



BASIC SCIENCE

Veno-venous extracorporeal blood phototherapy increases the rate of carbon monoxide (CO) elimination in CO-poisoned pigs

Anna Fischbach¹  | Steffen B. Wiegand^{1,2} | Luca Zazzeron¹ | Lisa Traeger¹ |
 Raffaele di Fenza¹ | Aranya Bagchi¹ | William A. Farinelli³ | Walfre Franco⁴  |
 Sandeep Korupolu³ | Jutta Arens⁵ | Luigi Grassi¹ | Francesco Zadek¹ |
 Donald B. Bloch^{1,6} | R. Rox Anderson³ | Warren M. Zapol¹

¹Anesthesia Center for Critical Care Research of the Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA

²Department of Anesthesiology, University Hospital, LMU Munich, Munich, Germany

³Department of Dermatology, Massachusetts General Hospital and Harvard Medical School, Wellman Center for Photomedicine, Boston, Massachusetts, USA

⁴Department of Biomedical Engineering, University of Massachusetts, Lowell, Massachusetts, USA

⁵Department of Biomechanical Engineering, Faculty of Engineering Technology, University of Twente, Twente, The Netherlands

⁶Division of Rheumatology, Allergy, and Immunology, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA

Correspondence

Warren M. Zapol, Anesthesia Center for Critical Care Research of the Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.
 Email: wzapol@mgh.harvard.edu

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Abstract

Background and objectives: Carbon monoxide (CO) inhalation is the leading cause of poison-related deaths in the United States. CO binds to hemoglobin (Hb), displaces oxygen, and reduces oxygen delivery to tissues. The optimal treatment for CO poisoning in patients with normal lung function is the administration of hyperbaric oxygen (HBO). However, hyperbaric chambers are only available in medical centers with specialized equipment, resulting in delayed therapy. Visible light dissociates CO from Hb with minimal effect on oxygen binding. In a previous study, we combined a membrane oxygenator with phototherapy at 623 nm to produce a “mini” photo-ECMO (extracorporeal membrane oxygenation) device, which improved CO elimination and survival in CO-poisoned rats. The objective of this study was to develop a larger photo-ECMO device (“maxi” photo-ECMO) and to test its ability to remove CO from a porcine model of CO poisoning.

Study design/materials and methods: The “maxi” photo-ECMO device and the photo-ECMO system (six maxi photo-ECMO devices assembled in parallel), were tested in an in vitro circuit of CO poisoning. To assess the ability of the photo-ECMO device and the photo-ECMO system to remove CO from CO-poisoned blood in vitro, the half-life of COHb ($\text{COHb-}t_{1/2}$), as well as the percent COHb reduction in a single blood pass through the device, were assessed. In the in vivo studies, we assessed the $\text{COHb-}t_{1/2}$ in a CO-poisoned pig under three conditions: (1) While the pig breathed 100% oxygen through the endotracheal tube; (2) while the pig was connected to the photo-ECMO system with no light exposure; and (3) while the pig was connected to the photo-ECMO system, which was exposed to red light.

Results: The photo-ECMO device was able to fully oxygenate the blood after a single pass through the device. Compared to ventilation with 100% oxygen alone, illumination with red light together with 100% oxygen was twice as efficient in removing CO from blood. Changes in gas flow rates did not alter CO elimination in one pass through the device. Increases in irradiance up to 214 mW/cm^2 were associated with an increased rate of CO elimination. The photo-ECMO device was effective over a range of blood flow rates and with higher blood flow rates, more CO was eliminated. A photo-ECMO system composed of six photo-ECMO devices removed CO faster from CO-poisoned blood than a single photo-ECMO device. In a CO-poisoned pig, the photo-ECMO system increased the rate of CO elimination without significantly increasing the animal's body temperature or causing hemodynamic instability.

