaOH. We performed six experiments to randomly test four sweep flows (100, 250, 500, 1000 mL × min^{-1}) for each fluid plus 10 L × min^{-1} oxygen. Alkaline liquid ventilation proved to be feasible and safe. No damages or hemolysis were detected. NaOH showed higher CO_2 removal capacity compared to oxygen for flows up to 1 L × min^{-1}. However, the highest CO_2 extraction power exerted by NaOH was comparable to that of 10 L × min^{-1} oxygen. Further studies with dedicated devices are required to exploit potential clinical applications of alkaline liquid ventilation.

Keywords: extracorporeal CO_2 removal; liquid ventilation; membrane lung

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1. Introduction

Extracorporeal carbon dioxide removal ($ECCO_2R$) clears CO_2 from the blood through an extracorporeal membrane lung (ML). This allows independent modulation of minute ventilation and arterial partial pressure of CO_2 ($PaCO_2$), which are otherwise physiologically linked [1]. $ECCO_2R$ has been proposed to facilitate ultra-protective ventilation [2–4] and to promote non-invasive ventilation [5]. This could be particularly beneficial in patients suffering from respiratory failure, including exacerbations of chronic obstructive pulmonary disease (COPD) [6], acute respiratory distress syndrome (ARDS) [7], and patients awaiting lung transplantation [8]. The amount of CO_2 removed by the extracorporeal

support is a crucial determinant of clinical efficacy [9,10]. However, the clinical benefits of $ECCO_2R$ are still under evaluation due to safety concerns, mainly related to hemorrhagic and thrombotic adverse events [9].

Several $ECCO_2R$ devices are clinically available. They are mainly characterized by a low extracorporeal blood flow (i.e., <500 mL $\times$ min^{-1}) to achieve minimally invasive approaches [11]. Indeed, although 500 mL of blood contain an amount of CO_2 comparable to the amount of CO_2 produced by the body in one minute ($\dot{V}CO_2$), the relatively low CO_2 transfer efficiency of conventional MLs significantly reduces the efficacy of these strategies [12].

The transmembrane gradient of PCO_2 is the driving force that moves CO_2 from blood to the sweeping gases. However, the use of high sweep gas flows, while maximizing the transmembrane gradient, does not increase CO_2 clearance significantly. Indeed, during $ECCO_2R$, most of the extracorporeal CO_2 removal capacity is achieved for sweep gas flows below 2 L $\times$ min^{-1} since, at higher flows, the system rapidly loses efficiency [13–16].

Several $ECCO_2R$ techniques are currently undergoing preclinical evaluations. The main aim is to overcome the present limitations to enhance CO_2 removal [17–21] effectively. To this purpose, our group has achieved high rates of CO_2 removal through acidification of the blood entering the ML [22–27]. This strategy reduced dissociated CO_2 (HCO_3^-) in favor of dissolved CO_2 (PCO_2), thus increasing the efficiency of $ECCO_2R$. Nevertheless, these approaches are still experimental, mainly due to safety and technical issues [28,29].

In the present study, we hypothesized that extracorporeal CO_2 removal could be enhanced through the ventilation of the ML with a sweep fluid with an extremely high CO_2 absorbing capacity (sodium hydroxide -NaOH- solutions), thereby preserving the transmembrane CO_2 gradient.

Indeed, when a high amount of CO_2 is added to dilute NaOH solutions, carbon dioxide first hydrates to carbonic acid (H_2CO_3), Equation (1), which will subsequently react with NaOH to form sodium bicarbonate ($NaHCO_3$), Equation (2).

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \quad (1)$$

$$NaOH + H_2CO_3 \leftrightarrow Na^+ + HCO_3^- + H_2O \quad (2)$$

Instead, when CO_2 is added to highly concentrated NaOH solutions, sodium bicarbonate is formed directly, Equation (3), which subsequently forms sodium carbonate, Equation (4).

$$NaOH + CO_2 \leftrightarrow NaHCO_3 \quad (3)$$

$$NaHCO_3 + NaOH \leftrightarrow Na_2CO_3 + H_2O \quad (4)$$

Consequently, highly concentrated NaOH solutions can absorb a conspicuous amount of CO_2 while keeping PCO_2 almost down to zero although the elevated pH of the solution causes safety concerns.

The aim of the present proof-of-principle study was to evaluate in-vitro the feasibility and the CO_2 transfer efficacy of membrane lung ventilation with a NaOH solution. This type of ventilation was named "alkaline liquid ventilation".

Different concentrations of NaOH were tested and the efficacy and efficiency in CO_2 removal of alkaline liquid ventilation were compared to conventional sweep gas flow.

2. Materials and Methods

An in vitro setting (Figure 1) was built to simulate a patient undergoing extracorporeal CO_2 removal.

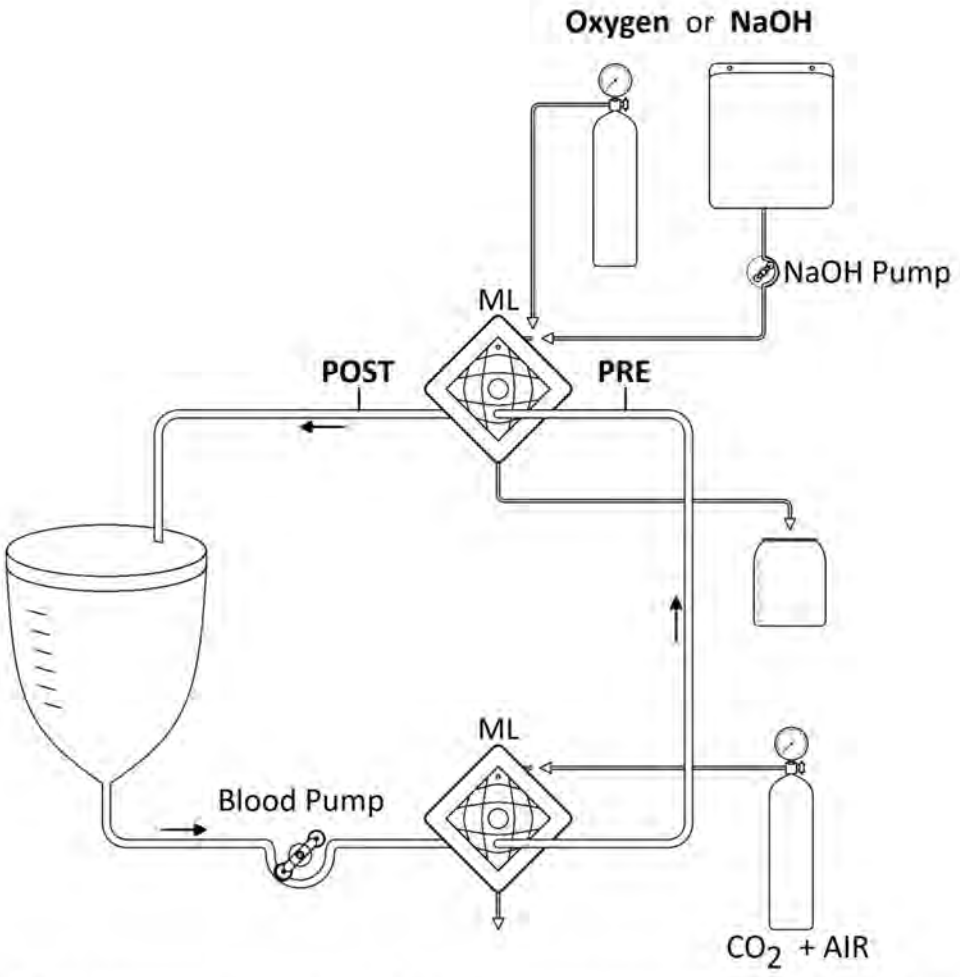


Figure 1. Schematic representation of the extracorporeal circuit. ML: membrane lung; PRE: blood sampling access upstream the ML for $ECCO_2R$; POST: blood sampling access downstream the ML for $ECCO_2R$.

