Extracorporeal Hyperoxygenation Therapy for Carbon Monoxide Poisoning: From In Vitro Proof of Principle to In Vivo Feasibility

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vorgelegt von

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Weitere Publikationen

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Article

Extracorporeal Hyperoxygenation Therapy (EHT) for Carbon Monoxide Poisoning: In-Vitro Proof of Principle

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Abstract: Carbon monoxide (CO) poisoning is the leading cause of poisoning-related deaths globally. The currently available therapy options are normobaric oxygen (NBO) and hyperbaric oxygen (HBO). While NBO lacks in efficacy, HBO is not available in all areas and countries. We present a novel method, extracorporeal hyperoxygenation therapy (EHT), for the treatment of CO poisoning that eliminates the CO by treating blood extracorporeally at elevated oxygen partial pressure. In this study, we proof the principle of the method in vitro using procine blood: Firstly, we investigated the difference in the CO elimination of a hollow fibre membrane oxygenator and a specifically designed batch oxygenator based on the bubble oxygenator principle at elevated pressures (1, 3 bar). Secondly, the batch oxygenator was redesigned and tested for a broader range of pressures (1, 3, 5, 7 bar) and temperatures (23, 30, 37 °C). So far, the shortest measured carboxyhemoglobin half-life in the blood was 21.32 min. In conclusion, EHT has the potential to provide an easily available and effective method for the treatment of CO poisoning.

Keywords: carbon monoxide; poisoning; extracorporeal therapy; hyperoxygenation; oxygenator; hollow fibre membrane oxygenator; bubble oxygenator

1. Introduction

Carbon monoxide (CO) poisonings are responsible for estimated 50,000 emergency department visits and an estimated economic burden of $1.3 billion in the USA annually [1,2]. It is still the most lethal poisoning occurring in industrial nations nowadays, although the first extensive study on CO poisoning and its effects was published by Douglas et al. already in 1912 [3]. They ascertained that CO binds to hemoglobin (Hb), forms carboxyhemoglobin (CO-Hb), and thereby blocks the physiological oxygen (O2) pathway. Small amounts of CO were found to be sufficient to cause severe hypoxia, because the affinity of hemoglobin for CO is substantially higher than for O2 (230–270 times [4]). Subsequent studies discovered more disruptive effects of elevated CO concentrations: CO causes an increase in nitric oxide formation, leading to hypotension; CO has a negative impact on myocardial function by binding to myoglobin; CO binds to platelet hem protein and cytochrome c oxidase, causing direct cellular damage by interrupting cellular respiration. This leads to neuronal necrosis and apoptosis [5]. Furthermore, increased CO concentrations lead to the formation of reactive oxide species and inflammation [4,6,7]. The clinical symptoms range from headaches and nausea to visual disturbances and cardiac arrhythmia, and ultimately to coma, seizures, and breathing and circulatory failures [8]. Long-term effects are common, they occur in up to 40% of the patients. Most common are neurological sequelae but also dementia, psychosis, disturbance of memory and movement disorders have been reported [6,8].

In room air, the half-life of carbon monoxide is approx. 320 min [5]. The current treatment of CO poisoning is normobaric oxygen (NBO) or hyperbaric oxygen (HBO), as the elimination of CO increases with increased O_2 concentrations [4,9], see Figure 1. NBO is rapidly available and usually immediately initiated, decreasing the CO half-life to approx. 74 min [5]. HBO increases the physically solved O_2 further, whereby the CO-Hb half-life is reduced drastically, resulting in a CO half-life of approx. 20 min [5]. The use of HBO compared to NBO was found to reduce the incidence of cognitive abnormalities from 33% to 18% [10]. However, while the rationale of the HBO treatment is compelling, up to date the use of HBO for the treatment of CO poisoning remains controversial, as there is no sufficient evidence of its benefits [9,11]. A recent review by Roderique et al. [7] even cautions for the use of HBO, as it further increases reactive oxygen species, potentially exacerbating the damage that is already occurring. Furthermore, in practice the limited number of hyperbaric chambers presents a major issue in therapy. A recent study by Chin et al. [12] concluded that only 11.9% of 361 surveyed hyperbaric centres in the USA were sufficiently equipped for dealing with emergency cases. Especially in remote areas the coverage is insufficient at best, resulting in transportation times of several hours to a hyperbaric chamber. Furthermore, the long preparation time of most available chambers further delays the rapidly needed therapy, even in metropolitan areas [5,9,13]. Additionally, a patient's comorbidity such as, e.g., acute respiratory distress syndrome (ARDS) or chronic obstructive pulmonary disease (COPD) may mitigate the use of NBO and HBO, as the CO elimination takes place in the lungs [14,15]. Therefore, novel treatment options are needed [5].

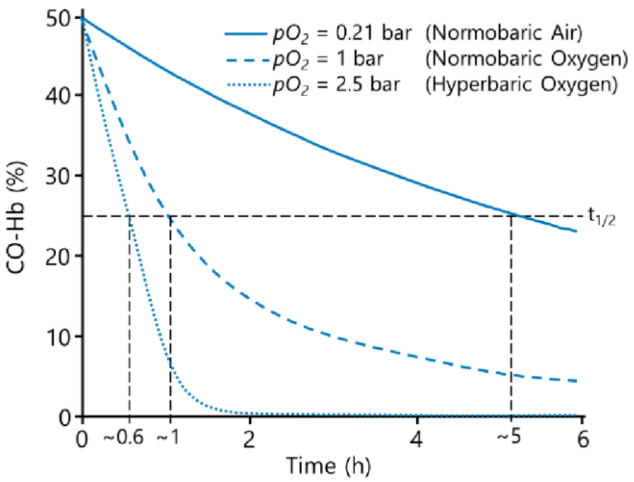

Figure 1. Influence of O_2 partial pressure on CO-Hb-level decrease, determined from [5,9].

Pharmacological treatment options that are currently being investigated include the use of a combination of hydroxocobalamin and ascorbic acid into a reduced form [16], a supramolecular complex (hemoCD) [17], and molecules based on bioengineered neuroglobin [18,19]. These drugs have shown a great affinity for CO and are used as scavenger molecules to eliminate carbon monoxide.

There are also non-pharmaceutical approaches, for example the ClearMate™ [20], which enhances the CO elimination by hyperventilation.

For many respiratory indications, such as COPD and ARDS, the use of extracorporeal membrane oxygenation (ECMO) has proven to be efficacious [21]. Within these systems, the native function of the lung, the uptake of oxygen and the removal of carbon dioxide, is taken over by an oxygenator [22]. As the elimination of CO takes place solely in the lungs, this approach could also be used for the cure of CO poisoning. However, ECMO is not designed for a rapid CO elimination. Therefore, the enhancement by phototherapy is investigated, whereby the dissociation of CO from hemoglobin is facilitated by light [23].

Our approach, the extracorporeal hyperoxygenation therapy (EHT) is based on applying the method of hyperbaric oxygenation to a gas exchanger, enhancing the elimination of CO by increasing the dissolved oxygen levels in the blood. As only the blood is affected, higher pressure levels can be reached than with HBO, because the patient is not exposed to the pressure. Additionally, an increase in reactive oxygen species would arise only there and not in the patient, as the half-life of most reactive oxygen species is in the range of micro or even nano seconds [24]. Moreover, the device could also be rather small and therefore portable. This would allow the start of the treatment directly at the site of the accident and also facilitate an area-wide, easily accessible coverage. Furthermore, patients suffering ARDS or COPD could be treated irrespective of the gas exchange performance of their lungs. Overall, we hypothesize that this approach provides a promising extension to the currently used treatment options.

To maximize the positive outcome of such a novel device, a high detoxification velocity is imperative. The CO elimination is presumably mainly depending on:

1. Velocity of chemical bonding processes;
2. Concentration of O_2 and CO in plasma;
3. Shift of carbon monoxide-hemoglobin dissociation curve;
4. Diffusion of O2 to Hb and of CO away from Hb.

In order to examine the feasibility and limitations of the EHT regarding the detoxification velocity, we performed two series of experiments. Preliminary experiments comparing the performance of hollow fibre membrane oxygenators (HFMO) versus a specifically designed batch oxygenator (BO) based on the bubble oxygenator principle [25] were conducted. Subsequently, the more promising option was revised, and the performance regarding the detoxification velocity was tested for a broader range of operating points. Test variables were temperature and pressure within the device, which we assumed to be the primary influencing factors. As elevated temperatures and pressures in combination with blood-air contact can also be related to blood damage, the finally performed evaluation included the rate of hemolysis occurring during the detoxification.

2. Materials and Methods

2.1. Poisoning of Blood In Vitro

The poisoning of blood in vitro was carried out in a bench top circulation loop consisting of a cardiotomy reservoir, a roller pump (HL-20, Maquet, Rastatt, Germany), and a membrane oxygenator (hilite 7000, Xenios AG, Heilbronn, Germany). The circuit was filled with fully heparinized porcine blood from the slaughterhouse. Blood parameters were set to meet the requirements of ISO 7199 for blood-gas exchangers [26]. Blood flow was set to 3 L/min while sweep gas flow was 0.5 L/min containing 3% CO and 97% N_2 (Linde AG, Pullach, Germany). When the CO-Hb-level reached the desired value, the poisoning was ended by stopping the blood flow and flooding the oxygenator fibres with pure nitrogen (N_2). Afterwards, the CO-poisoned blood was filled into a blood bag and placed on a platform shaker to keep the blood homogenously mixed. The blood was always used at day of retrieval for all experiments.

2.2. Measured Parameters and Method of Analysis

The parameter of main interest was the fraction of CO-Hb in percent. It was measured using a blood gas analyser (ABL800 Flex, Radiometer Medical ApS, Brønshøj, Denmark), which was also used for measuring the pH-value.

Hemolysis was determined by photometric measurement of the plasma free hemoglobin (Ultrospec 2100 Pro, Biochrom, Berlin, Germany). The analysis of hemolysis was performed according to DIN 58,931 [27] by means of the cyanmethemoglobin method

(Hemoglobin FS, DiaSys, Germany) according to manufacturers' instructions. For this, the plasma of each blood sample was separated from the cells by double centrifugation at 1500× *g* for 15 min.

2.3. Preliminary Experiments

We hypothesized that using a specifically designed batch oxygenator (BO) based on the bubble oxygenator principle provides faster CO elimination than the hollow fibre membrane oxygenator (HFMO), due to the lack of mass transfer resistance associated with the membrane that inhibits a diffusion of CO out of the blood. Furthermore, the short residence time inside the HFMO is unfavourable because of the binding kinetics of CO to hemoglobin. Therefore, we performed preliminary experiments, comparing these two approaches for CO elimination: a previously described HFMO [28] was compared to the specifically designed BO.

2.3.1. Test Set-Up Hollow Fibre Membrane Oxygenator

The experimental setup (Figure 2a) consisted of the HFMO (A), a blood bag (B), serving as a reservoir, a clamp-on flow sensor (C) (H9XL, Transonic Europe B.V., Elsloo, The Netherlands) connected to a flow meter (HT110, Transonic Europe B.V., Elsloo, The Netherlands), and a blood pump (D) (deltastream® DP3, XENIOS AG, Heilbronn, Germany). The sweep gas of the oxygenator was controlled by mass controllers (E–G) (MASS-VIEW® MV-304, Bronkhorst High-Tech B.V., AK Ruurlo, The Netherlands). For the experiments with excess pressure, the oxygenator and the blood bag were placed in a pressure resistant chamber (H), which was pressurized, using a pressure control valve (I) (VPPM-6L-L-1-G18-0L6H-V1PS1C1, Festo GmbH and Co. KG, Esslingen, Germany). Due to the flexibility of the blood bag, the applied pressure was existent across the whole test loop. To avoid a pressure drop across the hollow fibre membrane, the sweep gas was pressurized to the identical pressure as the pressure resistant chamber, by the pressure control valve. A Series RM Rate-Master® controller (J) (Dwyer Instruments Inc., Michigan City, IN, USA), connected to the chamber, was set to the same gas flow rate as the sweep gas, to prevent a pressure build-up inside the chamber. Samples were taken at the sampling port (K). The temperature was measured pre-oxygenator (L).

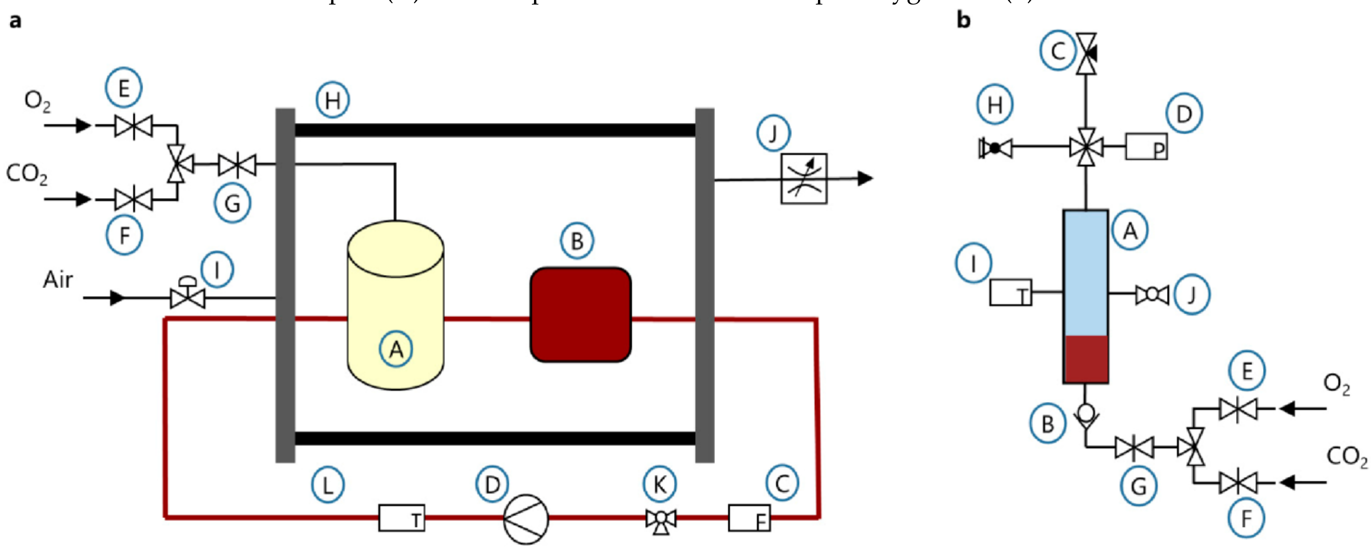

Figure 2. Experimental setups for the preliminary experiments (**a**) for the testing of a HFMO, (**b**) for the testing of a BO.

The test loop was primed with sterile 0.9% sodium chloride solution (B. Braun Melsungen AG, Melsungen, Germany) to wet the components. After draining the priming solution, the test loop was filled with 500 mL poisoned blood with a starting CO-Hb of ~30% (range: 29.5–32.7%) and 12 ± 1 g/dL of hemoglobin. The sweep gas was set to 5 L/min with a concentration of 5% carbon dioxode (CO_2) and 95% O_2. Thus, by maintaining a constant sweep gas flow throughout all experiments, the influence of the sweep gas flow on the CO elimination can be disregarded. The experiments were carried out at room temperature (Ø 24 ± 2 °C). Samples for the blood gas and hemolysis measurement were taken after 0 min, 5 min, 10 min, 15 min, and 30 min. The experimental matrix was set up by three different blood flow rates and two different pressure levels. The experiments were repeated two times. The test parameters are shown in Table 1.