A closed-loop circuit was assembled with 3/8 and 1/4 inch polyvinylchloride class IV medical tubes (Medtronic, Minneapolis, MN, USA), one 4 L reservoir (VHK 71000 venous hardshell cardiotomy reservoir, Getinge, Gothenburg, Sweden), two polypropylene oxygenators membrane gas exchangers (Quadrox-i Small adult HMO 50000, Getinge, Gothenburg, Sweden) and one peristaltic pump (Multiflow Roller Pump Module H10 series, Stöckert Shiley, München, Germany).

The circuit was primed with about 3 L of swine blood collected at a local abattoir during usual slaughtering processes in compliance with CE regulations (1069/2009), authorization number 0141051/19 provided by ATS Milano, Regione Lombardia. MultiBic® solution (Fresenius Medical Care Italia, Palazzo Pignano, Italy) was added to achieve a total volume of about 4 L. Sodium Heparin 25000 I.U. (Pfizer Italia S.r.l, Latina, Italy), anticoagulant-citrate-dextrose ACD 300 mL (Fresenius Kabi Italia, Isola Della Scala, Italy) and cefazolin 1 g (Teva Italia, Milano, Italy) were added to the blood.

The first gas exchanger downstream the reservoir was ventilated with a gas mixture of air and CO_2 to maintain a constant PCO_2 of 60 $\pm$ 2 mmHg at the inlet of the second oxygenator throughout all experiments. The second oxygenator was employed to remove CO_2 through either ventilation with oxygen or a continuous infusion of sodium hydroxide (NaOH) solution, "alkaline liquid ventilation", into the gas side of the membrane lung. Circuit accesses for blood sampling were positioned upstream (PRE) and downstream (POST) of the second oxygenator.

NaOH pellets (Sigma-Aldrich, Merck KGaA, Saint Louis, MO, USA) were diluted in distilled water to achieve the required concentrations. NaOH solutions were stored in disposable parenteral bags (Bertoni Nello S.r.l. Modena, Italy) and infused into the gas inlet port in the gas exchanger using a peristaltic pump (Multiflow Roller Pump Module H10 series, Stöckert Shiley, München, Germany). NaOH exiting the oxygenator was discarded.

The blood temperature was kept stable at 37 °C through heat exchangers connected to the membrane lungs.

The study was divided into four steps: (1) a mathematical modeling of NaOH and CO_2 interactions to evaluate the theoretical basis of the study; (2) a safety and feasibility test to evaluate the effects of increasing NaOH concentrations on the membrane lung integrity and CO_2 removal; (3) an efficiency test to compare the CO_2 removal of similar sweep flows (up to 1 L × min^{-1}) of oxygen vs. NaOH at the concentration selected following the feasibility test; (4) an efficacy test to compare the CO_2 removal of the best liquid ventilation flow, selected from the efficiency test, vs. 10 L × min^{-1} of oxygen.

All the in-vitro tests were performed with 500 mL × min^{-1} of blood flow.

2.1. Mathematical Modeling

Theoretical effects of CO_2 absorption by aqueous NaOH were computed solving a system of equations (MATLAB R2018b; The Math Works, Inc, Natick, MA, USA), including standard mass-action, mass-conservation and electroneutrality laws of the involved species: water, NaOH, CO_2 (see the Online Supplement for more details).

We simulated a closed system with aqueous NaOH at varying concentrations (from 0 to 100 by 20 mmol × L^{-1}) in which we introduced CO_2 at different concentrations (from 0 to 100 by 5 mmol × L^{-1}).

Of note, in the present mathematical model of a closed system, total pressure could exceed barometric pressure.

2.2. Definitions and Calculations

Bicarbonate ion concentration ([HCO_3^-]) was calculated from pH and PCO_2 modifying the Henderson-Hasselbalch equation

$$[HCO_3^-] = \alpha \times PCO_2 \times 10^{pH-pK} \quad (5)$$

where α = 0.0307 mmol × L^{-1} × $mmHg^{-1}$ (solubility of CO_2 in plasma) [30,31] and pK = 6.129 (negative logarithm of the equilibrium constant) [31–33].

Plasma carbon dioxide content PRE and POST membrane lung (expressed in mmol × L^{-1}) was calculated according to the method published by Douglas et al. [34]:

$$[TCO_2] = \alpha \times PCO_2 \times \left(1 + 10^{pH-pK}\right) \quad (6)$$

Carbon dioxide transfer across the membrane lung, $\dot{V}CO_2$ (in mL × min^{-1}), was calculated from the transmembrane lung TCO_2 difference [35]:

$$\dot{V}CO_2 = ([TCO_{2PRE}] - [TCO_{2POST}]) \times \text{blood flow} \times 25.45 \quad (7)$$

where TCO_{2PRE} represents CO_2 content before the membrane lung while TCO_{2POST} is the CO_2 content after the membrane lung, blood flow is measured in L × min^{-1}, and the conversion factor is in mL × $mmol^{-1}$.

2.3. Safety and Feasibility Test

Possible macroscopic detrimental effects on the membrane lung were evaluated. The effect on CO_2 removal of alkaline liquid ventilation at increasing concentrations of NaOH (10, 30, 60, 90, 100 mmol × L^{-1}) and increasing ventilating flows (100, 250, 500, 1000 mL × min^{-1}) was likewise evaluated. Each combination of NaOH concentration and sweep fluid flow was

tested once and for 15 min. At the end of each step, PRE and POST blood samples were collected for blood gas analysis (BGA) (Radiometer abl800 flex, Copenhagen, Denmark).

In addition, the integrity of the oxygenator was evaluated through visual inspection of the membrane lung, evaluation of the presence of blood in the NaOH solution exiting the oxygenator, and through analysis of blood sodium, potassium, and methemoglobin as indirect markers of hemolysis. The time-course of methemoglobin was evaluated at 4 time points (15, 30, 45, and 60 min) while testing aqueous NaOH at different sweep flows during the efficiency and efficacy tests.

The CO_2 removal efficiency was estimated by computing PCO_2 differences across the membrane lung and $\dot{V}CO_2$.

At the end of the feasibility test, we selected the highest NaOH concentration endured by the membrane lung to perform the subsequent efficiency and efficacy tests.

2.4. Efficiency and Efficacy Tests

We performed six experiments with blood from 4 pigs. For each experiment, we tested, in random order, two different sweeping fluids, pure oxygen (FiO_2 equal to 1) and aqueous NaOH at 100 mmol $\times$ L^{-1} (the concentration selected from the feasibility test). Four sweep flows (100, 250, 500, 1000 mL $\times$ min^{-1}) for each fluid were randomly tested. We also randomized and tested 10 L/min of oxygen flow. Each combination of sweep fluid and flow was applied once during the single experiment.

The target PRE PCO_2 was 60 $\pm$ 2 mmHg.

At the end of each step lasting about 15 min, we collected PRE and POST blood samples for BGA.

CO_2 removal efficiency and efficacy were evaluated from PCO_2 differences across the membrane lung and $\dot{V}CO_2$.

The highest CO_2 removal achieved with alkaline liquid ventilation was compared with the CO_2 removal achieved with conventional gaseous ventilation performed with 10 L $\times$ min^{-1} of oxygen.

2.5. Statistical Analysis

Data are reported as median and interquartile range (IQR). Two-way repeated measures ANOVA or two-way repeated measures ANOVA on ranks was used, as appropriate, to test safety, feasibility (PRE and POST values), and efficiency.

One-way repeated measures or Friedman repeated measures was used, as appropriate, to test safety and feasibility (POST–PRE differences) and to compare methemoglobin values at different time points.

Paired *t*-test or Wilcoxon signed rank test was used, as appropriate, to test efficacy. Post-hoc analyses were performed with Bonferroni or Tukey corrections. Statistical significance was defined as $p < 0.05$. Analysis was performed with SAS software 9.4 (SAS Institute, Inc., Cary, NC, USA) and SigmaPlot v.11.0 (Systat Software Inc, San Jose, CA, USA).

3. Results

3.1. Mathematical Modeling

The PCO_2 of gas/oxygen or distilled water, in a closed system, at increasing concentrations of CO_2 raises linearly, see Figure 2, although the slope is steeper in water relative to gas/oxygen. Instead, if NaOH is added to water, the solution PCO_2 remains close to zero as long as the added CO_2 is lower than the amount of added NaOH. When similar amounts of CO_2 and NaOH are added, almost all CO_2 reacts forming HCO_3^- and the solution pH is around 8.220–8.230.

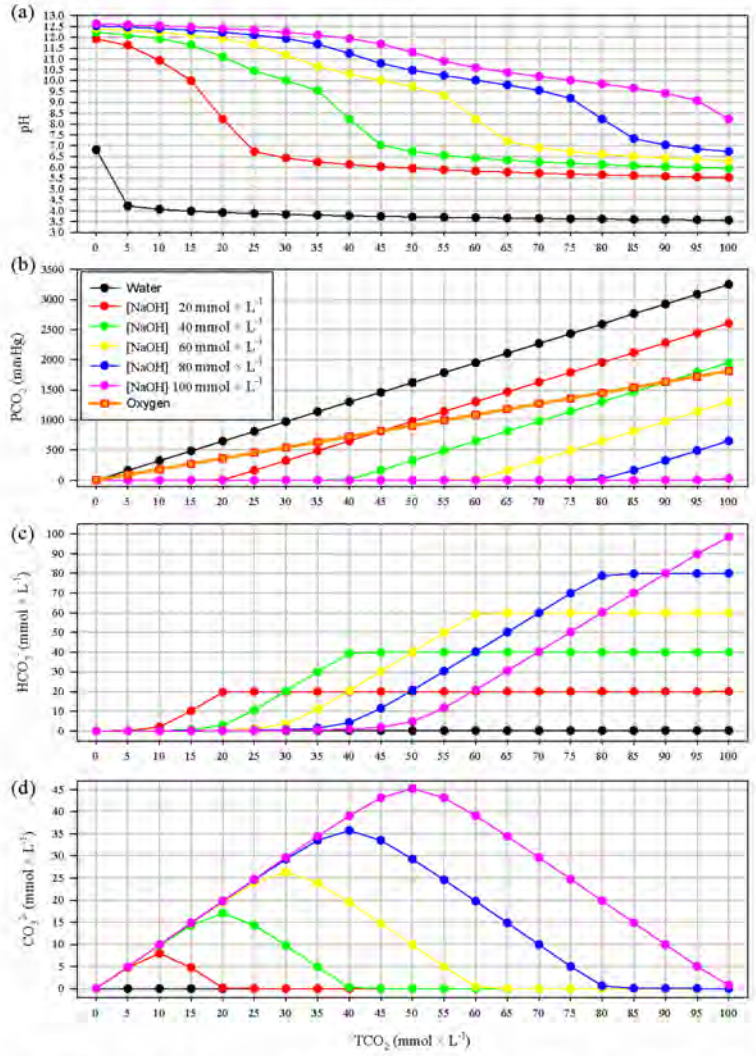


Figure 2. Simulated effects of increasing TCO_2 from 0 to 100 by 5 mmol × L^{-1} in a *closed* system with aqueous NaOH at varying concentrations (from 0 (water) to 100 by 20 mmol × L^{-1}). Panel (**a**) represents pH; panel (**b**) represents PCO_2, the orange line with red squares represents PCO_2 values of one *closed* liter of oxygen/gas containing increasing TCO_2; panel (**c**) represents HCO_3^-; panel (**d**) represents CO_3^{2-}. Abbreviations: PCO_2, partial pressure of carbon dioxide; HCO_3^-, bicarbonate; CO_3^{2-}, carbonate; TCO_2, total CO_2 content.

Otherwise, if the added CO_2 is lower than NaOH, carbonic acid dissociates to HCO_3^- which, due to the alkaline milieu, further dissociates to CO_3^{2-}, thus reducing the concentration of HCO_3^-. When CO_2 is near half or lower than NaOH, almost all CO_2 forms CO_3^{2-} and the solution pH is above 11. Instead, if the TCO_2 is higher than NaOH, all hydroxide reacts with CO_2 forming HCO_3^- and the pH decreases below 8.

Interestingly when the added CO_2 is higher than twice the NaOH, the PCO_2 in the NaOH solution will be higher than the one in a similar gas volume containing the same amount of CO_2.

For example, one liter of gas containing 200 mL (7.86 mmol) of CO_2, the theoretical $\dot{V}CO_2$ of an adult, would have a PCO_2 of 143 mmHg (713 mmHg × 0.2), while 1 L of water would have a higher PCO_2 of 255 mmHg. On the contrary, the same amount of CO_2 could be stored in 1 L of NaOH 10 mmol × min^{-1} solution with a PCO_2 close to zero.

3.2. Feasibility and Safety Test

No detectable damages to the membrane lung were observed. Moreover, no blood was found in the sweep fluid exiting the oxygenator.

Figure 3 and Table 1 report the BGAs of PRE and POST blood. PCO_{2PRE} was stable throughout the entire test, 59.0 (58.0–60.0) mmHg. Delta PCO_2 across the membrane lung was significantly lower at 10 mmol × L^{-1} (−32.2 (−38.6–−23.1) mmHg). Otherwise, it showed small increases as NaOH concentration increases (−41.4 (−43.1–−36.8), −47.7 (−49.5–−44), −47.8 (−48.6–−47) and −48.2 (−48.4–−46.6) mmHg at 30, 60, 90, and 100 mmol × L^{-1} respectively). PCO_{2POST} was reduced to about 12 mmHg with NaOH concentration ≥ 60 mmol × L^{-1} (12.0 (11.0–15.0), 11.4 (10.2–12.4) and 12.4 (11.3–13.1) mmHg at 60, 90, and 100 mmol × L^{-1} respectively), subsequently pH_{POST} increased up to 7.913 (7.885–7.943) at NaOH concentration equal to 100 mmol × L^{-1}. The lowest $\dot{V}CO_2$ was also recorded at the lowest NaOH concentration 73.9 (54.3–91.8) mL × min^{-1}. PRE blood sodium and potassium concentration were stable (see Supplementary Table S1 for details). POST chloride concentrations were higher than PRE values while sodium concentrations were lower. Moreover, a simultaneous decrease in potassium and calcium POST concentrations was observed. These results are similar to the observations of Langer et al. in couples of measurements of blood entering and leaving the ML in 20 critically ill patients [36]. Methemoglobin values were not different over the time during the experiments (median (IQR) values 1.100 (0.950–2.850) at 15 min, 1.100 (1.050–2.150) at 30 min, 1.100 (1.050–2.400) at 45 min, 1.200 (1.050–2.900) at 60 min; $p = 0.606$).