Table 1. Test parameters for the preliminary experiments.

HFMO					**BO**				
Blood Flow Rate (mL/min)			**Pressure (bar)**		**Gas Flow Rate (L/min)**			**Pressure (bar)**	
100 (low)	500 (medium)	1000 (high)	1	3	0.4 (low)	2.21 (medium)	4 (high)	1	3

2.3.2. Test Set-Up Batch Oxygenator

The experimental setup can be seen in Figure 2b. A standard process engineering bubble column (A) (SCHOTT AG, Mitterteich, Germany) was used as a batch oxygenator. The blood inlet and a porosity 2 VitraPOR® micro-immersion filter (ROBU® GlasfilterGeräte GmbH, Hattert, Germany) for gas dispersion and pressure build-up were located in the bottom of the column. The filter was connected via tubing and a check valve (B) to MASS-VIEW® MV-304 controllers (E–G) (Bronkhorst High-Tech B.V., AK Ruurlo, The Netherlands), controlling the gas flow into the bubble column. A needle valve (C) to regulate the gas outflow was placed at the top of the column. Thus, by matching inand outflow, a constant excess pressure could be maintained. The corresponding pressure sensor (D) (A-10, WIKA SE and Co. KG, Klingenberg, Germany) and a safety relief valve (H) (855811318001, ESSKA, Hamburg, Germany) were also connected to the top of the column. Furthermore, the top of the column was filled with filters from a cardiotomy reservoir (MVC 4030, XENIOS AG, Heilbronn, Germany) to prevent foam from rising. A temperature sensor (I) and a sampling port (J) were mounted in the middle of the column.

The BO was filled with 500 mL poisoned blood with a starting CO-Hb of ~30% (range: 29.7–33.5%) and 12 ± 1 g/dL of hemoglobin. For the pressure build-up, the gas flow was set to 0.1 L/min and the needle valve was closed until the operating pressure was reached (10 min). For experiments at ambient pressure, the same time was waited without gas flow. Then, the gas flow was increased to the respective test parameter. The needle valve was adjusted accordingly to maintain a constant operating pressure. Samples for the blood gas and hemolysis measurement were taken at the start of the CO elimination (0 min), at 15 min, and 30 min. The experimental matrix was set up by three different gas flow rates and two different pressure levels. The experiments were repeated three times. The test parameters are shown in Table 1.

2.4. Main Experimental Setup

After the preliminary tests, the batch oxygenator was revised and scaled down to allow a temperature management and the operation at higher pressure levels, see Figure 3a. The main component of every BO was a 200 mm long cylinder of borosilicate glass with a diameter of 40 mm (Mennes, Selm, Germany). One glass cylinder was sealed between a bottom plate and a top plate serving as lids and was clamped with three threaded rods.

The bottom plate, made from PVC, served as inlet for the sweep gas and held a biplane filter disc, series 16, porosity 2, VitraPOR® (ROBU® Glasfilter-Geräte GmbH, Hattert, Germany) for gas distribution and bubble formation. The filter discs, which have pore sizes between 40 and 100 µm, were glued into the notches of the bottom plates with a solvent-free 2-component epoxy resin adhesive (UHU endfest 300, UHU GmbH and Co. KG, Bühl/Baden, Germany).

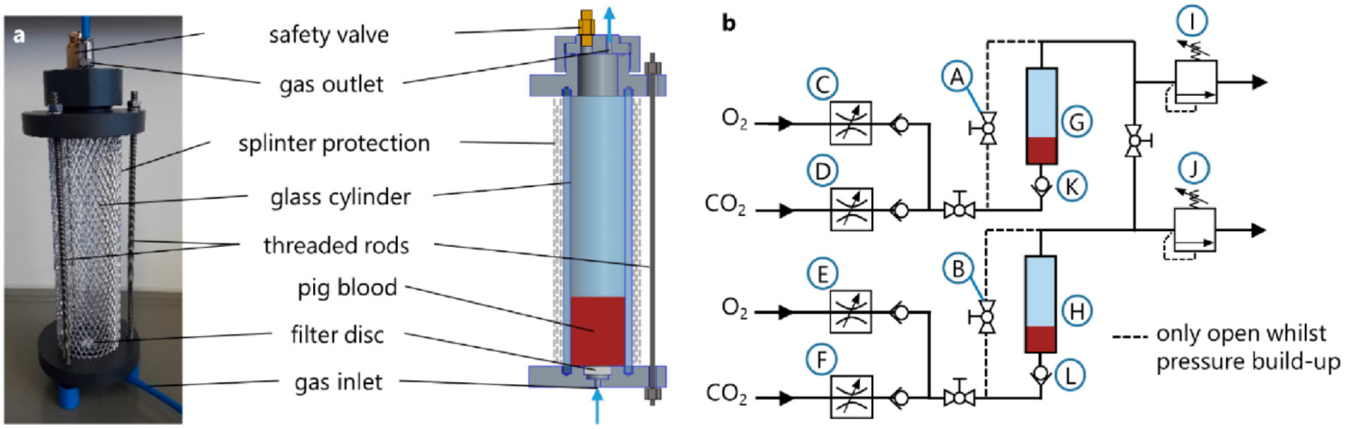

Figure 3. Structure of pressure-resistant BO (**a**) and main experimental setup for the operation of two BOs (**b**).

The top plate was also made from PVC and contained a connection panel, which held the gas outlet fitting and a safety valve. For safety reasons, an aluminium splinter protection was placed around the glass cylinder.

The pneumatic circuit for the operation of the BOs was realized by pneumatic fittings, tubing, and several ball valves (QH-QS-6, Festo GmbH and Co. KG, Esslingen, Germany) and check valves (H-QS-6, Festo GmbH and Co. KG, Esslingen, Germany). The circuit structure of two BOs, which were used in parallel for the testing of one operating point, is shown in Figure 3b. Two identical BOs (G, H) were used throughout the experiments. The gas flow was adjusted by four digital gas flow controllers (C–F) (ANALYT-MTC GmbH, Müllheim, Germany). In order to prevent any backwards flow of blood through the filter into the tubing system, check valves (K, L) (H-QS-6, Festo GmbH and Co. KG, Esslingen, Germany) were installed directly prior to the gas inlet of each BO. The two pressure regulators (I, J) (DVU01-100000, ESSKA, Hamburg, Germany) were used for controlling the pressures inside the BOs.

The temperature regulation of the blood inside the BOs was realized by a water bath, which was tempered by means of a temperature control unit E 100 (Lauda, LaudaKönigshofen, Germany). The two BOs were placed in the water bath during test runs.

2.5. Main Experimental Procedure

For preparation of the CO elimination, 50 mL of poisoned blood with an Hb-CO of ~42.5% (range: 41.3–44.8%) and 12 ± 1 g/dL of hemoglobin was drawn from the bag and filled in each BO. In order to prevent the formation of foam during the elimination, 50 µL of Antifoam 204 (Sigma Aldrich, St. Louis, MO, USA) were added to each BO by pipetting it directly onto the blood surface. After closing the BOs, both BOs were transferred into the already tempered water bath.

The pressure build-up in the BOs took place while the two ball valves (A, B) to the gas-outlet were open (Figure 3). This inhibited the gas passage through blood before the pressure had reached the target value. To keep the pH-value as constant as possible, a fixed amount of CO_2 in the blood is desired. This was achieved by preconditioning the blood according to ISO 7199 and using a sweep gas with a defined CO_2 concentration of 0.03 standard litre per minute.

The total gas volume flow (O_2 plus CO_2) inside the BOs was kept constant, regardless of the pressure level, and was set to 0.3 L/min per BO. Thereby, a constant influence of the gas flow rate on CO elimination and hemolysis was maintained.

The CO elimination was started when the pressure in the BOs had reached the target value. By closing the ball valves (A, B) to the gas-outlet, the gas was forced to pass through the check valves (K, L), the dispersers and into the blood in both BOs. Both BOs were set to identical pressure levels. Solely, the test duration was varied: for the first BO the elimination was stopped after 5 min, for the second BO after 15 min. Directly after turning off the gas supply of a BO, the pressure in the BO was released quickly. For the calculation of the CO-Hb half-life it was assumed that the measurements of both BOs are part of the same curve progression. By using two BOs with different termination times the blood was depressurized in the BOs and not the sample syringe, eliminating the problem of failed measurements due to bubbles in the samples.

Samples for the blood gas and hemolysis measurement were taken

- before CO elimination,
- shortly after filling of the BO with blood (0 min), and
- after CO elimination, directly after the pressure release at 5 or 15 min.

Three different examined temperatures of 23 °C, 30 °C and 37 °C and four pressure levels of 1 bar, 3 bar, 5 bar, and 7 bar absolute pressure set up the experimental matrix. This adds up to a total of 12 different test conditions carried out in this study. Every experiment was performed three times.

2.6. Calculations and Statistics

The CO-Hb level is measured using a blood gas analyser, which does not allow a continuous monitoring of CO-Hb, but only discrete measurement points. Therefore, it is difficult to poison the blood to exactly the same starting value $\text{CO-Hb}_{\text{start}}$ for each series of tests. Hence, the absolute detoxification velocity (ΔCO-Hb per minute) is not a suitable parameter for a comparison, since it is dependent on the starting value.

Pace et al. [29] described CO elimination to be following the simple exponential rate expression:

$$\text{CO-Hb}(t) = \text{CO-Hb}_{\text{start}} \times e^{-kt}$$

CO-Hb(t) stands for the percentage of CO-Hb at time t. $\text{CO-Hb}_{\text{start}}$ is the percentage before the CO elimination. The CO-Hb half-life is therefore a suitable parameter that describes the velocity of the CO elimination independent of the start value. The half-life was calculated using a nonlinear regression with the assumption that the plateau equals 0% CO-Hb. Subsequently, we performed a two-way ANOVA followed by Tukey's multiple comparison test.

For each experiment, the plasma free hemoglobin (pfHb) was measured at the start and the end of the CO elimination. The values were subtracted to calculate the delta pfHb. Subsequently, we performed a two-way ANOVA followed by Tukey's multiple comparison test.

To analyse the pH-value, an average value for each experiment was calculated, using the measurement at each time point. With these average values, we performed a two-way ANOVA followed by Tukey's multiple comparison test. Zeitlicher verlauf.

All statistical analyses were performed with GraphPad Prism 9.

3. Results

3.1. Comparison of CO Elimination of HFMO and BO

The CO-Hb half-life of the preliminary experiments are shown in Figure 4. The entire measurement data set is provided as supplementary information (Tables S1 and S2). Therein, failed measurements are identified. The characterization by blood gas analysis of some samples was impossible due to bubbles, which formed in the syringe during the release of excess pressure. Therefore, only intermediate samples were affected. The measurements of start and end samples were always successful.

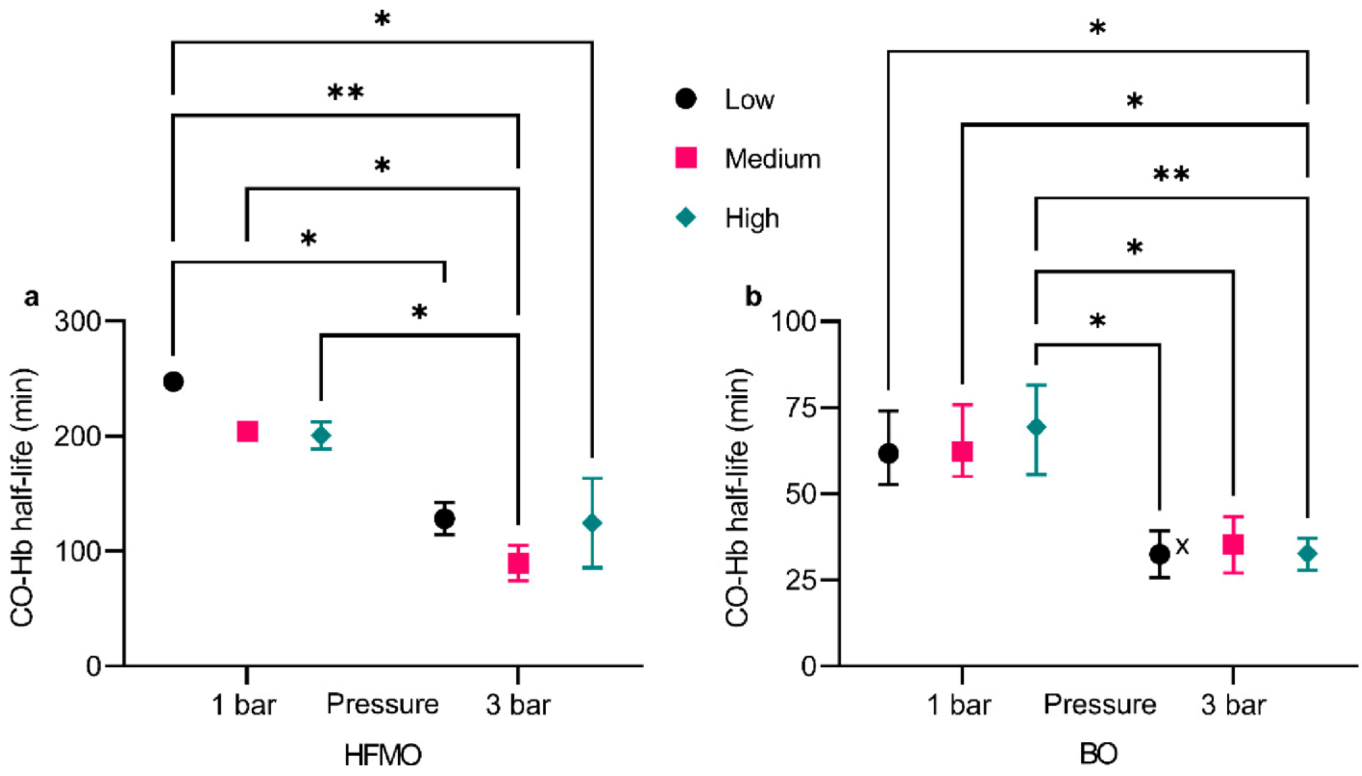

Figure 4. CO-Hb half-life with (**a**) HFMO and (**b**) BO dependent on pressure and flow rate. The whiskers represent the highest and lowest values of the corresponding experiments. Flow rate stands for blood flow rate for HFMO experiments and for gas flow rate for BO experiments. Note the different scale of the axes. (*) $p \leq 0.05$, (**) $p \leq 0.01$. (x) During one experiment, bubbles inside the samples prevented the analysis.

In both the HFMO and the BO, a variation of the blood flow rate or the gas flow rate, respectively, does not affect the CO-Hb half-life significantly. An increase in pressure results in a faster CO elimination in both oxygenators, as expected. The overall effect is considered significant ($p \leq 0.0001$). However, a superior performance of the specifically designed batch oxygenator, especially at an operating pressure of 3 bar, can be observed. The use of a BO leads to an increased performance by a factor of more than 3.5 compared to an HFMO, when comparing the averaged CO-Hb half-lives at a pressure of 3 bar. Furthermore, the increased pressure level entails a stronger effect on the half-life for the BO than for the HFMO. Based on these preliminary results, we decided to proceed using the BO, which we redesigned and evaluated in the main experiments.

3.2. Performance of Extracorporeal CO Elimination with a Revised Batch Oxygenator

Figure 5 shows the CO-Hb half-life in relation to different temperatures and varying pressure levels. The values were averaged from the results of three identical measurements. For the original data, see supplementary information (Table S3).