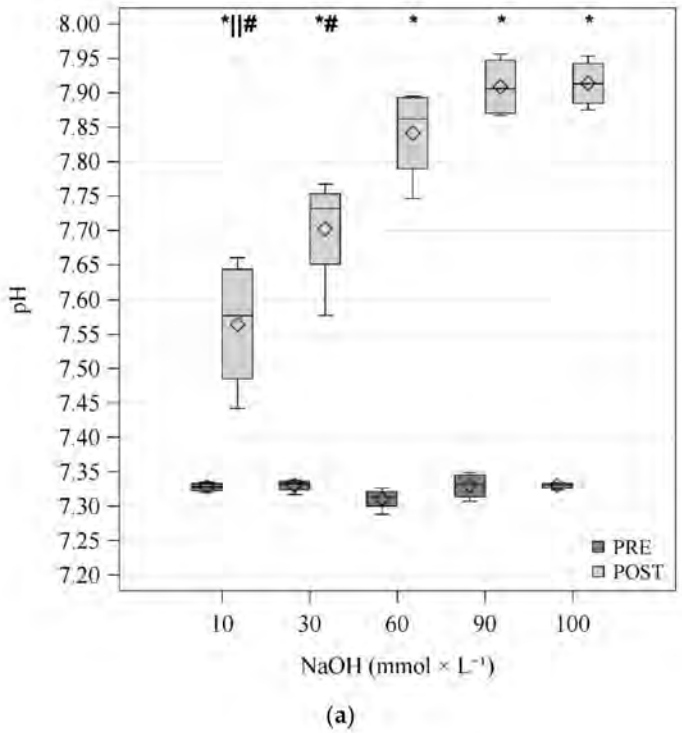
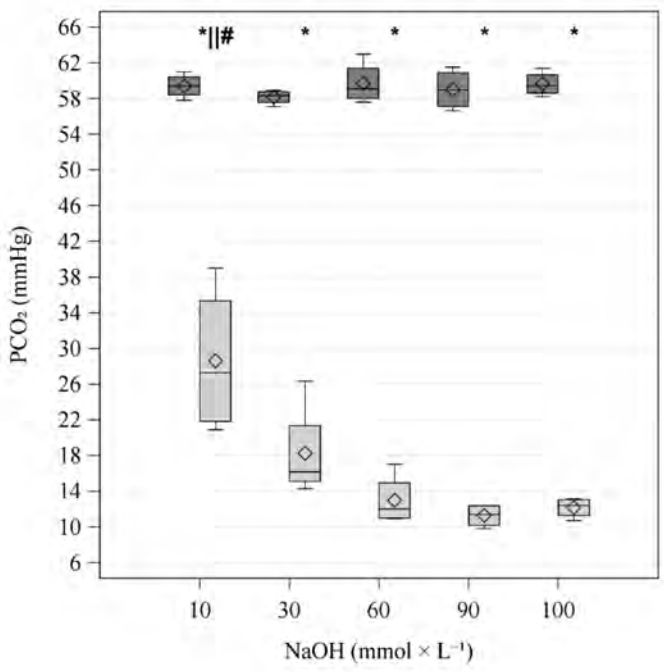
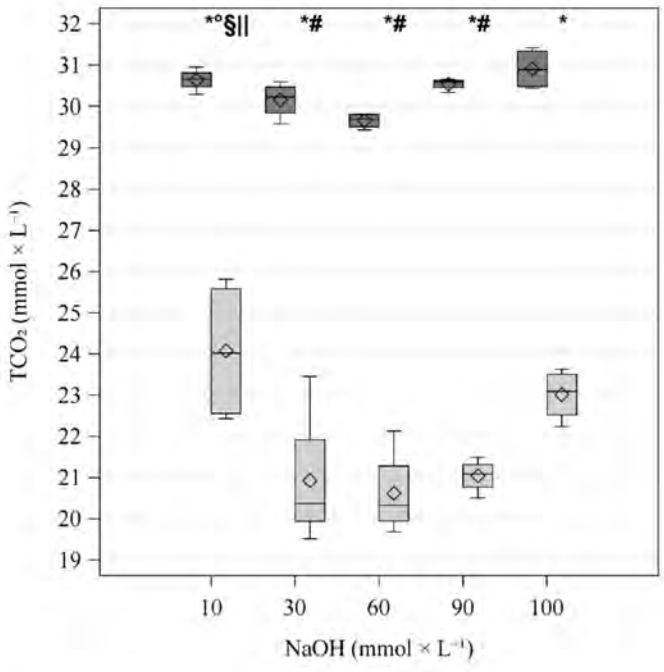


Figure 3. *Cont.*



(**b**)



(**c**)

Figure 3. Figures display the distribution of data by using a rectangular box plot and whiskers, the bottom and top edges of the box indicate the intra-quartile range (IQR) between the first and third quartiles (the 25th and 75th percentiles). The diamond marker inside the box indicates the mean value. The line inside the box indicates the median value. Whiskers indicate the range of values outside of the intra-quartile range but at a distance lower than the upper and lower fences ($\pm 1.5 \times$ IQR). Dark grey represents PRE blood sampling. Light grey represents POST blood sampling. Statistical analysis: Two-way ANOVA RM (TCO_2) or two-way ANOVA RM on ranks (pH and PCO_2). * $p < 0.05$ vs. PRE; ° $p < 0.05$ vs. 30; § $p < 0.05$ vs. 60; || $p < 0.05$ vs. 90; # $p < 0.05$ vs. 100. (**a**) pH distribution according at different NaOH concentrations; (**b**) PCO_2 (partial pressure of carbon dioxide) distribution at different NaOH concentrations. (**c**) TCO_2 (Carbon dioxide content) distribution at different NaOH concentrations.

Table 1. Efficiency tests results.

Variable		Ventilation	Flow (L × min^{-1})				*p* Vent.	*p* Flow	*p* Int.
			100	250	500	1000			
pH	**PRE** $	NaOH	7.346 (7.337–7.374)	7.351 (7.333–7.359)	7.356 (7.333–7.363)	7.336 (7.334–7.366)	**0.027**	0.999	**0.020**
		O_2	7.325 (7.306–7.333) *	7.313 (7.311–7.349) *	7.321 (7.301–7.34) *	7.325 (7.318–7.346)			
	POST $	NaOH	7.972 (7.968–8.057) #	7.987 (7.977–8.077) §#	7.964 (7.932–8.040)	7.938 (7.902–8.008)	**<0.001**	**<0.001**	**<0.001**
		O_2	7.352 (7.333–7.379) *°§#	7.405 (7.374–7.439) *§#	7.481 (7.435–7.514) *#	7.616 (7.612–7.654) *			
	Difference $	NaOH	0.628 (0.597–0.683) #	0.643 (0.624–0.718) §#	0.606 (0.597–0.673)	0.591 (0.565–0.635)	**<0.001**	**<0.001**	**<0.001**
		O_2	0.028 (0.011–0.041) *°§#	0.094 (0.063–0.101) *§#	0.145 (0.124–0.186) *#	0.295 (0.268–0.326) *			
PCO_2 (mmHg)	**PRE**	NaOH	59.7 (59.2–60.1)	59.5 (59.0–59.7)	59.4 (58.4–60.2)	60.0 (59.2–60.4)	0.909	0.882	0.332
		O_2	59.0 (58.4–59.9)	59.0 (58.7–60.5)	60.6 (59.0–61.0)	59.7 (59.5–59.8)			
	POST	NaOH	11.2 (11.0–13.0)	10.5 (10.3–11.1)	11.7 (11.3–12.1)	13.1 (12.8–13.1)	**<0.001**	**<0.001**	**<0.001**
		O_2	54.6 (53.7–56.2) *°§#	46.2 (45.4–49.7) *§#	40.4 (39.0–41.6) *#	28.2 (26.9–29.1) *			
	Difference	NaOH	−48.3 (−48.9–−47.1)	−48.5 (−50.5–−48.3)	−47.5 (−49.0–−46.3)	−46.5 (−47.6–−45.3)	**<0.001**	**<0.001**	**<0.001**
		O_2	−4.4 (−6.2–−2.0) *°§#	−13.1 (−13.8–−9.5) *§#	−19.1 (−22.8–−17.4) *#	−31.7 (−32.9–−30.7) *			
PO_2 (mmHg)	**PRE**	NaOH	138.0 (136.0–139.0) #	137.0 (137.0–143.0) #	137.5 (136.0–146.0)	141.5 (137.0–153.0)	0.231	0.460	**0.002**
		O_2	144.0 (141.0–162.0)	143.0 (140.0–159.0)	143.5 (138.0–156.0)	143.0 (139.0–154.0)			
	POST $	NaOH	125.0 (120.0–130.0)§#	130.5 (128.0–140.0) #	148.5 (142.0–157.0)	161.5 (159.0–169.0)	**<0.001**	**<0.001**	0.700
		O_2	595.5 (591.0–602.0) *§#	608.5 (603.0–623.0) *#	616.0 (611.0.–6260) *#	648.0 (632.0–654.0) *			
	Difference	NaOH	−13.0 (−16.0— −11.0)§#	−6.5 (−9.0–−3.0) §#	9.0 (6.0–11.0) #	18.0 (14.0–21.0)	**<0.001**	**<0.001**	0.105
		O_2	451.5 (429.0–461.0) *§#	455.5 (445.0–471.0) *§#	462.5 (453.0–477.0) *#	497.5 (487.0–508.0) *			
K^+ (mEq × L^{-1})	**PRE**	NaOH	4.1 (4.0–4.4)	4.1 (4.1–4.5)	4.2 (4.1–4.4)	4.2 (4.1–4.5)	0.127	0.594	0.299
		O_2	4.1 (3.9–4.2)	4.0 (4.0–4.2)	4.0 (4.0–4.2)	4.1 (4.0–4.2)			
	POST	NaOH	4.1 (4.0–4.3)	4.1 (4.0–4.4)	4.1 (4.0–4.3)	4.1 (4.0–4.4)	0.265	0.709	0.363
		O_2	4.1 (3.9–4.2)	4.0 (4.0–4.2)	4.0 (4.0–4.1)	4.0 (3.9–4.2)			
	Difference	NaOH	0.0 (0.0–0.1)	0.1 (0.0–0.1)	0.1 (0.1–0.1)	0.1 (0.1–0.1)	**0.009**	0.337	0.86
		O_2	0.0 (0.0–0.0) *	0.0 (0.0–0.0) *	0.0 (0.0–0.0) *	0.0 (0.0–0.1) *			
Na^+ (mEq × L^{-1})	**PRE**	NaOH	143.0 (142.0–144.0)	143.0 (143.0–144.0)	143.5 (142.0–145.0)	143.5 (143.0–144.0)	**0.038**	0.233	0.973
		O_2	139.0 (138.0–143.0) *	139.0 (139.0–143.0) *	139.5 (138.0–144.0) *	139.5 (138.0–145.0) *			
	POST	NaOH	141.0 (139.0–142.0)	140.0 (140.0–142.0)	140.5 (140.0–141.0)	141.5 (140.0–142.0)	0.407	0.524	0.096
		O_2	139.0 (138.0–143.0)	138.5 (137.0–143.0)	139.0 (138.0–143.0)	138.5 (137.0–143.0)			
	Difference	NaOH	−2.0 (−3.0–−2.0)	−3.0 (−3.0–−2.0)	−3.0 (−4.0–−2.0)	−2.0 (−3.0–−2.0)	**<0.001**	0.215	**0.012**
		O_2	0.0 (0.0–0.0) *#	−1.0 (−1.0–0.0) *	−1.0 (−1.0–−1.0) *	−1.0 (−1.0–−1.0) *			

Table 1. *Cont.*

Variable		Ventilation	Flow (L × min^{-1})				*p* Vent.	*p* Flow	*p* Int.
			100	250	500	1000			
Ca^{++} (mEq × L^{-1})	**PRE**	NaOH	1.3 (1.3–1.4)	1.4 (1.3–1.4)	1.4 (1.3–1.4)	1.4 (1.3–1.4)	0.755	0.854	0.769
		O_2	1.4 (1.2–1.4)	1.3 (1.2–1.4)	1.3 (1.2–1.4)	1.3 (1.2–1.4)			
	POST	NaOH	1.2 (1.1–1.3)	1.2 (1.1–1.2)	1.2 (1.2–1.3)	1.2 (1.2–1.3)	**0.066**	0.110	**<0.001**
		O_2	1.4 (1.2–1.4) *§#	1.3 (1.2–1.4) *§#	1.3 (1.2–1.3)	1.3 (1.2–1.3)			
	Difference [$]	NaOH	−0.1 (−0.1–−0.1)	−0.2 (−0.2–−0.1)	−0.1 (−0.1–−0.1)	−0.1 (−0.1–−0.1)	**<0.001**	**0.032**	**0.002**
		O_2	0.0 (0.0–0.0) *§#	0.0 (0.0–0.0) *§#	0.0 (0.0–0.0) *	−0.1 (−0.1–−0.1) *			
Cl^{-} (mEq × L^{-1})	**PRE**	NaOH	111.5 (111.0–113.0)	111.5 (111.0–113.0)	111.5 (111.0–113.0)	111.5 (110.0–113.0)	0.232	0.529	0.529
		O_2	111.0 (111.0–112.0)	111.0 (111.0–112.0)	111.0 (110.0–112.0)	111.0 (110.0–112.0)			
	POST	NaOH	114.0 (114.0–115.0)	114.5 (114.0–115.0)	114.0 (114.0–115.0)	114.0 (114.0–115.0)	**0.002**	**0.042**	**0.002**
		O_2	111.5 (111.0–113.0) *#	111.0 (111.0–113.0) *#	112.0 (111.0— 113.0) *#	112.5 (112.0–114.0) *			
	Difference	NaOH	2.5 (2.0–3.0)	3.0 (2.0–3.0)	2.5 (2.0–3.0)	2.5 (2.0–3.0)	**0.007**	**0.002**	**0.001**
		O_2	0.5 (0.0–1.0) *#	0.0 (0.0–1.0) *§#	1.0 (1.0–1.0) *#	2.0 (1.0–2.0)			
Lac (mEq × L^{-1})	**PRE** [$]	NaOH	1.4 (0.5–2.3)	1.4 (0.5–2.4)	1.3 (0.5–2.3)	1.4 (0.5–2.5)	0.180	0.361	0.614
		O_2	1.1 (0.4–2.5)	1.2 (0.4–2.6)	1.1 (0.4–2.6)	1.1 (0.4–2.5)			
	POST [$]	NaOH	1.4 (0.5–2.3)	1.5 (0.5–2.3)	1.4 (0.5–2.4)	1.4 (0.5–2.4)	0.197	0.459	0.850
		O_2	1.0 (0.4–2.6)	1.1 (0.4–2.6)	1.2 (0.4–2.6)	1.1 (0.4–2.5)			
	Difference	NaOH	0.0 (0.0–0.0)	−0.1 (−0.1–0.1)	0.1 (0.0–0.1)	0.0 (0.0–0.0)	1.000	0.297	0.922
		O_2	0.0 (0.0–0.0)	0.0 (−0.1–0.0)	0.0 (0.0–0.1)	0.0 (0.0–0.0)			
Hb (g × dL^{-1})	**PRE**	NaOH	6.45 (5.50–8.20)	6.80 (5.30–8.20)	6.70 (5.30–8.30)	6.70 (5.20–8.10)	0.643	0.641	0.511
		O_2	6.55 (5.50–8.30)	6.60 (5.30–8.20)	6.55 (5.30–8.40)	6.55 (5.40–7.90)			
	POST	NaOH	6.60 (5.50–8.30)	6.70 (5.40–8.20)	6.75(5.40–8.30)	6.60 (5.30–8.20)	0.547	0.083	0.893
		O_2	6.55 (5.50–8.40)	6.60 (5.30–8.20)	6.60 (5.30–8.50)	6.55 (5.40–7.90)			
	Difference	NaOH	0.05 (0.00–0.10)	0 (−0.10–0.00)	0.05 (0.00–0.10)	0.05 (0.00–0.10)	**0.025**	0.661	0.154
		O_2	0.00 (−0.10–0.00) *	0.00 (0.00–0.00) *	0.00 (0.00–0.10) *	0.00 (0.00–0.00) *			
HCO_3^{-} (mmol × L^{-1})	**PRE**	NaOH	30.1 (29.5–32.2)	30.2 (29.3–31.8)	30.3 (29.4–31.7)	29.8 (29.0–31.8)	0.050	0.508	0.165
		O_2	28.3 (27.6–29.9) *	28.2 (27.6–29.8) *	28.1 (27.5–30.4) *	28.5 (28.2–30.2) *			
	POST	NaOH	25.7 (23.3–29.1)	25.9 (23.2–28.1)	26.4 (23.2–29)	26.3 (23.7–29.7)	0.577	**0.043**	**0.003**
		O_2	28.0 (27.2–28.6) §#	27.5 (26.7–28.6) #	27.0 (26.3–28.2)	26.6 (26.1–27.2)			
	Difference	NaOH	−4.4 (−6.3–−2.4) #	−4.5 (−6.8–−2.4) #	−4.2 (−6.2–−1.3)	−3.9 (−5.7–−1.7)	**0.018**	**0.003**	**<0.001**
		O_2	−0.3 (−0.5–−0.2) *§#	−0.8 (−0.9–−0.6) *§#	−1.3 (−1.7–−1) *#	−2.1 (−2.8–−1.4)			