For the comparison of different pressures at constant temperatures it is notable that higher pressures result in a shorter half-life; the overall effect of the pressure is considered significant ($p \leq 0.0001$). The increase from ambient pressure to 5 bar and 7 bar results in a significant decrease of the half-life for every temperature. The increase from ambient pressure to 3 bar is only significant at 30 °C. Additionally, the general decrease of the CO-Hb half-life seems to increase linearly with increasing pressures.

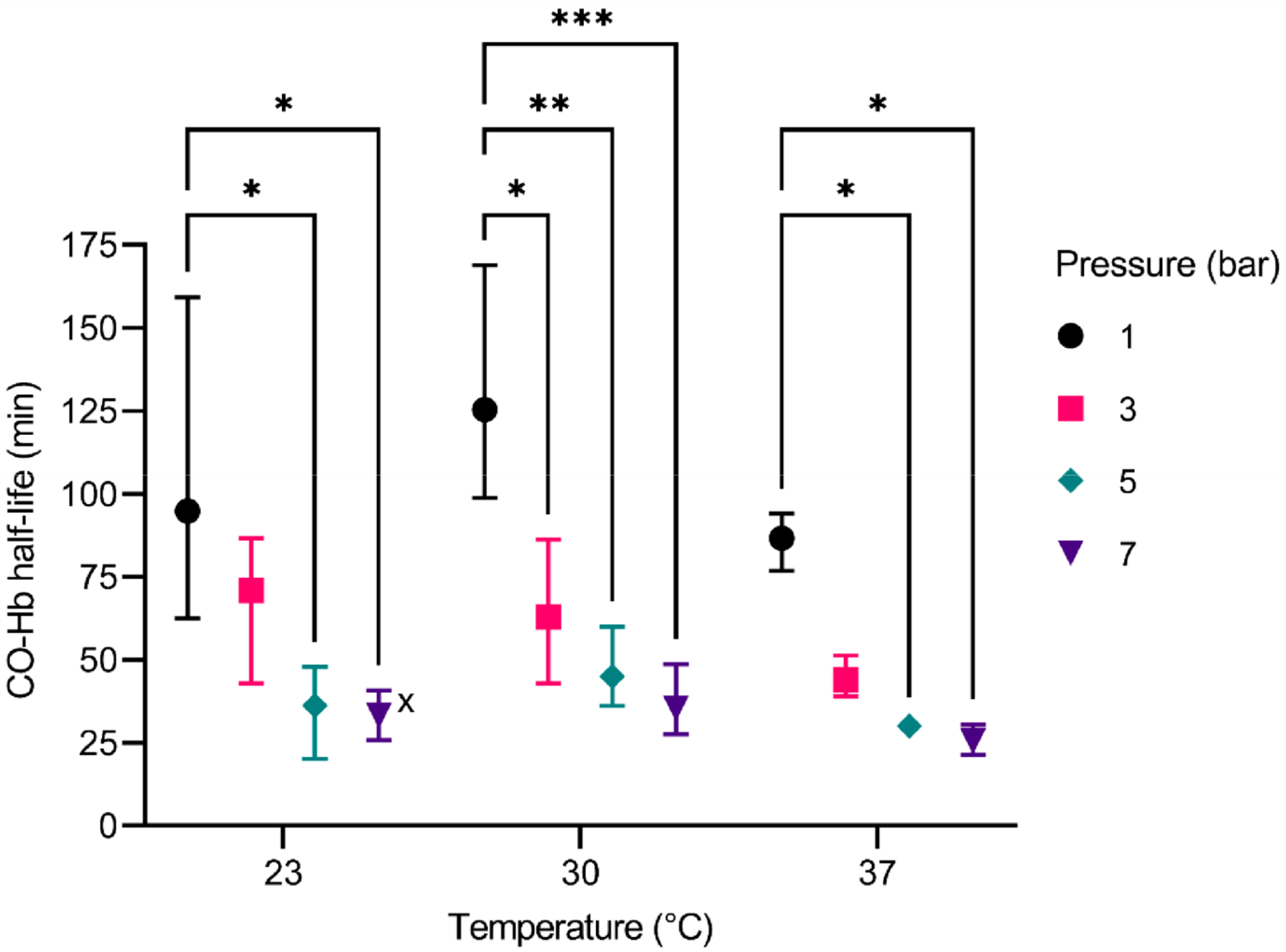

Figure 5. CO-Hb half-life of the revised BO dependent on temperature, and pressure. The whiskers represent the highest and lowest values of the corresponding experiments. (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$. (x) One experiment was excluded due to extensive foam formation inside the BO.

An increase in temperature, does not show a significant effect on the CO-Hb half-life. For an increase in temperature from 23 °C to 30 °C, for example, the half-life increases for a pressure of 1, 5, and 7 bar. Yet, for a pressure of 3 bar, a decrease of the half-life from 30 °C to 37 °C is visible. At the physiological temperature of 37 °C, the CO-Hb half-life reaches the lowest value at 37 °C and 7 bar. Finally, it is also notable that the variances of the measurements are distinctively lower at 37 °C.

3.3. Hemolysis

The increase in plasma free hemoglobin during the experiments dependent on temperature and pressure is displayed in Figure 6. For the original data, see supplementary information (Table S4). The overall influence of the temperature is considered significant ($p \leq 0.001$); experiments at higher temperatures generally result in higher concentrations of plasma free hemoglobin than experiments at lower temperatures. For experiments at 5 bar and 7 bar for example, the concentration of plasma free hemoglobin doubles with an increase in temperature from 23 °C to 37 °C. The overall effect of the pressure on the plasma free hemoglobin is considered not significant.

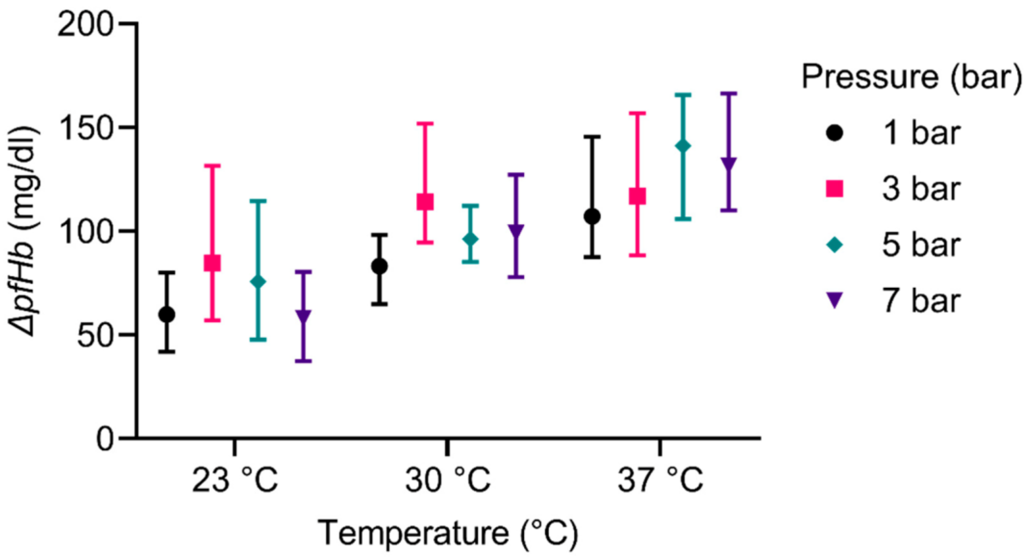

Figure 6. Plasma free hemoglobin dependent on temperature and pressure. The whiskers represent the highest and lowest values of the corresponding experiments.

3.4. pH-Value

Figure 7 shows the pH-values during the experiments dependent on temperature and pressure. For the original data, see Supplementary Information (Table S5). The results show high deviations, especially for lower temperatures, which decrease slightly at higher temperatures. For the experiments at 30 °C and 37 °C, increasing mean pH-values with increasing pressures can be observed. Overall, pH-values and temperature show a reciprocal relationship, which is considered significant ($p \leq 0.0001$). The overall effect of the pressure is considered not significant.

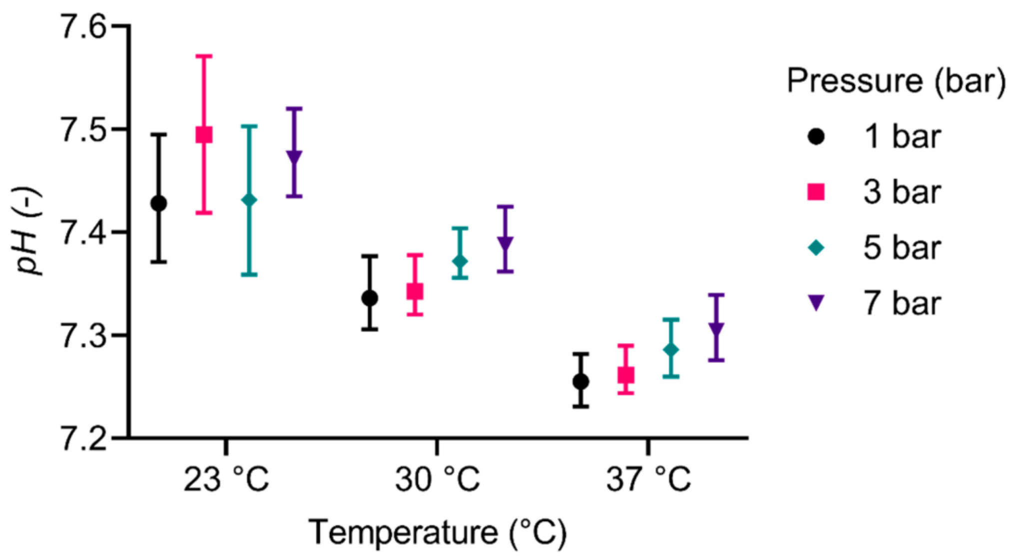

Figure 7. pH-value dependent on temperature, and pressure. The whiskers represent the highest and lowest values of the corresponding experiments.

4. Discussion

A novel treatment option for carbon monoxide poisonings is urgently needed, as demonstrated by the numerous research activities described in the introduction. However, the only approved therapy is the ClearMate, which has not gained clinical acceptance. The other approaches are still at the in-vivo stage, which means a failure rate of over 90% [30], in case of the pharmaceutical approaches. The light-enhanced ECMO is also a medical device with an extracorporeal approach. However, to achieve a pervasive illumination of the opaque blood, a vast surface area is required.

The overall aim of this study was to achieve a proof of principle of a novel therapeutical method that eliminates CO extracorporeally from blood. The underlying approach aims at enhancing the current therapies or at providing a novel therapy option where the current therapies are unavailable or of no avail. The novel method is realized by extracorporeal hyperoxygentation of the patient's blood. The EHT allows for higher pressures than the HBO therapy, because the patient is not exposed to the pressure and therefore, no side effects such as oxygen toxicity etc. have to be considered. Furthermore, the resulting device could be small and portable, essentially bringing the HBO therapy to the patient.

In the first part of the study, the CO elimination performances of HFMO and BO were compared. The performance of a BO was increased by a factor of more than 3.5. Although the different flow rates, blood flow rate for the HFMO and gas flow rate for the BO lack in comparability, no influence on the performance is observable. Therefore, the comparison of the flow rates provides no bias towards the interpretation of the results.

We hypothesize that the mass transfer resistance due to membranes in the HFMO results in a slower CO elimination, compared to the BO. This is supported by the results of the preliminary experiments. Additionally important is the difference in residence time within HFMO and BO. On the one hand, the HFMO needs a continuous flow-through, to provide ample mixing of the blood. On the other hand, in the BO, the mixing is realized by the rising gas bubbles. It is therefore possible, to implement a batch process using a BO, thus providing higher residence times. This is especially important because of the binding kinetics: CO unbinds from hemoglobin at 1/2000 the speed of oxygen [31]. Therefore, a process with a higher residence time leads to a higher performance. Thus, the batch process of the utilized BO, which ensures a high residence time, might lead to the increased performance. The flow-through concept of the HFMO, while able to sufficiently oxygenate blood, does not provide enough residence time for an efficient elimination of CO. Hence, for the subsequent experiments, the concept of using a BO with a batch process was further investigated. Additionally, this supports our assumption of the velocity of the bonding processes as an influence on the CO elimination.

The overall effect of pressure on the elimination of CO has already been studied in vivo by numerous scientists working with HBO [5]. However, there has been no research concerning a practical approach with pressures higher than used in HBO therapy (2.5 bar–3 bar) and different temperatures, since such parameters cannot be safely investigated in vivo due to, e.g., oxygen toxicity. Nevertheless, our tests on the elimination of CO dependent on pressure and temperature provide evidence that a further increase in pressure and the alteration of the blood temperature might enhance the elimination of CO.

The decrease of the CO-Hb half-life due to increased pressure is shown by all experiments. This can be explained by Le Chatelier's principle [32], by which the response of a stressed equilibrium system can be predicted. The basic concept of the elimination of CO from blood can be described as an equilibrium reaction, as first stated by Douglas et al. [3]:

$$O_2\text{-Hb} + CO \rightleftharpoons CO\text{-Hb} + O_2$$

here, the stress on the equilibrium system caused by the increased pressure manifests in two ways:

First, because the equilibrium of both sides of the reaction is dependent on the respective concentrations; an increase in O_2 concentration in the plasma results in a shift of the equilibrium to the left side: more CO is unbound and dissolved in the plasma, which can then be eliminated. The increased O_2 concentration is achieved by raising the overall pressure level and supplying high amounts of oxygen. This effect was seen in all our experiments, as an increase in pressure always led to an improved elimination of CO. The experiment at 23 °C and 5 bar, which showed a CO-Hb half-life of 7.72 min (see Supplementary Table S3), is regarded as an outlier. Overall, the effect is consistent with the experiences made with HBO [9].

Second, an increased pressure favours conditions that are less volumetric. In the literature, the activation volume of O_2 binding to hemoglobin has been described as being positive, whereas the activation volume of CO binding to hemoglobin shows negative activation volumes. Therefore, as the pressure is increased, the equilibrium system experiences a stress that leads to the binding of more O_2, as this state requires less volume [33–35]. This effect has a negative impact on the elimination of CO, yet it seems to be minor compared to the effect of the increased O_2 concentration described before. An increase in pressure achieved with inert gases would probably make this effect more apparent but was not focus of our study.

Although the realistic operating temperature of our method would be 37 °C, we tested the CO elimination at a broader range of temperatures to determine the dependencies. In theory, varying the temperature during the CO elimination influences the elimination threefold:

First, with increasing temperature, the oxygen dissociation curve is shifted to the right, which decreases the affinity of hemoglobin for oxygen [36,37]. This effect is also true for CO, as the binding mechanism to the hemoglobin is similar [3,38]. The decreased affinity of hemoglobin for CO yields a faster CO elimination, whereas a decreased affinity for O_2 results in a slower CO elimination. This is because of the lower affinity for O_2, the chances of CO binding to hemoglobin are increased. However, unbound CO is eliminated from the plasma due to a concentration gradient towards the CO-free membranes or gas bubbles. O_2 on the other hand is not eliminated, because the membranes are flushed with oxygen and the bubbles consist mainly of oxygen.