Table 1. *Cont.*

Variable		Ventilation	Flow (L × min^{-1})				*p* Vent.	*p* Flow	*p* Int.
			100	250	500	1000			
plasma TCO_2 (mmol × L^{-1})	**PRE**	NaOH	31.9 (31.4–34.1)	32.0 (31.1–33.6)	32.1 (31.3–33.5)	31.7 (30.9–33.6)	0.051	0.476	0.194
		O_2	30.1 (29.5–31.8)	30.1 (29.4–31.5)	30.0 (29.4–32.3)	30.3 (30.1–32.0)			
	POST	NaOH	26.0 (23.6–29.5)	26.3 (23.5–28.4)	26.7 (23.6–29.3)	26.7 (24.1–30.1)	0.258	**0.009**	**<0.001**
		O_2	29.7 (28.9–30.1) §#	29.1 (28.1–30.0) #	28.3 (27.4–29.5) #	27.5 (26.9–28.1)			
	Difference	NaOH	−5.9 (−7.8–−3.9) #	−6 (−8.3–−4.0) #	−5.7 (−7.6–−2.8)	−5.3 (−7.1–−3.3)	**0.006**	**<0.001**	**<0.001**
		O_2	−0.5 (−0.6–−0.3) *°§#	−1.1 (−1.3–−0.9) *§#	−1.9 (−2.2–−1.7) *#	−3.0 (−3.8–−2.3)			
$\dot{V}CO_2$ (mL × min^{-1})		NaOH	65.3 (43.3–86.7) #	67.0 (44.3–92.2) #	63.5 (31.6–84.5)	59.1 (36.4–79)	**0.006**	**<0.001**	**<0.001**
		O_2	5.4 (3.7–6.7) *°§#	12.5 (10.5–14.6) *§#	20.7 (18.6–24.9) *#	33.6 (26.1–42.6)			

Abbreviations: PCO_2, partial pressure of carbon dioxide; PO_2, partial pressure of oxygen; Na^+, sodium; K^+, potassium; Ca^{++}, calcium; Cl^-, chloride; Lac, Lactate; Hb, hemoglobin; HCO_3-, bicarbonate, TCO_2, total CO_2 content, $\dot{V}CO_2$, amount of carbon dioxide removed by the membrane lung. Data are expressed median (IQR); Differences were computed as POST values–PRE values. *p*: *p* values of two-way ANOVA RM or two-way ANOVA RM on ranks ($) for NaOH vs. O_2 comparison (*p* Ventilation), Flow effect (*p* Flow) and interaction (*p* int.); Post-hoc analysis with Bonferroni or Tukey corrections: * $p < 0.05$ vs. NaOH; ° $p < 0.05$ vs. 250 mL/min; § $p < 0.05$ vs. 500 mL/min; # $p < 0.05$ vs. 1000 mL × min^{-1}.

As the highest delta PCO_2 was observed when 100 mmol × L^{-1} NaOH was used, this concentration was employed for the efficiency and efficacy tests.

3.3. *Efficiency Test*

Blood gas analyses of PRE and POST blood with NaOH and oxygen are reported in Table 1. PCO_{2PRE} was stable throughout the entire test, 59.6 (58.9–60.4) mmHg. Increasing oxygen flows showed increasing CO_2 removal, both as delta PCO_2 across the membrane lung and $\dot{V}CO_2$, see Figure 4. Conversely, all NaOH flows showed similar CO_2 removal, except for a lower $\dot{V}CO_2$ at 1000 mL × min^{-1} compared to 100 and 250 mL × min^{-1} (see Figure 3). When comparing $\dot{V}CO_2$ achieved with liquid and gaseous ventilation, liquid ventilation achieved significantly higher CO_2 removals for 100, 250, and 500 mL × min^{-1} of flow. On the contrary, while the median value was higher also for 1000 mL × min^{-1}, this difference did not reach statistical significance.