Second, a higher temperature facilitates diffusion, which becomes apparent in the Einstein-Smoluchowski relation [39,40]:

$$D = \frac{k_B \cdot T}{6 \cdot \pi \cdot \eta \cdot R_0}$$

with k_B as Boltzmann constant, T as Temperature, η as viscosity, and R_0 as particle radius. Thus, the mobility of CO and O_2 in blood is increased, whereby the exit of CO out if the hem pocket and the subsequent diffusion towards the membranes and the gas bubbles is facilitated. For O_2 on the other hand, this entails an easier diffusion into the hem pocket, once it is free from CO. Thereby, a rebinding of the CO is prohibited. Additionally, the reaction rate of O_2 and hemoglobin is solely limited by the migration process of O_2 through the Hb, as binding itself occurs so quickly [31,33]. The Einstein-Smoluchowski relation also implies that the diffusion is increased with lower viscosity of the liquid. In our case, the liquid is blood plasma, which shows a decrease in viscosity with higher temperatures, further enhancing the elimination of CO [41,42]. Furthermore, the rapid and uniform solution of O_2 during the pressure build-up and thereby the increase in O_2 concentration is also enhanced. This is favourable for the elimination of CO as well, because a high concentration (or partial pressure) of O_2 yields the highest influence on the CO-Hb half-life (Figure 5).

Third and contrarily, higher temperatures lead to a lower solubility of gases in liquids. For blood, this was measured by Christoforides et al. [43], who ascertained that an increase of temperature from 23 °C to 37 °C entails a ca. 20% lower Bunsen solubility coefficient. This coefficient linearly correlates with the concentration of gas in the liquids. Therefore, the O_2 concentration in the blood is reduced at high temperatures. This diminishes the effects of higher pressures on the CO-Hb half-life, compared to experiments at equal pressure levels but lower temperatures. The influence of the temperature on the Bunsen solubility coefficient is slightly regressive for increasing temperatures, which makes the reduction of the CO elimination less distinct.

In summary, the increase of the temperature yields opposing effects on the CO elimination. This may be the reason that the impact of the temperature shows no significant effect on the elimination of CO.

Bubble oxygenators, on which our revised design is based, are generally associated with high rates of hemolysis [44,45]. Pearson and McArdle [46] compared the hemocompatibility of several membrane and bubble oxygenators. The rise in plasma free hemoglobin (pfHb) caused by the cardiopulmonary bypass ranged from 13 mg/dl to 34 mg/dl for membrane oxygenators and from 9 mg/dl to 46 mg/dl for bubble oxygenators. The lowest measured values of pfHb in our study are ~60 mg/dl, the highest >140 mg/dl for a 15 min duration. Our presented results are also contradictory to the study of Bücherl [47], who reported that the hemolysis in a bubble oxygenator decreases with rising temperatures. These differences could be accounted to several factors: The design of our device was not focused on hemocompatibility, we even used non-hemocompatible materials such as borosilicate safety glass for the proof of principle. We also performed in-vitro experiments, whereas the cited articles studied the effects in vivo. Nakahara and Yoshida [48] studied the hemolysis in a bubble column in vitro and reported rates comparable to the results shown here. They also investigated the impact of antifoam agents on hemolysis and reported a dependency on the type and the concentration of antifoam. In our study, the increase of the temperature could entail an increased hemolytic activity and therefore be responsible for the deviation to Bücherl's experiments. The pressure, contrarily, showed no distinct influence on the hemolysis, as seen in the results (Figure 6). This is in accordance with the literature [49–53]. Pohlmann et al. [54] even reported no impact of the air interface on the hemolysis at comparable ratios of blood volume and gas flow rates.

Even though it has been tried to maintain a constant CO_2 partial pressure and therefore pH-value during all experiments by varying the CO_2 fractions in the feed, the results indicate that this has not been successful (Figure 7). For an application in vivo, this is not tolerable and therefore further studies are needed. However, for the present study, the influence of the pH-value has to be analysed regarding the CO elimination. Most likely, the influence of temperature on the acid dissociation constant of the bicarbonate buffer system was not correctly considered. This could entail the decreasing pH-values with increasing temperatures. The pH-value is known to be of great importance for gas transport in blood. Lowering the pH-value leads to a right shift of the oxygen binding curve [36]. Since the binding of CO to Hb is similar to the one of O_2 [3,38], a lower pH-value is therefore expected to accelerate the elimination of CO.

The in-vitro experiments were conducted two times for the HFMO and three times for each BO, as they were highly complex, especially for high pressure levels. Therefore, we present the entire data set in the results as well as in the supplementary information online. Nevertheless, the results proof the principle of extracorporeal elimination of CO at elevated pressure levels as aspired. For further development, the results can be interpreted as general tendencies, but more extensive studies still have to be conducted.

As discussed, the studied parameters influence the elimination of CO in many different, mostly opposing ways. From our experiments it is not possible to conclude the magnitude of the different influences. However, the focus of our study was to proof the principle of extracorporeal CO elimination using a novel concept. As of yet, no efforts have been made to optimize the CO elimination. For this subsequent optimization a more comprehensive study of the various influences is needed.

For a clinical applicability, not only the CO elimination has to be considered, but also the safety of the patient. With our concept, two main aspects result: First, the impact of the blood-air contact on the hemocompatibility. Bubble oxygenators have been associated with blood trauma [25] and are no longer clinically used. However, in our approach is has to be taken into account that, compared to approx. 6 h for bubble oxygenators, the blood is only treated for a very limited amount of time and then returned to the patient, where after a different volume of blood is drained and treated. The presented results regarding hemolysis seem promising considering that the device was not hemocompatibly designed. Nevertheless, other aspects of hemocompatibility, such as thrombogenicity and inflammatory reactions, have to be examined as well. Second, the device has to be designed in a way that the application of high pressures in the device does not endanger the patient or medical staff.

5. Conclusions

In conclusion, the presented experiments show that the extracorporeal elimination of CO from blood is possible. The shortest CO-Hb half-life at a physiological temperature of 37 °C was achieved at a pressure of 7 bar with a value of 20.4 min. This is in the range of the HBO therapy. However, because the filling and emptying, as well as the pressure build-up and release take time and as only a fraction of the whole blood can be treated at once, a further increase of the detoxification velocity is necessary. As mentioned, no effort has been made yet to optimize the device and therefore, the necessary improvement seems realistic. Following the redesign and optimization for detoxification velocity, and the validation of clinical safety, the presented concept has the potential to enhance the current therapy chain for CO poisoning by providing an exhaustive coverage more easily than HBO, and by offering a novel treatment option for paramedics in the field and physicians in the hospital.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/membranes12010056/s1, Table S1: Entire data set for the CO elimination with the HFMO; Table S2: Entire data set for the CO elimination with the BO; Table S3: Entire data set for the CO elimination with the revised BO; Table S4: Entire data set for the hemolysis of the experiments with the revised BO; Table S5: Entire data set for the pH-value of the experiments with the revised BO.

Author Contributions: Conceptualization, N.B.S., P.C.S., T.S.-R., R.K., U.S., G.W. and J.A. Formal analysis, N.B.S., P.C.S., A.H. and S.S. Investigation, N.B.S., P.C.S., A.H. and S.S. Methodology, N.B.S., P.C.S., A.H., S.S. and A.J. Supervision, A.J., T.S.-R., R.K., U.S. and J.A. Visualization, N.B.S. and A.H. Writing—original draft, N.B.S. Writing—review and editing, P.C.S., A.H., S.S., A.J., T.S.-R., R.K., U.S., G.W. and J.A. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: RWTH Aachen University has filed a patent application entitled “System for the extracorporeal elimination of carbon monoxide” (WO2017153034A1), which is pending; the inventors are J.A., P.C.S., T.S.-R., G.W. and U.S., N.B.S. and P.C.S. are co-founders of a company to commercialize the technology described in this manuscript. The other authors declare that they have no conflict of interest.

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Supplementary Materials

Extracorporeal Hyperoxygenation Therapy (EHT) for Carbon Monoxide Poisoning: In-Vitro Proof of Principle

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Table S1: Entire data set for the CO elimination with the HFMO

Sample			CO-Hb (%)		CO-Hb half life (min)	
p (bar)	Flow rate	t (min)	n = 1	n = 2	n = 1	n = 2
		0	30.1	31.2		
		5	28.5	30.9		
		10	28.9	30.3		
		15	28.2	29.8		
1	low	30	27.3	28.8	243.3	252.1
		0	29.9	31.4		
		5	28.8	30.5		
		10	28.3	30	199.1	209.5
		15	27.8	29.7		
1	medium	30	26.8	28.3		
		0	30	31.1		
		5	28.7	30.2		
		10	28.3	29.8		
		15	27.7	29.3		
1	high	30	26.7	28.1	188.9	212.7
		0	31.6	31.5		
		5	30.1	30.6		
		10	28.9	28.7	142.3	114.4
		15	28.8	28.5		
3	low	30	27.1	26.3		
		0	30.1	31.4		
		5	29.5	28.8		
		10	26.7	28		
		15	25.8	27.9		
3	medium	30	23.1	25.3	74.28	105.2
		0	32.1	31.3		
		5	29.9	30.3		
		10	28.7	29.4	85.61	163.4
		15	28.6	28.9		
3	high	30	24.8	27.5		

Table S2: Entire data set for the CO elimination with the BO

Sample			CO-Hb (%)			CO-Hb half-life (min)		
p (bar)	Flow rate	t (min)	n = 1	n = 2	n = 3	n = 1	n = 2	n = 3
		0	32.4	32.8	31			
		15	27.4	25.2	24.6			
1	low	30	24.6	23.5	21.1	74.02	58.52	52.68
		0	32	32.8	31.1			
		15	26.9	26.1	25			
1	medium	30	24.5	22.9	21.5	75.82	56.04	55.08
		0	32.6	33.3	31			
		15	26.7	N/A	24.5			
1	high	30	24.6	25.8	21.6	71.04	81.49	55.51
		0	29.4	30.1	N/A			
		15	24.1	N/A	N/A			
3	low	30	16.8	13.4	N/A	39.25	25.7	N/A
		0	29.6	25.7	26.8			
		15	26.1	N/A	N/A			
3	medium	30	15	11.9	16.6	35.43	27.01	43.41
		0	29.2	30.9	27.4			
		15	20.9	21.5	N/A			
3	high	30	13.5	18.3	14.6	27.9	37.16	33.03

Table S3: Entire data set for the CO elimination with the revised BO

Sample			CO-Hb (%)			CO-Hb half-life (min)		
p (bar)	T (°C)	t (min)	n=1	n=2	n=3	n=1	n=2	n=3
1	23	0 5 15	43.1 41.7 40.3	41.5 40.4 35.3	44.8 43 38	159.2	62.99	62.53
1	30	0 5 15	43 41.8 39.1	41.5 40.4 37.4	44.4 43.2 41.7	108.70	98.80	168.90
1	37	0 5 15	43 41.6 38.3	41.3 39.7 36.1	44.5 43.3 39.9	89.2	76.85	94.11
3	23	0 5 15	43.1 40.9 33.9	41.5 39.6 36.6	44.5 42.2 39.4	42.95	83.22	86.70
3	30	0 5 15	43.1 41.2 33.9	41.3 37.9 34.6	44.4 42.2 39.3	42.86	59.75	86.25
3	37	0 5 15	42.9 39.9 32.9	41.3 38.4 32.2	44.5 42.2 36.4	39.05	41.66	51.41
5	23	0 5 15	43.1 39 25.5	41.4 36.8 33.2	44.5 41.6 34.5	20.18	47.91	40.68
5	30	0 5 15	43.1 39.7 33	41.3 38.6 31	44.4 41.6 37.3	38.89	36.10	59.94
5	37	0 5 15	43 39.3 30.8	41.4 37.5 28.6	44.3 41 31.7	31.14	28.14	31.04
7	23	0 5 15	43.1 38.7 12	41.4 36.6 32	44.5 40.9 29.7	(9,94)	40.73	25.84
7	30	0 5 15	42.9 39.3 29.4	41.3 36.7 29.1	44.3 41.4 35.8	27.58	29.7	48.74
7	37	0 5 15	43 39.2 26.2	41.3 35.1 27.1	44.3 39.6 31.5	21.32	24.61	30.49

Table S4: Entire data set for the hemolysis of the experiments with the revised BO

Sample			pfHb (mg/dl)		
p (bar)	T (°C)	t (min)	n = 1	n = 2	n = 3
		0	20.7	27.49	25.505
		5	52.07	64.51	66.5
1	23	15	62.575	107.65	83.265
		0	24.455	30.915	26.15
		5	59.55	72.365	70.825
1	30	15	111.05	95.905	124.475
		0	30.56	31.475	25.875
		5	74.765	82.595	76.67
1	37	15	118.9	177.033	113.5
		0	23.445	28.595	24.405
		5	45.605	88.745	75.85
3	23	15	80.45	160.1	89.62
		0	23.81	30.005	27.2
		5	65.505	81.77	76.53
3	30	15	175.65	126.15	121.7
		0	27.255	33.515	27.715
		5	79.13	107.9	77.64
3	37	15	184.1	139.45	116.05
		0	24.565	29.43	26.025
		5	52.72	71.48	69.8
5	23	15	72.295	144	91.155
		0	27.865	31.12	27.51
		5	78.32	85.93	78.83
5	30	15	118.95	116.3	139.75
		0	27.77	36.575	30.06
		5	86	86.61	78.915
5	37	15	133.65	188.75	195.75
		0	24.165	33.46	26.91
		5	44.075	73.82	70.65
7	23	15	61.5	113.85	83.625
		0	25.525	31.32	25.355
		5	68.97	73.47	77.8
7	30	15	152.7	123.45	103.3
		0	30.045	34.935	28.175
		5	74.99	96.695	91.05
7	37	15	196.45	145	146.25

Table S5: Entire data set for the pH-value of the experiments with the revised BO

Sample			pH (-)		
p (bar)	T (°C)	t (min)	n = 1	n = 2	n = 3
		0	7.844	8.042	7.841
		5	7.256	7.285	7.250
1	23	15	7.013	7.159	7.166
		0	7.635	7.801	7.650
		5	7.146	7.191	7.176
1	30	15	7.138	7.139	7.152
		0	7.468	7.602	7.496
		5	7.104	7.113	7.128
1	37	15	7.120	7.132	7.136
		0	7.818	8.010	7.824
		5	7.721	7.271	7.240
3	23	15	7.175	7.204	7.193
		0	7.619	7.784	7.638
		5	7.194	7.184	7.191
3	30	15	7.146	7.166	7.162
		0	7.467	7.587	7.487
		5	7.139	7.136	7.113
3	37	15	7.125	7.146	7.154
		0	7.766	7.986	7.806
		5	7.256	7.261	7.256
5	23	15	7.053	7.261	7.233
		0	7.608	7.765	7.626
		5	7.270	7.239	7.193
5	30	15	7.190	7.208	7.250
		0	7.446	7.576	7.477
		5	7.172	7.175	7.154
5	37	15	7.161	7.194	7.218
		0	7.756	7.971	7.787
		5	7.269	7.299	7.299
7	23	15	7.280	7.289	7.288
		0	7.587	7.749	7.614
		5	7.309	7.241	7.214
7	30	15	7.191	7.285	7.294
		0	7.449	7.564	7.474
		5	7.204	7.218	7.132
7	37	15	7.174	7.235	7.287

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Extracorporeal hyperoxygenation therapy (EHT) for CO poisoning: in vitro and in vivo feasibility of a full-scale batch system

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Carbon monoxide (CO) poisoning is one of the most common causes of injury and death from poisoning. The primary objective of therapy is to eliminate CO from the patient as quickly as possible to prevent acute and long-term effects. The ideal treatment is hyperbaric oxygen in a pressure chamber. However, pressure chambers are scarce, and therefore, the most common treatment is normobaric oxygen (NBO), which, however, has limited efficacy. Here, we present a full-scale batch system for extracorporeal hyperoxygenation therapy (EHT), which facilitates CO elimination extracorporeally by increasing the dissolved oxygen concentration in the blood. The EHT was characterized in vitro, resulting in a minimum carboxyhemoglobin half-life of 3.26 ± 0.11 min. In large animal trials the EHT reduced the median carboxyhemoglobin half-life by 42% (29.77 min EHT vs. 70.8 min control (NBO)). However, the EHT also induced oscillations in hemodynamic pressures due to changes in the animals' circulatory volume during operation. After optimization, the EHT could be a promising option for treating CO poisoning.

Carbon monoxide (CO) poisoning is one of the most common causes of injury and death due to poisoning worldwide[1]. The global incidence has remained stable over the past 25 years and is estimated at 137 cases per million, with a mortality rate of 4.6 cases per million[2]. In the US, accidental, non-fire related CO poisoning results in an annual societal burden of $ 1.3 billion[3].

CO is produced during incomplete combustion. The colorless, odorless, and tasteless gas is undetectable by humans and, once inhaled, enters the bloodstream via the lungs. There it binds, competitively with oxygen (O_2), to hemoglobin (Hb), creating carboxyhemoglobin (COHb)[4]. Unfortunately, the affinity of hemoglobin for CO is more than 200 times greater than that for O_2. For example, this means that a concentration of only 0.022% in the breathing air results in 30% of hemoglobin in the blood being occupied with CO (30% COHb)[5]. Consequently, oxygen delivery to organs and tissues is inhibited, resulting in hypoxia. In addition, the oxygen dissociation curve is shifted to the left, further inhibiting oxygen release from hemoglobin into the tissue[4]. CO also binds to cytochrome c oxidase, inhibiting mitochondrial respiration and causing additional apoptosis and neural necrosis[6]. Furthermore, CO causes platelet activation, an increase in reactive oxygen species and inflammation, contributing to neurological and cardiac injury[6]. Clinical symptoms of CO poisoning are nonspecific and range from headaches and dizziness to unconsciousness, coma, and death. Long-term symptoms occur in up to 50% of patients and include impairment of concentration, memory, learning, and speech, as well as depression, dementia, and psychosis[7,8]. Within 5 years, 10% of patients suffer a heart attack[9], the risk of diabetes mellitus is increased[10], and long-term mortality increases from 1.6 to 8.4%[11].

The primary goal of therapy is to eliminate the CO from the organism to prevent acute and long-term effects[12]. CO poisoning is usually treated by administering normobaric oxygen (NBO) via face mask, which increases the elimination of carbon monoxide, decreasing the COHb half-life. Compared to breathing room air, which results in a COHb half-life of approx. 320 min, NBO reduces the COHb half-life to approx. 74 min[6]. A further decrease in the COHb half-life to approx. 20 – 40 min can be achieved by administering hyperbaric oxygen (HBO) inside a pressure chamber[6,13]. The underlying mechanism of CO removal is the increase in dissolved oxygen in the plasma, which shifts the equilibrium reaction towards more dissolved CO that can be

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eliminated. The basic concept was first described by Douglas et al.[14]. In a double-blind, randomized study by Weaver et al.[7], HBO also reduced the incidence of cognitive sequelae from 33% to 18%. In contrast, Annane et al.[15] found that in comatose patients, two HBO sessions worsened the outcome compared to one session. Buckley et al.[16] could not establish an efficacy of HBO compared to NBO for cognitive sequelae in a Cochrane systematic review. A review by Roderique et al.[17] even cautioned against HBO as potentially harmful due to a further increase in reactive oxygen species (ROS). In practice, the therapy is limited by the number of pressure chambers available, and the time required to prepare them before treatment. This can result in delays of several hours before treatment can begin[6,18,19]. In the US, for example, only 11.9% of 361 surveyed pressure chambers are quipped for dealing with emergencies[20]. In conclusion, new therapies and treatment options for patients with acute CO intoxication are needed[6].

Currently, several new therapeutic options are under investigation. On the one hand, there are pharmacological approaches, including a combination of hydroxocobalamin and ascorbic acid in a reduced form[21], a supramolecular complex (hemoCD)[22], and molecules based on bioengineered neuroglobin[23,24]. They use scavenger molecules with high affinity for CO to eliminate CO from the organism. On the other hand, treatments based on extracorporeal membrane oxygenation (ECMO) are being developed. ECMO is traditionally used in severe cases of acute respiratory distress syndrome (ARDS) and chronic obstructive pulmonary disease (COPD) to support or take over the function of the lungs by exchanging respiratory gases extracorporeally[25]. Therefore, the use of ECMO to remove CO seems obvious. However, due to the high affinity of hemoglobin for CO, elimination must be enhanced to achieve sufficient efficacy. The approach of Fischbach et al.[26], termed "photo-ECMO", centers on harnessing light irradiation to augment CO elimination. Fischbach et al. demonstrated the efficacy of their method by reducing the COHb half-life from 42.6 ± 1.5 min in the absence of light to 21.4 ± 1.4 min with the introduction of light. By increasing the blood flow rate and assembling six photo-ECMO devices in parallel, the COHb half-life was decreased even further to a minimum of 6.3 ± 1.2 min. Subsequently, Fischbach et al.[26] amplified the partial oxygen pressure within their system to enhance CO elimination. This augmentation was achieved by elevating the sweep gas pressure to 1.33 atm (1.35 bar), yielding a COHb half-life of 5.2 ± 0.4 min when employing light irradiation in four parallel devices.

Our approach, extracorporeal hyperoxygenation therapy (EHT), is based on enhancing extracorporeal CO elimination by further increasing the dissolved oxygen concentration. Since treatment is applied only to the extracorporeally pooled blood, higher pressures can be achieved than in a pressure chamber, and the increase in ROS only occurs extracorporeally. In addition, the EHT system can be less expensive and more mobile than a pressure chamber, resulting in better availability and accessibility. In previous in vitro experiments, we investigated the approach in a small-scale batch system[27]. Here, we describe the newly developed full-scale model and the in vitro experiments as well as in vivo testing of feasibility in a large animal model. The high-pressure gas exchanger is the key component of the EHT system and is based on a bubble oxygenator[28], in which bubbles are introduced into the blood to provide oxygenation and decarboxylation. For our high-pressure application, we developed a gas exchanger suitable for batch operation (see Fig. 6). A batch operation is favorable as it is possible to separate the gas exchanger from the extracorporeal circuit and therefore the patient during high-pressure detoxification. A standard batch operation with consecutive filling and emptying of the gas exchanger with blood would cause a circulatory volume shift for the patient each time, which is potentially harmful. We solved this problem by equipping the high-pressure gas exchanger with a blood loop containing two pumps and four valves (see Fig. 7). With different valve settings, the gas exchanger can be filled and emptied simultaneously while maintaining a constant blood flow in the extracorporeal circuit without circulatory volume shifts for the patient. Here, we describe the in vitro tests of a full-scale system and in vivo testing of feasibility in a large animal model.

Results

In vitro experiments

The results of the in vitro experiments are shown in Fig. 1. The original data can be found as Supplementary Table S1 online. A gas flow rate of 20 standard liters per minute (SLPM) entailed the shortest COHb halflife (3.47 ± 0.36 min). Gas flow rates of 10 SLPM and 5 SLPM resulted in significantly longer COHb half-lives (5.84 ± 0.67 min and 5.4 ± 0.14 min, respectively), while the difference between both was not significant.

To quantify the amount of hemolysis caused by the treatment, plasma free hemoglobin (PfHb) was measured. Figure 2 shows the difference in PfHb from pre- to posttreatment of a single batch with the EHT system. The original data can be found as Supplementary Table S2 online. Overall, PfHb was increased after treatment in a single batch, but there was no apparent dependence on gas flow. For example, for a gas flow of 5 SLPM, a difference in PfHb of 8.59 mg/dL was measured for the first experiment and 41.76 mg/dL for the second experiment. A similar variance was seen for the operating points with the other gas flows.

In vivo experiments

The results of the verification of the EHT in vivo showed a substantial decrease in the COHb half-life compared to NBO treatment alone (see Fig. 3). With the EHT, the COHb half-life was 29.77 (28.46, 43.72) min, whereas NBO treatment resulted in a COHb half-life of 70.8 (50.31, 83.91) min. Hence, the EHT system increased the CO elimination rate by a factor of 2.04. There was no distinct difference in median plasma free hemoglobin, although higher levels of plasma free hemoglobin were measured in one animal receiving EHT during and immediately after treatment. The results of the last measurement at the end of the trials showed comparable PfHb levels of the EHT group to those of the NBO group (see Fig. 4). The original data can be found as Supplementary Table S3 and S4 online.

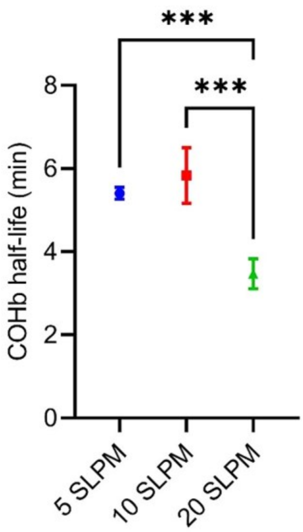

Fig. 1. COHb half-life dependent on gas flow rate of oxygen through the gas exchanger. SLPM = standard liter per minute. The data is presented as mean ± standard deviation. (*) $p \le 0.05$, (**) $p \le 0.01$, (***) $p \le 0.001$.

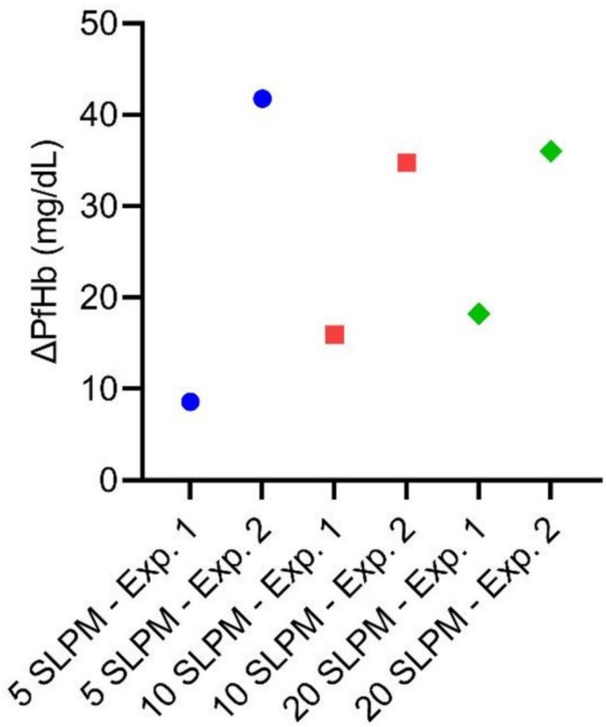

Fig. 2. Difference of plasma free hemoglobin from pre- to posttreatment in a single batch dependent on gas flow rate depicted as data from single experiments.

As shown in Fig. 5, the EHT resulted in periodic oscillations in mean pulmonary arterial pressure (PAP) that correlated in frequency with the withdrawal and return of the blood. The same effects shown here for PAP were also visible in the arterial and central venous pressure values (see Supplementary Fig. S1 and S2 online). In the first two animals treated with the EHT system, this phenomenon resulted in significantly increased PAP levels, which normalized only slowly towards the end of the experiment. The amplitude of the pressure oscillations decreased with each treated animal. In the PAP values of the third animal, only very small oscillations could be detected, and the pressure normalized quickly after the end of EHT.

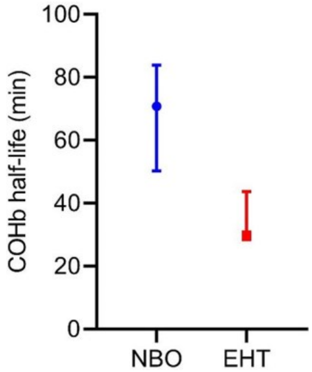

Fig. 3. COHb half-life for treatment with NBO and EHT system in CO-poisoned pigs ($N = 6$; 3 – NBO, 3 - EHT). The data is presented as median and range.

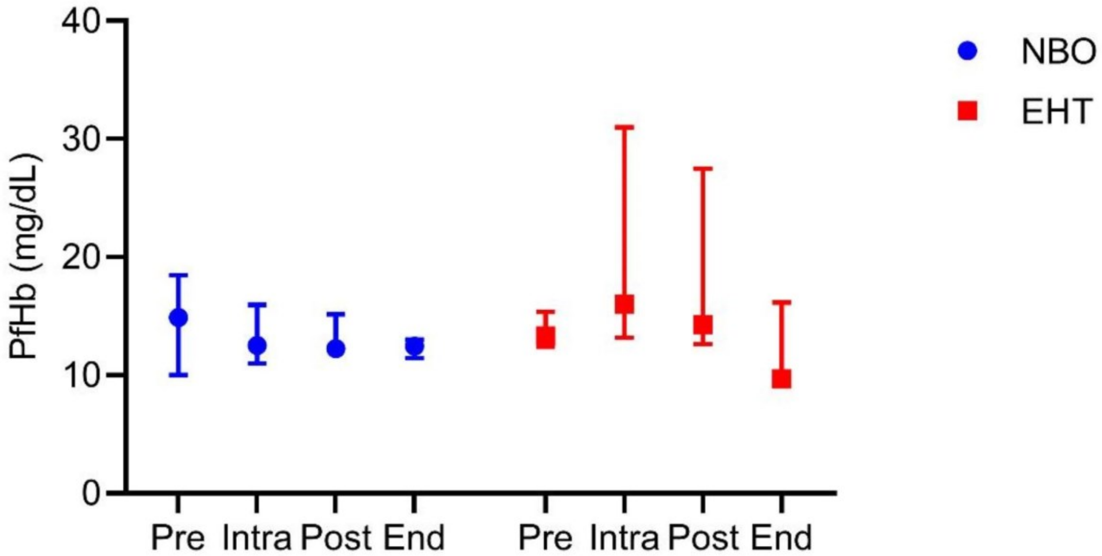

Fig. 4. Plasma free hemoglobin (PfHb) at different times during the in vivo experiments: "Pre" at start of recovery phase, "Intra" at 60 min of recovery phase, "Post" directly after recovery phase, and "End" at termination of animal trial (360 min after start of recovery phase). ($N = 6$; 3 – NBO, 3 - EHT). The data is presented as median and range.

Discussion

In this study, we present the prototype of a newly developed extracorporeal hyperoxygenation therapy system for the treatment of CO poisoning. The EHT system is designed to increase the dissolved oxygen concentration in the blood to accelerate the elimination of CO. This is accomplished in an extracorporeal device that treats one batch of blood at a time. In this batch, the dissolved oxygen concentration is greatly increased, facilitating the removal of CO. Subsequently, the treated blood is returned, and the process is repeated until the COHb value reaches physiological levels. The EHT system can be less expensive and more mobile than a pressure chamber, allowing for greater availability and accessibility. In previous in vitro experiments, we investigated the approach in a small-scale batch system[27]. Here, we describe the upscaled version of the system and a feasibility study. In the first part of the study, we characterized the CO elimination and blood damage of the EHT system in vitro. In the second part, we tested the EHT system in vivo in a large animal model imitating the clinical situation.