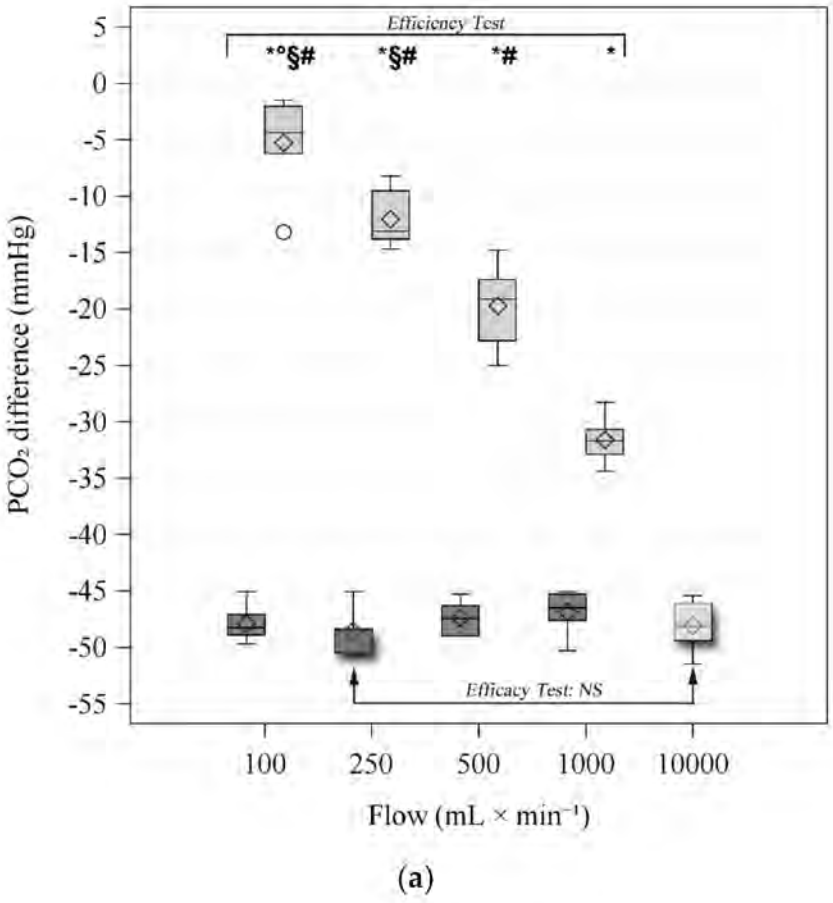


Figure 4. *Cont.*

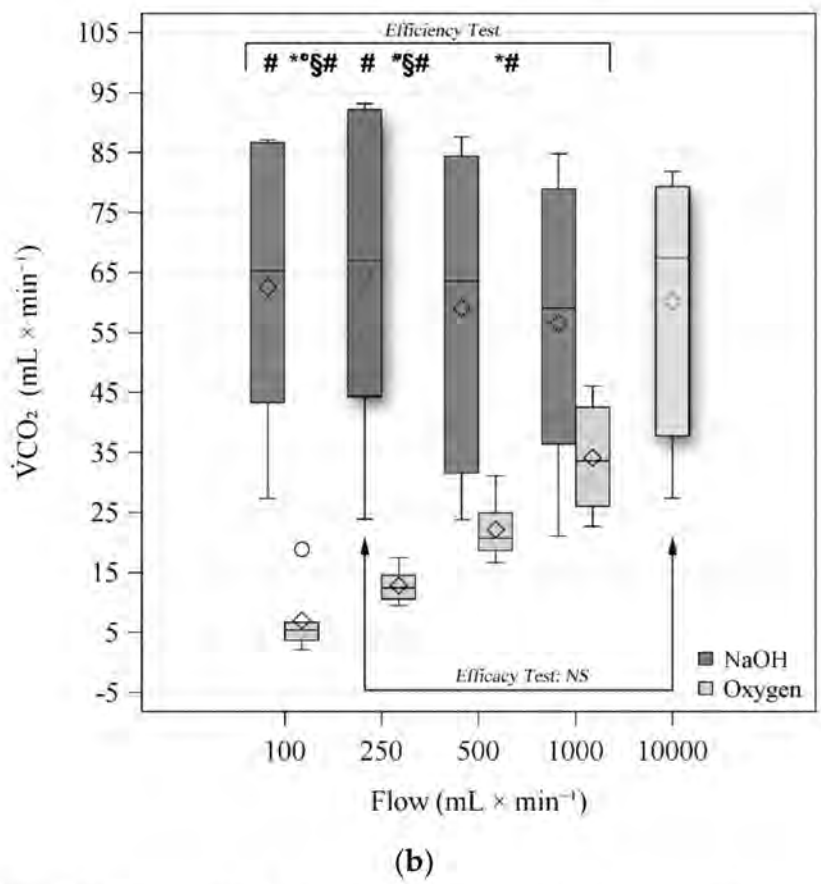


Figure 4. Figures display the distribution of data by using a rectangular box plot and whiskers, the bottom and top edges of the box indicate the intra-quartile range (IQR) between the first and third quartiles (the 25th and 75th percentiles). The diamond marker inside the box indicates the mean value. The line inside the box indicates the median value. Whiskers indicate the range of values outside of the intra-quartile range but at a distance lower than the upper and lower fences ($\pm 1.5 \times$ IQR) Dots represent outliers (observations that are more extreme than the upper and lower fences). Dark grey represents NaOH at 100 mmol $\times$ L^{-1} concentration. Light grey represents Oxygen. Efficiency test statistical analysis: Two-way ANOVA RM. * $p < 0.05$ vs. NaOH; ° $p < 0.05$ vs. 250 mL $\times$ min^{-1}; § $p < 0.05$ vs. 500 mL $\times$ min^{-1}; # $p < 0.05$ vs. 1000 mL $\times$ min^{-1}. Efficacy test statistical analysis: Paired t-test between NaOH at 100 mmol $\times$ L^{-1} concentration and 250 mL $\times$ min^{-1} sweep flow and oxygen at 1000 mL $\times$ min^{-1} sweep flow (boxes highlighted by outside shadow and arrows). (**a**) PCO_2 (partial pressure of carbon dioxide) difference (POST values–PRE values) distribution according to different sweep flows of NaOH and Oxygen. (**b**) $\dot{V}CO_2$ (Carbon dioxide transfer across the membrane lung) distribution according to different sweep flows of NaOH and Oxygen.

Blood pH_{POST} increased, according to the PCO_2 reduction, reaching values as high as 7.987 with NaOH at 250 mL $\times$ min^{-1}.

PO_{2PRE} was stable around 141.0 (137.0–147.0) mmHg both during NaOH and oxygen steps while PO_{2POST} increased up to 470.5 (452.3–507.0) mmHg only during oxygenation use, while it remained unchanged during liquid ventilation.

Blood electrolytes and lactate concentrations were stable throughout the experiment.

3.4. Efficacy Tests

In agreement with the highest $\dot{V}CO_2$ and delta PCO_2, NaOH 250 mL $\times$ min^{-1} was selected as the most performant NaOH flow and compared with 10 L $\times$ min^{-1} of oxygen in the efficacy test. Table 2 reports blood gas analyses of PRE and POST blood. Both delta

PCO_2 and $\dot{V}CO_2$ were similar, suggesting similar extracorporeal CO_2 removal (Figure 4, shadowed boxes).

Table 2. Efficacy tests results.

Variable		Ventilation		
		NaOH 250 mL × min^{-1}	**O_2 10,000 mL × min^{-1}**	***p***
pH	**PRE**	7.351 (7.333–7.359)	7.328 (7.322–7.355)	**0.032**
	POST	7.987 (7.977–8.077)	7.966 (7.921–8.013)	**0.020**
	Difference	0.643 (0.624–0.718)	0.627 (0.599–0.685)	0.094
PCO_2 (mmHg)	**PRE**	59.5 (59–59.7)	60 (59.3–60.5)	0.254
	POST	10.5 (10.3–11.1)	11.5 (10.8–13.9)	0.106
	Difference	−48.5 (−50.5–−48.3)	−48.1 (−49.4–−46.1)	0.522
PO_2 (mmHg)	**PRE** [$]	137 (137–143)	139 (137–165)	0.625
	POST	130.5 (128–140)	661.5 (649–677)	**<0.001**
	Difference	−6.5 (−9–−3)	518.5 (509–536)	**<0.001**
K^+ (mEq × L^{-1})	**PRE**	4.1 (4.1–4.5)	4.2 (4–4.4)	0.611
	POST [$]	4.1 (4–4.4)	4.1 (4–4.3)	0.438
	Difference	0.1 (0–0.1)	0.1 (0–0.1)	1.000
Na^+ (mEq × L^{-1})	**PRE**	143 (143–144)	142 (141–145)	0.516
	POST	140 (140–142)	139.5 (139–143)	1.000
	Difference	−3 (−3–−2)	−2 (−2–−2)	0.102
Ca^{++} (mEq × L^{-1})	**PRE**	1.4 (1.3–1.4)	1.3 (1.3–1.4)	0.927
	POST	1.2 (1.1–1.2)	1.2 (1.1–1.3)	0.413
	Difference	−0.2 (−0.2–−0.1)	−0.1 (−0.1–−0.1)	0.067
Cl^- (mEq × L^{-1})	**PRE** [$]	111.5 (111–113)	111.5 (111–112)	0.813
	POST	114.5 (114–115)	115 (114–115)	1.000
	Difference	3 (2–3)	3 (2–3)	0.611
Lac (mEq × L^{-1})	**PRE** [$]	1.4 (0.5–2.4)	1.3 (0.5–2.5)	0.375
	POST	1.5 (0.5–2.3)	1.2 (0.4–2.5)	0.233
	Difference	−0.1 (−0.1–0.1)	0 (−0.1–0)	1.000
Hb (g × dL^{-1})	**PRE**	6.80 (5.30–8.20)	6.55 (5.50–8.40)	0.499
	POST	6.70 (5.40–8.20)	6.55 (5.40–7.90)	0.590
	Difference	0.00 (−0.10–0.00)	0.00 (0.00–0.00)	0.363
HCO_3^- (mmol × L^{-1})	**PRE**	30.2 (29.3–31.8)	29.4 (28.7–30.6)	0.205
	POST	25.9 (23.2–28.1)	25.1 (23.1–28.1)	0.652
	Difference	−4.5 (−6.8–−2.4)	−4.6 (−5.7–−1.8)	0.185
plasma TCO_2 (mmol × L^{-1})	**PRE** [$]	32 (31.1–33.6)	31.3 (30.6–32.5)	0.313
	POST	26.3 (23.5–28.4)	25.5 (23.5–28.4)	0.695
	Difference	−6 (−8.3–−4)	−6.1 (−7.1–−3.4)	0.191
$\dot{V}CO_2$ (mL × min^{-1})		67 (44.3–92.2)	67.4 (37.8–79.4)	0.191