In general, the results of the in vitro experiments show that different gas flow rates influenced COHb half-life. The highest gas flow rate of 20 SLPM resulted in the shortest COHb half-life of 3.47 ± 0.36 min. This is most likely due to the continuously low concentration difference of CO between the blood and the gas phase. A higher gas flow rate removes the CO that diffuses into the gas phase more quickly so that a higher concentration difference between the blood and gas phases is maintained, compared to lower gas flow rates.

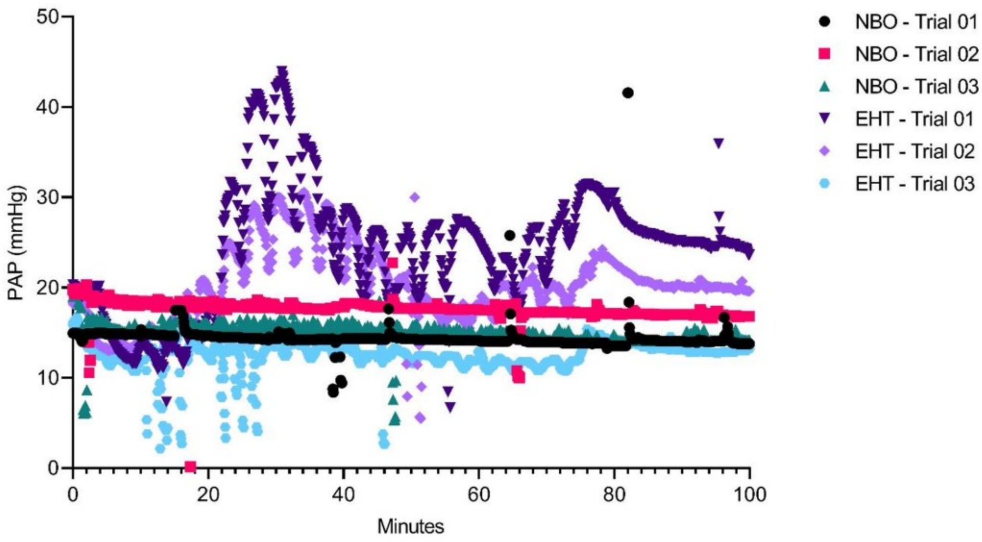

Fig. 5. Mean pulmonary artery pressures (PAP) during the first 100 min of the recovery phase of the control (NBO) and the test (EHT) group. The EHT group was treated for 80 min with the EHT system.

The non-significant difference in COHb half-life for the gas flow rates of 5 SLPM and 10 SLPM is likely caused by a plateau or inflection point in the correlation. Since the CO elimination rate is indirectly proportional to the residence time, it can be assumed that the COHb half-life will decrease again at lower gas flows, indicating an inflection point. A corresponding trend can be seen in the results, although the difference is not significant.

In a previous study, in which we tested the CO elimination of a small-scale EHT system, the shortest COHb half-life was 21.32 min in a blood volume of 50 mL at a pressure of 7 bar and a gas flow rate of 0.3 SLPM. In comparison, in this study, the shortest COHb half-life was 3.26 ± 0.11 min in a blood volume of 500 mL at a pressure of 7 bar and a gas flow rate of 20 SLPM. The increase in CO elimination may be attributed to two effects. First, in the upscaled EHT system, the gas flow rate, as well as the ratio of gas flow rate and blood volume, was higher, which resulted in more gas bubbles in the blood and hence a larger surface area for the diffusion of CO out of the blood. Second, in the previous study with the small-scale system, the antifoam agent was applied directly to the surface of the blood, limiting the formation of bubbles. In the upscaled EHT system, we applied the antifoam agent to the filter foam, which was placed at the top of the inner cylinder. This created an empty space between the blood surface and the foam, in which bubbles consisting of a thin layer of blood filled with oxygen were generated by the gas flow. These bubbles also increased the surface area for the elimination of CO, which could account for the increased CO elimination of the upscaled EHT.

The most similar approach to our EHT system is the “photo-ECMO” described by Fischbach et al.[26]. By harnessing light irradiation and increasing the sweep gas pressure to 1.33 atm (1.35 bar) they enhanced the CO elimination in an extracorporeal hollow-fiber membrane oxygenator. Employing four devices in parallel, yielded a COHb half-life of 5.2 ± 0.4 min, compared to a half-life of 3.26 ± 0.11 min at 7 bar with our EHT system.

The hemolysis measurements indicate that the hemolysis of the EHT system varies widely. Our results show the system’s capability to operate with minimal generation of PfHb in certain instances, while concurrently demonstrating higher PfHb values in other experiments. These variations could also be attributed to variations in blood handling procedures, including the filling and emptying of the gas exchanger, as well as the treatment of individual samples. Notably, we observed no discernible correlation between hemolysis and gas flow rates, so that hemolysis considerations did not impact the determination of gas flow rates for the subsequent in vivo experiments.

The in vitro experiments have certain limitations that need to be considered. The range of gas flows investigated in the experiments was limited, and the observed results indicate the existence of potential inflection and plateau points. This implies that more favorable operating points for CO elimination may exist at gas flow rates not explored in this study. Additionally, the manual blood handling introduced variability, particularly evident in the hemolysis results. Furthermore, the limited number of hemolysis experiments conducted and the potential variations, e.g., in blood properties and experimental days, necessitate cautious interpretation. However, the EHT system was not specifically optimized for minimal hemolysis, and the intention of these experiments was to demonstrate the feasibility of operating at acceptable rates of hemolysis.

An animal study was conducted to further validate the feasibility of the EHT system, simulating the dynamics of clinical applications. When comparing the COHb half-life of the NBO treatment of the control group to values reported in the literature, our results show a COHb half-life of 70.8 (50.31, 83.91) min compared to 74 min reported in the literature[6]. Despite differences due to biological variation inherent to in vivo experiments, the established intoxication protocol provided a reproducible model for evaluating different treatments.

For the in vivo experiments, we selected a gas flow rate of 10 SLPM as our operating point. This was because a flow rate of 20 SLPM, which resulted in the lowest COHb half-life, would have reduced the detoxification time but made the manual part of the handling difficult during the experiments. This could have led to errors and failed experiments. Additionally, when considering the time it takes for the first batch of treated blood to be returned to the animal, a gas flow rate of 10 SLPM was more favorable than 5 SLPM due to the shorter detoxification time. In the in vivo experiments, the animals were mechanically ventilated, which continuously eliminates CO. Hence, the animal's COHb continuously decreased while a batch of blood was being treated in the EHT system. Therefore, the following batch of blood drawn had a lower initial COHb level, resulting in a decrease in the amount of CO eliminated in the gas exchanger.

The in vivo performance of the EHT system resulted in a substantial reduction of the COHb half-life, indicating improved elimination compared to the control group. Importantly, CO elimination was twice as fast as with NBO alone, successfully translating the in vitro results to an in vivo setting. Fischbach et al.[26] also achieved improved CO elimination rates with their photo-ECMO in in vivo trials, resulting in a 36% reduction in COHb half-life. However, comparison of our results with the in vivo results of Fischbach et al. is limited by differences in animal size, CO poisoning protocol (repeated poisoning cycles in a single pig vs. only one poisoning cycle per pig), and differences in baseline COHb levels. A comparison with pharmacological approaches is difficult, as the underlying mechanisms are different. In addition, the pharmacological approaches may be used in combination with extracorporeal systems to further enhance CO elimination.

Hemolysis is an important concern in the clinical application of extracorporeal circulation, as e.g. in ECMO it is associated with increased rates of endothelial failure, renal failure, thrombotic events, transfusions, nonhemorrhagic stroke, pulmonary and systemic hypertension, decreased organ perfusion and mortality[29,30]. In extracorporeal circulation, hemolysis occurs due to unphysiological shear stress that damages red blood cells, leading to the release of free hemoglobin into the blood plasma. PfHb scavenges nitric oxide (NO), which is essential for maintaining endothelial function and vasodilation; thus, its depletion can result in vasoconstriction, platelet activation, and an increased risk of thrombosis[29–31]. Furthermore, hemolysis entails the formation of reactive oxygen species that damage cell membranes, lipids, proteins, and DNA, inducing systemic inflammatory response syndrome (SIRS), and thus lead to tissue and organ damage[31]. In our experiments, hemolysis levels were maintained below the critical threshold of 50 mg/dL[32] throughout the trials. In addition, hemolysis levels returned to normal after the EHT system was disconnected, indicating the system's ability to operate without causing sustained blood damage. However, considering the risks associated with hemolysis, future development of the EHT system should aim to optimize hemocompatibility.

The oscillations in hemodynamic pressures, shown in Fig. 5 for pulmonary artery pressure, raise significant concerns about the clinical application of the EHT system in its current state. These oscillations were most pronounced in the first trial and showed a decreasing trend in subsequent tests. Therefore, we attribute the observed oscillations to the manual control of the pump speed during simultaneous filling and emptying of the reactor. This manual control resulted in a certain difference in filling and emptying speed of the inner and outer cylinder that may have caused a variation in volume in the animals' circulation and thus the described oscillations. As the trials progressed, more experience was gained with the system, and the pumps were better synchronized, which explains the improvement between trials. Despite these improvements, small oscillations remained in the final trial, indicating the need for further optimization.

The nature of the EHT treatment includes dissolving large amounts of oxygen in the blood. As excess amounts of oxygen form bubbles during pressure release, there is a risk for introduction of these bubbles into the patient's circulation, causing gas embolisms. In arterial circulation, gas embolisms may lead to ischemic events, neurological deficits, and, in severe cases, circulatory failure, cardiac arrest, and coma[33]. To reduce the risk of gas embolisms, hypobaric oxygenators have been developed for CPB and ECMO. These hypobaric oxygenators use sub-atmospheric pressures on the gas side to decrease gas concentration in the blood before reinfusion into the patient's circulation[34]. Venous gas embolism is typically filtered by the lungs, but larger gas volumes can still cause cardiac arrhythmias, pulmonary hypertension, and right ventricular strain[33]. Due to the lower risk of venous gas embolisms, for dialysis machines Polaschegg and Levin[35] do not classify a continuous infusion of air below 0.03 mL/(kg*min) and the infusion of a bolus of 0.1 mL/kg as dangerous. The EHT system also returns the treated blood into the venous circulation. Additionally, the debubbling protocol to prevent gas bubbles from entering circulation was developed. However, further studies are needed to verify its safety across different application scenarios.

An important limitation of this study relates to the animal model used, which was configured to mimic the standard therapeutic setup for humans, with a COHb half-life of approximately 70 min. While this approach provides a basic framework, it does not fully capture the range of scenarios encountered in real-life situations. The inherent variability in the severity and duration of CO exposure in clinical cases may affect the generalizability of the findings beyond the specific conditions simulated in this study. In addition, the study involved only 3 animals per group. While this size was considered acceptable for establishing the feasibility of the EHT system, a larger sample size would have contributed to improved statistical robustness and generalizability of the results. The inherent biological variability in animal models introduces an additional layer of complexity, as individual animals may have different responses to CO poisoning and treatment. In addition, we did not measure recirculation of previously treated blood back into the system during simultaneous filling and emptying. Increased recirculation would have negatively impacted the performance of the EHT system. Although the use of small cannulae and low flow rates potentially minimized the likelihood of significant recirculation, this should be considered when interpreting the study results.

In conclusion, the animal study represents an important step in validating the feasibility of the EHT system for CO elimination. The in vivo experiments demonstrated a substantial reduction in the COHb half-life with EHT compared to NBO treatment alone. The hemolysis results are acceptable, while there are still opportunities for improvement, for example regarding design, choice of materials, and production processes. Nevertheless, other aspects of hemocompatibility, such as thrombogenicity and inflammatory response should also be investigated in the future. However, the oscillations in hemodynamic pressures present a significant challenge for clinical translation. While improvements have been observed with increasing operator experience and adjustments, further optimization is essential to eliminate the oscillations. These optimizations could include servocontrolled pumps to match the blood flows or the redesign of EHT towards a continuous system. Following the optimizations, the EHT system, with greater availability and accessibility than HBO, could provide a promising option for managing CO poisoning. EHT might also be combined with veno-arterial extracorporeal membrane oxygenation (VA ECMO) in severe cases of CO poisoning where cardiac function is compromised, and VA ECMO is used to support heart and lung function[36]. The EHT system could be added to VA ECMO similar to how CRRT is combined with ECMO[37,38], enhancing CO elimination while preserving hemodynamic stability. This combined approach could provide a safer, more feasible alternative to transporting ECMO-supported patients to hyperbaric oxygen chambers. Future studies could evaluate the practicality and efficacy of combining EHT with VA ECMO.

Methods

The system for extracorporeal hyperoxygenation therapy

The high-pressure gas exchanger (see Fig. 6) had two coaxial compartments. The outer compartment was defined by the outer hollow cylinder with a length of 280 mm, an outer diameter of 100 mm and a wall thickness of 5.3 mm. The outer cylinder was fastened and sealed between two caps made from polyoxymethylene. Coaxial to the outer cylinder, a second inner cylinder (length 250 mm, outer diameter 75 mm, wall thickness 1.8 mm) was mounted on the lower cap, defining the inner compartment. The inner cylinder was closed at the top with a plug, and just below were six rectangular openings. A polyurethane filter foam (30 PPI, Filteron GmbH, Solingen, Germany) coated with Simethicone USP (Dow Corning® Q7-2243 LVA, DuPont de Nemours, Inc., Wilmington, Delaware, USA) was wrapped around these openings on the outside of the cylinder. The bottom cap was equipped with a blood inlet and outlet, as well as a gas inlet. Above the gas inlet, a perforated silicone disc (OXYFLEX MT 300, Supratec Gesellschaft für Umwelt- und Verfahrenstechnik mbH, Simmern, Germany (cut to size)) was installed that dispersed the gas into the blood. The gas and blood inlets were connected to the inner compartment, and the blood outlet was connected to the outer compartment. The top cap was equipped with a gas oulet, a gas connector for pressure build-up and a gas connector for the debubbling line, which was a perforated Heidelberger line (B. Braun SE, Melsungen, Germany) that extended into the blood in the outer compartment. The gas section consisted of a pressure reducer connected to an oxygen bottle, two three-way valves, a gas flow controller, and a needle valve (see Fig. 7).

In the high-pressure gas exchanger, one batch at a time was treated according to the following procedure: 500 mL of blood was pumped through the blood inlet into the inner compartment, after which the inlet was clamped off via two-way valves and the pressure inside the gas exchanger was quickly increased by 6 bar by introducing oxygen into the system via the pressure build-up line. Subsequently, a gas flow (100% oxygen) was supplied via the gas inlet. The gas flow was dispersed by the silicone disc into the blood, creating bubbles in the blood in the inner compartment. The resulting dispersion rose inside of the inner cylinder until it was diverged by the plug through the openings. Once the dispersion contacted the filter foam outside of the openings, gas and blood were separated: the blood was collected in the outer compartment, while the gas escaped through the gas outlet. After the blood was carried from the inner to the outer compartment, the pressure inside the gas exchanger was released over 60 s. During the release of the pressure, 4 short (0.25 s) bursts of gas were passed through the debubbling line and dispersed into the blood to force nucleation and consequent release of excess dissolved oxygen. Once the gas exchanger had reached ambient pressure, a waiting period of 30 s succeeded to ensure the complete release of excess oxygen from the blood in the outer chamber. The debubbling procedure was optimized, until no bubbles were detectable with an ultrasonic bubble counter (BC100, GAMPT mbH, Merseburg, Germany).

To allow an automatic treatment of multiple batches successively without causing a shift in the patients' circulatory volume each time, the high-pressure gas exchanger was expanded by a blood loop (see Fig. 7), controlled by a custom-made controller. The blood loop consisted of a 13 Fr double-lumen cannula inserted into a femoral vein (Achim Schulz-Lauterbach Vertrieb medizinischer Produkte GmbH, Iserlohn, Germany), a heat exchanger (ECMOtherm-II, Medtronic plc, Dublin, Ireland), two peristaltic blood pumps (15QQ, Boxer GmbH, Ottobeuren, Germany), an arterial filter (Baby Sherlock, EUROSETS S.r.l., Medolla, Italy), and four pinch valves. Two of the pinch valves (numbers 1 and 2) were regular pinch valves (ASCO S170XA01 × 2900VU, Emerson, Saint Louis, Missouri, USA), while the other two (numbers 3 and 4) were designed for high-pressure resistance (ASCO S206.05-Z130A-24VDC, Emerson, Saint Louis, Missouri, USA). The valves could be switched between two states: detoxification and simultaneous filling and emptying. During detoxification, valves 3 and 4 were closed to separate the high pressure in the gas exchanger from the blood loop. Valves 1 and 2 were open, and the blood low rate of both pumps was combined to provide the total blood flow rate of 300 mL/min, circulating the blood through the loop and the cannula. During simultaneous filling and emptying, valves 1 and 2 were closed to stop the blood circulation through the loop. Valves 3 and 4 were open, and the rotational speed

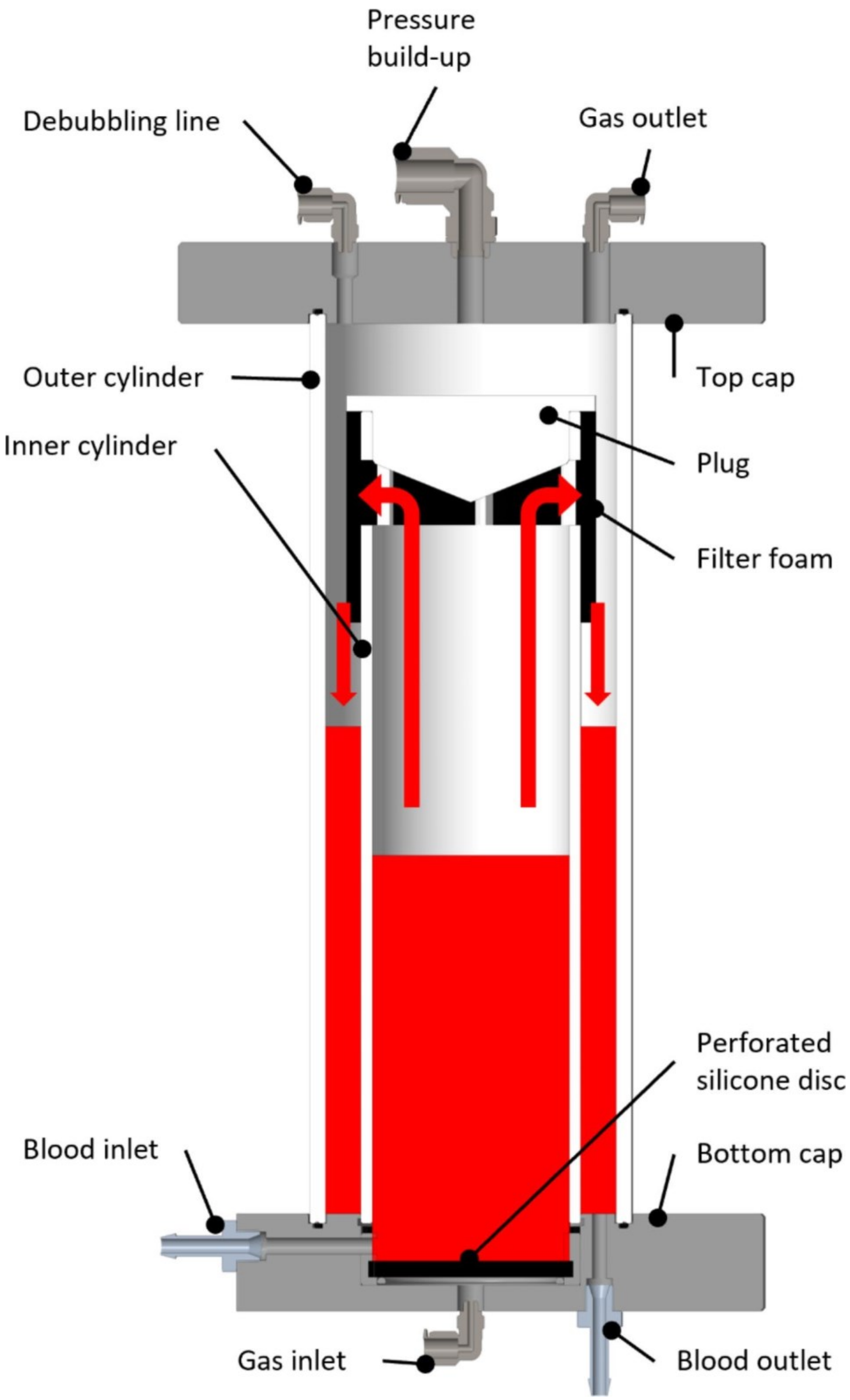

Fig. 6. Detailed sectional view of the high-pressure gas exchanger. During operation, the blood is carried from the inner compartment along the red arrows into the outer compartment by oxygen bubbles that are introduced via the gas inlet.

of each pump was set individually to achieve the total blood flow rate each. Hence, the gas exchanger was filled and emptied simultaneously, while the total blood flow rate through the cannula remained constant. Once the inner compartment was filled and the outer compartment was empty, the simultaneous filling and emptying was stopped manually, and the system switched to detoxification.

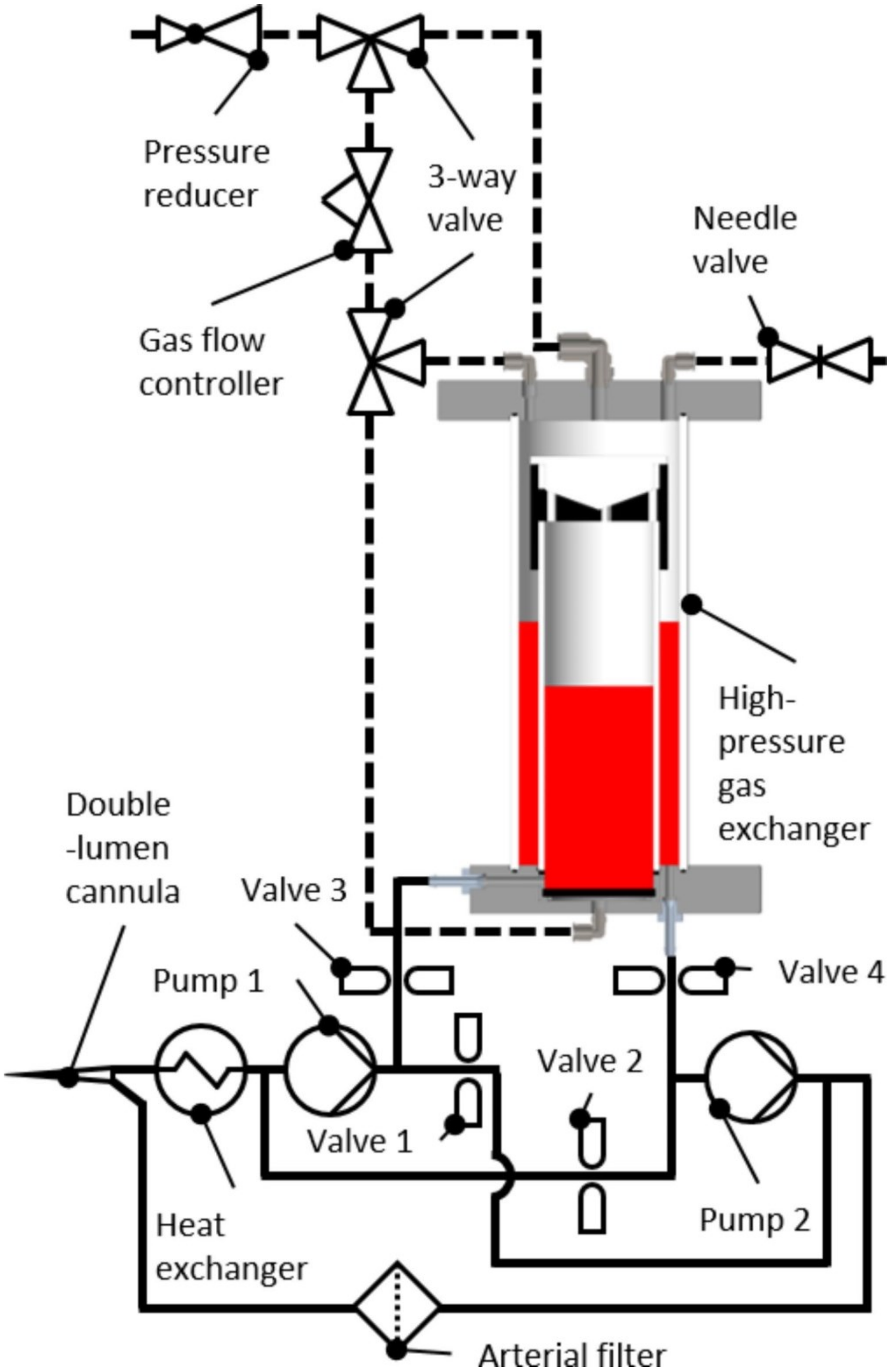

Fig. 7. Schematic of the setup used in the in vivo experiments. The components were connected to a control system, allowing multiple batches to run automatically in sequence.

In vitro experiments

On the day of the experiment, pooled porcine blood was obtained from the slaughterhouse and fully heparinized. We used a CPB machine (HL-20, Maquet, Rastatt, Germany) to pump the blood through a circulation loop consisting of a cardiotomy reservoir and an oxygenator (hilite 7000, Xenios AG, Heilbronn, Germany). The blood was conditioned according to ISO 7199[39], and afterwards, sweep gas containing 3% CO and 97% N_2 (Linde AG, Pullach, Germany) was used to poison the blood to a target value of 40 − 45% COHb.

Gas flow rate (SLPM)	Residence time (s)	Number of replicates (–)
5	240	6
10	120	11
20	60	6

Table 1. Gas flow rate during the experiments and resulting residence times.

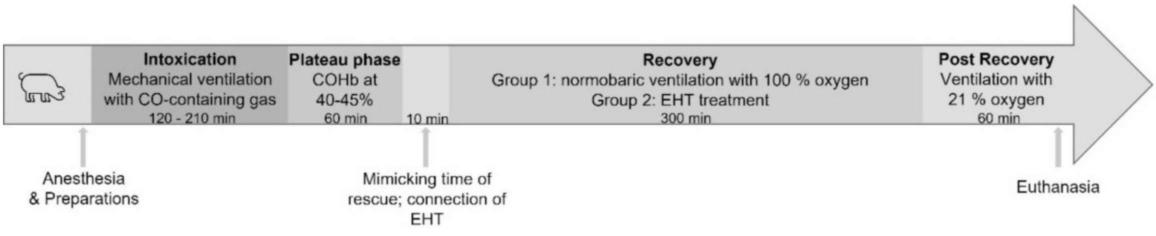

Fig. 8. Experimental procedure for the in vivo experiments.

For the in vitro experiments, the high-pressure gas exchanger was operated without the blood loop. For every batch, we used freshly poisoned blood. After every batch, we collected the blood through the blood outlet and measured the COHb. During the experiment, the whole gas exchanger was placed in a water bath at 37°C to ensure a constant temperature. We varied the gas flow rate in the experiments to investigate the effect on detoxification. The variation in gas flow rates also resulted in different residence times because with lower gas flow rates, the blood rises more slowly in the inner cylinder and vice versa. The blood flow rates, resulting residence times, and number of replicates are shown in Table 1. All other parameters were kept constant. To measure hemolysis, we performed two experiments for each gas flow and analyzed the blood before and after. Each experiment was performed with fresh porcine blood.

In vivo experiments

Following the in vitro experiments, we verified the feasibility of the EHT for CO poisoning in living organisms via in vivo experiments with single pigs. All experiments were performed in accordance with relevant guidelines and regulations, followed the principles of laboratory animal care and the authors complied with the ARRIVE guidelines. The experiments and the experimental protocol were approved by the governmental animal care committee (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, Recklinghausen, Germany). The experiments were performed on six female pigs of the German landrace with a weight of 76.9 ± 5.27 kg. Three pigs served as a reference to measure the elimination of CO under physiological conditions with normobaric high oxygen ventilation. The other three pigs in the test group received EHT for the treatment of CO poisoning. The animals arrived two weeks before the experiments for acclimatization and were examined by a veterinarian. 12 h before the start of the experiment, the animals were kept sober with free access to water. For premedication, they received 1 mL Atropin 1%, 4 mg/kg Azaperon i.m. and 10 mg/kg Ketamin i.m. Afterwards, intravenous access was placed in an ear vein, the animals were orally intubated, and anesthesia was maintained with 5 – 10 mg/kg propofol and 8 – 20 µg/kg fentanyl. Additionally, they received 1 ml/kg/h crystalloid fluids, and a urinary catheter was placed. The mechanical ventilation was set to a target $paCO_2$ of 35 – 45 mmHg with a PEEP of 5 mbar, a respiratory rate of 10 – 18/min and a tidal volume of 6 – 10 ml/kg. An arterial catheter was placed into one femoral artery to continuously measure the arterial blood pressure and take blood samples, and a high-flow 13 Fr double lumen Shaldon catheter and a 4-lumen central vein catheter were placed into the femoral veins to connect the EHT system and to have central access to inject medication. Additionally, a pulmonary artery catheter was placed in the right internal jugular vein to continuously measure the pulmonary artery pressure and discontinuously measure the pulmonary capillary wedge pressure, and the cardiac output, as well as to take blood samples. Heparin was injected continuously with a target activated clotting time (ACT) of > 150 s; if necessary, boli were given to reach the target ACT.

The experimental protocol included five phases: intoxication, plateau, rescue, recovery and post recovery (see Fig. 8). For intoxication, we connected a gas cylinder containing 940 ppm CO, 20% O_2 and the rest nitrogen to the ventilator. FiO_2 was set to 0.2 – 0.21. Blood samples for blood gas analysis were taken every 15 min to control the progress of intoxication, until the target COHb of 40 – 45% was reached. During the following plateau phase, the COHb was kept at 40 – 45% for 60 min by mixing air with the CO-containing gas and monitoring the COHb values via blood gas analysis every 10 min. Subsequently, the animals of both groups were ventilated with air (FiO_2 0.21) for 10 min, mimicking the rescue period of CO poisoned patients. During this phase, a bolus of 25,000 IU of heparin was administered, and the EHT system was connected to the animals of the test group via the Shaldon catheter. Before the connection to the cannula, the tubing and the outer compartment were primed with 0.9% saline solution to account for the additional extracorporeal volume of the EHT system. During the recovery phase, the control group received high oxygen ventilation (FiO_2 1.0) for 300 min, whereas

Sequence	Duration (s)
Filling and emptying	100
Detoxification	90
Pressure release	60
Waiting	30

Table 2. Different sequences and the respective durations for the in vivo experiments.

in the test group, the elimination of CO was enhanced by the EHT. The target blood flow rate was 300 mL/min, resulting in a time of 100 s for simultaneous filling and emptying. The gas flow during detoxification was set to 10 SLPM. The different sequences and the respective durations for the in vivo experiments are summarized in Table 2. The EHT was continued for 75 min or until a target COHb of 5% was reached. Afterwards, the animals were ventilated with an FiO_2 of 1.0 until the end of the recovery phase. Blood samples were taken every 15 min. Another period of 60 min was established as the post recovery phase to verify the lasting reduction of CO in the animals' blood. During this period, the animals were ventilated with air. Blood samples for measuring COHb were taken every 15 min. Blood samples for measuring plasma free hemoglobin were taken every 60 min. Vital data was logged at a frequency of 0.2 Hz (temperature, heart rate, arterial pressure, central venous pressure, pulmonary artery pressure, central venous oxygen saturation) or recorded manually every 15 min (breathing rate, respiratory minute volume, inspiratory oxygen fraction, fractional end-tidal oxygen concentration, cardiac output, medication, pulmonary capillary wedge pressure).