Abbreviations: PCO_2, partial pressure of carbon dioxide; PO_2, partial pressure of oxygen; Na^+, sodium; K^+, potassium; Ca^{++}, calcium; Cl^-, chloride; Lac, Lactate; Hb, hemoglobin; HCO_3^-, Bicarbonate, TCO_2, total CO_2 content, $\dot{V}CO_2$, amount of carbon dioxide removed by the membrane lung. Data are expressed median (IQR); Differences were computed as POST values–PRE values. *p*: *p* values of Paired *t*-test or Wilcoxon Signed Rank Test ([$]) for NaOH (250 mL × min^{-1}) vs. O_2 (10,000 mL × min^{-1}) comparison.

4. Discussion

This in-vitro study shows that continuous infusion (from 100 to 1000 mL × min^{-1}) of highly concentrated sodium hydroxide solutions into the gas side of conventional polypropylene oxygenators is feasible, despite pH values of the sweeping solution above 12. At low sweep flows, alkaline liquid ventilation showed significantly higher CO_2 removal capacity than conventional gaseous ventilation. However, the maximum CO_2 removal efficiency achieved through liquid ventilation was not superior to the one achieved with 10 L × min^{-1} of sweep gas flow.

The working hypothesis underlying this study was to exploit the high CO_2 absorbing capacity of NaOH solutions. Indeed, in our experimental context, the concentration of NaOH was always significantly higher than the amount of CO_2 extracted from the ML. The PCO_2 of the alkaline sweep fluid was persistently very close to 0 mmHg, as the added CO_2 was instantly hydrated and dissociated to bicarbonate and carbonate. This allowed to keep the PCO_2 close to zero and thus optimize the transmembrane PCO_2 gradient, favoring the efficiency of extracorporeal CO_2 removal.

Indeed, a solution containing 10 mEq of NaOH could absorb 200 mL of CO_2 while maintaining PCO_2 close to zero. On the contrary, the same amount of CO_2 added to 10 L of gas would result in a PCO_2 around 15 mmHg, therefore reducing the blood-gas CO_2 gradient.

However, the data showed that increasing NaOH flow did not lead to a linear increase in CO_2 removal. Instead, for NaOH flows greater than 250 mL × min^{-} there was an unexpected reduction in CO_2 removal. This reduced efficiency could depend on the density of the sodium hydroxide solution and the mechanics of the membrane lung. Therefore, a clinical application of alkaline liquid ventilation does not seem exploitable using the current technology. The technical complexity and safety profile require further evaluations, although the present tests have recorded no damage to the membrane lung.

Another important difference between gaseous and liquid ventilation needs to be discussed. Although the oxygenation capacity of low-flow devices using conventional gaseous ventilation is limited by the amount of blood reaching the ML, a certain amount of oxygen is added to the blood. On the contrary, the NaOH infusion does not oxygenate the extracorporeal blood, limiting its potential clinical application to patients with isolated hypercapnic respiratory failure, i.e., able to oxygenate properly through their native lungs.

Although devices with higher CO_2 extraction capacity resulted more effective [3,9,10], numerous studies confirm the ability of $ECCO_2R$ to achieve physiological targets. Nevertheless, the clinical application of $ECCO_2R$ is still limited and no conclusive indications have been identified mainly because of safety concerns [37,38]. Indeed, a consistently high rate of complications has been reported, mostly related to hemolysis, bleeding, and thrombosis. In this context, the present study aim was to achieve a highly efficient $ECCO_2R$ technique to ensure a clinical efficacy with limited extracorporeal blood flows, potentially enabling regional anticoagulation [39,40]. The tested technology, which was not developed for alkaline liquid ventilation, did not meet such expectations. However, we cannot exclude that a dedicated device could achieve more satisfying results.

This study presents several limitations. First, we could not perform any gas analysis of the sodium hydroxide solution. The CO_2 extraction capacity was estimated both as differences in PCO_2 and TCO_2 between the blood samples upstream and downstream of the artificial lung [41]. $\dot{V}CO_2$ showed higher variability than PCO_2, as shown in Figure 4, possibly due to the baseline different blood composition. Indeed, we can speculate that this phenomenon may be explained by the wide range in hemoglobin concentrations (see Table 1), which affects the ML $\dot{V}CO_2$ [42]. Secondly, the alkaline liquid ventilation was tested only in vitro and for a limited time consequently we cannot exclude different effects and safety issues in vivo scenarios. Thirdly, we only tested one type of polypropylene membrane lung. Further tests with different devices may be required.

5. Conclusions

This in-vitro study showed that $ECCO_2R$ through alkaline liquid ventilation of the ML is feasible and safe. The CO_2 removal efficiency of alkaline liquid ventilation was higher than conventional gaseous ML ventilation only for low sweep flows. Indeed, at high sweep gas flow, the CO_2 removal efficiency was comparable between the two techniques.

The development of a dedicated device may be necessary to exploit the potential of this technology. Further studies will be required before any possible clinical application.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/membranes11070464/s1, The Online Supplement: Alkaline_liquid_ventilation_online_supplement.docx.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Abbreviations

ACD, anticoagulant-citrate-dextrose; ARDS acute respiratory distress syndrome; Ca^{++}, calcium; Cl^-, chloride; CO_2, carbon dioxide; CO_3^{2-}, carbonate; COPD, chronic obstructive pulmonary disease; $ECCO_2R$, extracorporeal carbon dioxide removal; H_2CO_3, carbonic acid; H_2O, water; Hb, hemoglobin; HCO_3^-, bicarbonate; IQR, interquartile range; K^+, potassium; Lac, Lactate; ML, membrane lung; Na^+, sodium; Na_2CO_3, sodium carbonate; NaHCO3, sodium bicarbonate; NaOH, sodium hydroxide; $PaCO_2$, arterial partial pressure of CO_2; PCO_2, partial pressure of carbon dioxide; PO_2, partial pressure of oxygen; TCO_2, total CO_2 content; $\dot{V}CO_2$, amount of carbon dioxide removed by the membrane lung in one minute.

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