Analyses and statistics

The main parameter of interest was the fraction of COHb in percent. It was measured using a blood gas analyzer (ABL800 Flex, Radiometer Medical ApS, Brønshøj, Denmark). Hemolysis was determined by photometric measurement of the plasma free hemoglobin (Ultrospec 2100 Pro, Biochrom, Berlin, Germany). The analysis of hemolysis was performed according to DIN 58931[40] by means of the cyanmethemoglobin method (Hemoglobin FS, DiaSys, Germany) according to the manufacturers' instructions. For this, the plasma of each blood sample was separated from the cells by double centrifugation at 1,500×g for 15 min. The vital signs in the in vivo experiments were recorded at a frequency of 0.2 Hz with a custom-made data logger.

The COHb half-life was calculated using a nonlinear regression with a one phase exponential decay model and the assumption that the plateau equals 0% COHb. Subsequently, for the results of the in vitro experiments, we performed a one-way ANOVA followed by Tukey's multiple comparison test. Normality was confirmed by Shapiro-Wilk test. Values are presented as mean ± standard deviation. For the results of the in vivo experiments, the variables are presented as median (range). All statistical analyses were performed with GraphPad Prism 9.

Data availability

The datasets generated and/or analyzed during the current study are available in this published article and its supplementary information files and from the corresponding author on reasonable request.

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Conceptualization: N.B.S., P.C.S., M.F.M., C.H., T.S.-R., S.V.J., U.S., and R.K. Formal analysis: N.B.S., H.L., P.C.S., and M.F.M. Investigation: N.B.S., H.L., P.C.S., M.F.M., C.H., C.L., and R.K. Methodology: N.B.S., P.C.S., M.F.M., C.H., and R.K. Supervision: T.S.-R., S.V.J., U.S., and R.K. Visualization: N.B.S., H.L., and M.F.M. Writing—original draft: N.B.S. Writing—review and editing: H.L., P.C.S., M.F.M., C.H., C.L., T.S.-R., S.V.J., U.S., and R.K.

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Declarations Competing interests

RWTH Aachen University has filed a patent application entitled "System for the extracorporeal elimination of carbon monoxide" (WO2017153034A1), the inventors are P.C.S., T.S.-R., and U.S.. N.B.S., P.C.S., and M.F.M. are co-founders of a company to commercialize the technology described in this manuscript. The other authors declare that they have no conflict of interest.

Additional information

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Supplementary Information

for

Extracorporeal hyperoxygenation therapy (EHT) for CO poisoning: in vitro and in vivo feasibility of a full-scale batch system

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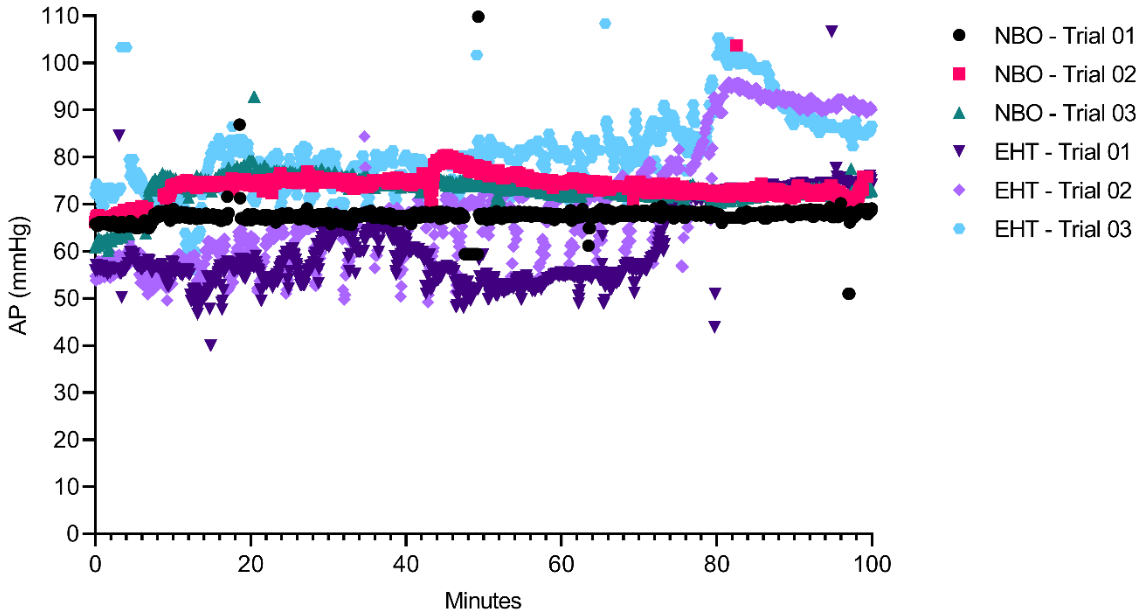
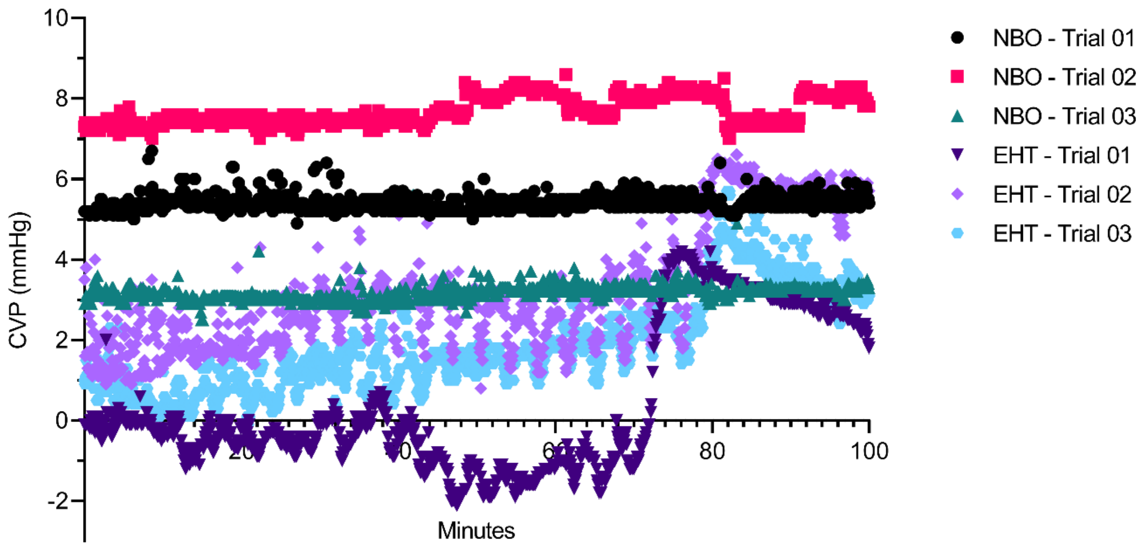


Supplementary Figure S1: Mean arterial pressures (AP) during the first 100 minutes of the recovery phase of the control (NBO) and the test (EHT) group. The EHT group was treated for 80 minutes with the EHT system.



Supplementary Figure S2: Mean central venous pressures (CVP) during the first 100 minutes of the recovery phase of the control (NBO) and the test (EHT) group. The EHT group was treated for 80 minutes with the EHT system.

Supplementary Table S1: Carboxyhemoglobin (COHb) before and after the treatment in the EHT system in vitro with 3 different gas flow rates. Each gas flow rate resulted in the indicated treatment time of the batch.

Gas flow rate		Time (min)	COHb (%) n=1	n=2	n=3	n=4	n=5	n=6	n=7	n=8	n=9	n=10	n=11
5 SLPM	pre	0	43,8	43,7	43,8	42,8	42,8	42,9	-	-	-	-	-
	post	4	26,3	25,6	26	26,1	25,7	25,8	-	-	-	-	-
10 SLPM	pre	0	42,3	42,3	42,3	40,9	40,9	43,7	43,7	43,8	42,9	42,9	42,8
	post	2	34	33,7	32,8	32	31,2	35,9	35,3	34,2	34,4	32,8	32,3
20 SLPM	pre	0	43,8	43,8	43,8	41,6	41,6	41,6	-	-	-	-	-
	post	1	34,8	34,9	35,9	34,7	34,6	34,5	-	-	-	-	-

Supplementary Table S2: Plasma free hemoglobin (PfHb) before (Pre) and after (Post) the treatment in the EHT system in vitro with 3 different gas flow rates.

	PfHb (mg/dL)					
Gas flow rate	5 NLPM		10 NLPM		20 NLPM	
Pre	55,84	65,945	51,48	67,755	55,84	82,68
Post	64,425	107,7	67,4	102,55	74,075	118,7

Supplementary Table S3: Arterial carboxyhemoglobin (COHb) of the control (NBO) and the test (EHT) group during the recovery phase of the in vivo experiments. The values marked with an asterisk were not included in the calculation of the carboxyhemoglobin half-life of the EHT system, because the EHT system was stopped after 80 min.

	Arterial COHb (%)					
Time in recovery phase	NBO			EHT		
0:00:00	35,4	42,5	41,1	42	42,3	39,8
0:15:00	30,2	35	31	31,1	29,3	29
0:30:00	26,2	31	25,4	24,9	21,7	21,5
0:45:00	23,3	27,3	21,6	20,9	12,9	13
1:00:00	20,4	24,6	14,7	15,3	9,4	9,5
1:15:00	15,1	22,5	12,5	13,5	7,5	6,9
1:30:00	12,9	20,2	10,6	12,4*	5,7*	5,4*
1:45:00	11,4	15,3	9,2	10,8*	4,3*	4,1*
2:00:00	10	13,8	7,8	10,2*	3,5*	3,2*
2:15:00	9,3	12,3	6,7	8,8*	2,5*	2,7*
2:30:00	8,1	11,2	5,9	7,5*	2,5*	1,8*
2:45:00	7,3	10,2	5	6,7*	1,7*	1,3*
3:00:00	6,4	9,2	4,3	6*	1,6*	1*
3:15:00	5,6	8,4	3,9	5,5*	1,3*	0,5*
3:30:00	5	7,7	3,2	4,8*	0,9*	0,4*
3:45:00	4,6	6,9	2,7	4,3*	0,8*	0,1*
4:00:00	4,1	6,5	2,5	3,9*	0,3*	-0,1*
4:15:00	3,5	5,7	2	3*	0*	-0,3*
4:30:00	3,1	5,4	1,6	2,8*	-0,1*	3*
4:45:00	2,9	5	1,5	2,4*	0,1*	2,9*
5:00:00	2,6	4,4	1,1	2,5*	-0,3*	2,7*

Supplementary Table S4: Plasma free hemoglobin (PfHb) of the control (NBO) and the test (EHT) group at different times during the in vivo experiments: "Pre" at start of recovery phase, "Intra" at 60 minutes of recovery phase, "Post" directly after recovery phase, and "End" at termination of animal trial (360 minutes after start of recovery phase).

	PfHb (mg/dL)					
		NBO			EHT	
Pre	18,45	14,885	10,026	15,365	13,31	12,425
Intra	12,515	15,96	10,97	30,98	13,185	16,02
Post	12,245	15,145	11,59	27,47	12,63	14,27
End	12,45	13,015	11,465	16,15	9,444	9,669

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Erklärung § 5 Abs. 1 zur Datenaufbewahrung

Hiermit erkläre ich, dass die dieser Dissertation zu Grunde liegenden Originaldaten im Institut für Angewandte Medizintechnik, Lehr- und Forschungsgebiet Kardiovaskuläre Technik des Universitätsklinikums Aachen hinterlegt sind.

Erklärung gemäß § 5 Abs. (1) und (2), und § 11 Abs. (3) 12. der Promotionsordnung

Hiermit erkläre ich, **Niklas Balduin Steuer,** an Eides statt, dass ich den wesentlichen Anteil an der Publikation:
Niklas B. Steuer, Peter C. Schlanstein, Anke Hannig, Stephan Sibirtsev, Andreas Jupke, Thomas Schmitz-Rode, Rüdger Kopp, Ulrich Steinseifer, Georg Wagner and Jutta Arens**:** Extracorporeal Hyperoxygenation Therapy (EHT) for Carbon Monoxide Poisoning: In-Vitro Proof of Principle; *Membranes*; 2022; 12(1):56 geleistet habe.
Die Anteile an der Arbeit waren wie folgt:

	Niklas B. Steuer	Peter C. Schlanstein	Anke Hannig	Stephan Sibirtsev	Andreas Jupke	Thomas Schmitz-Rode	Rüdger Kopp	Ulrich Steinseifer	Georg Wagner	Jutta Arens	Summe (%)
Studienüberwachung					20	20	20	20		20	100
Studiendesign/Konzeption	60	15				5	5	5	5	5	100
Visualisierung	85		15								100
Durchführung der Experimente	70	15	10	5							100
Entwicklung der Methodik	65	15	10	5	5						100
Datenanalyse und Interpretation	75	15	5	5							100
Verfassung des Manuskripts	100										100
Korrektur des Manuskripts		20	10	10	10	10	10	10	10	10	100

Aus diesem wesentlichen Anteil ergibt sich selbstverständlich die Stellung als Erstautor.

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Als Doktorvater bestätige ich die Angaben von Niklas Balduin Steuer

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Niklas B. Steuer, Hannah Lüken, Peter C. Schlanstein, Matthias F. Menne, Christiane Hoffmann, Cavan Lübke, Thomas Schmitz-Rode, Sebastian Victor Jansen, Ulrich Steinseifer and Rüdger Kopp**:** Extracorporeal hyperoxygenation therapy (EHT) for CO poisoning: in vitro and in vivo feasibility of a full-scale batch system; *Scientific Reports*; 2025; 15, 4066 geleistet habe.
Die Anteile an der Arbeit waren wie folgt:

	Niklas B. Steuer	Hannah Lüken	Peter C. Schlanstein	Matthias F. Menne	Christiane Hoffmann	Cavan Lübke	Thomas Schmitz-Rode	Sebastian Victor Jansen	Ulrich Steinseifer	Rüdger Kopp	Summe (%)
Studienüberwachung							20	20	20	40	100
Studiendesign/Konzeption	60		10	5	5		5	5	5	5	100
Visualisierung	80	15		5							100
Durchführung der Experimente	60	5	10	10	10	5					100
Entwicklung der Methodik	65		10	10	5					10	100
Datenanalyse und Interpretation	65	10	15	10							100
Verfassung des Manuskripts	100										100
Korrektur des Manuskripts		20	10	10	10	10	10	10	10	10	100

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