Conclusion: In this study, we developed a photo-ECMO system and demonstrated its ability to remove CO from CO-poisoned 45-kg pigs. Technical modifications of the photo-ECMO system, including the development of a compact, portable device, will permit treatment of patients with CO poisoning at the scene of their poisoning, during transit to a local emergency room, and in hospitals that lack HBO facilities.

KEYWORDS

blood phototherapy, carbon monoxide, carbon monoxide poisoning, extracorporeal membrane oxygenation, photodissociation

INTRODUCTION

Carbon monoxide (CO) inhalation and poisoning leads to more than 50,000 visits to emergency departments in the United States each year¹ and is the main cause of poisoning-related deaths.² The mortality rate from CO poisoning is 1%–3%,^{3,4} and the rate of morbidity is nearly 40%.⁵ Patients who survive CO poisoning often suffer from neurologic symptoms, including cognitive dysfunction, motor deficits, depression, and anxiety.⁶ Cardiac sequelae after CO poisoning include myocardial infarction,⁷ reduced left ventricular function,⁸ and arrhythmias.⁹ The neurologic and cardiac sequelae of CO poisoning increase morbidity and mortality.¹⁰ The economic burden of CO poisoning in the United States, if one considers both hospital costs and lost earnings, is approximately 1.3 billion dollars each year.¹¹

The toxicity associated with inhalation of CO is caused by impairment of oxygen (O₂) delivery to and inhibition of ATP generation in mitochondria.¹² Hemoglobin (Hb) has a 250-fold higher affinity for CO than for oxygen.¹³ Carboxyhemoglobin (COHb) reduces the oxygen-carrying capacity of blood, resulting in tissue hypoxia.¹⁴ The main goal of treatment for CO poisoning is, therefore, to remove CO from the body as quickly as possible. The efficacy of CO removal can be measured in terms of the half-life of COHb (COHb-*t*_{1/2}), which represents the time needed to remove 50% of the COHb from blood. The standard treatment for CO poisoning is to breathe either normobaric or hyperbaric 100% O₂.^{15,16} By increasing the partial pressure of O₂, CO is displaced from Hb and exhaled through the lungs. When a patient with CO poisoning breathes air, the COHb-*t*_{1/2} is 4–5 h.¹⁷ Breathing 100% O₂ reduces COHb-*t*_{1/2} to 1.5–2 h.¹⁸ Hyperbaric oxygen (HBO) can further reduce COHb-*t*_{1/2} to 30 min.¹⁹ However, oxygen therapy for the treatment of CO poisoning has important limitations. Because HBO chambers are only available in medical centers with specialized equipment, this therapy is delayed by the time needed for patient transport. In addition, when CO poisoning is associated with lung injury, which can be produced by inhalation of smoke or other chemicals,^{20,21} oxygen therapy to remove CO from the lungs becomes less effective due to limited gas exchange.

Haldane and Smith²² reported in 1896 that visible light dissociates CO from COHb without altering the ability of Hb to bind O₂. In a recent study, we reported the development of an extracorporeal veno-venous membrane oxygenator with optimized characteristics to facilitate exposure of blood to visible light.²³ We tested this “mini” photo-ECMO (extracorporeal membrane oxygenation) device in a rat model of CO poisoning, with and without concomitant lung injury, and observed that the device significantly increased the rate of CO removal from blood and improved the survival rate of CO-poisoned rats. However, a larger (“maxi” photo-ECMO) device is required before photo-ECMO can be considered a potential option for treating CO poisoning in humans. In this study, we report the development and characterization of a maxi photo-ECMO device and show that the device can efficiently remove CO from CO-poisoned blood *in vitro*. We further demonstrate that six maxi photo-ECMO devices, combined within a single “photo-ECMO system,” increases the rate of CO elimination in a porcine model of CO poisoning.

MATERIALS AND METHODS

Study design

The aim of this study was to assess the feasibility and efficacy of extracorporeal blood phototherapy in a large animal model of CO poisoning. We developed a membrane oxygenator suitable for blood phototherapy in a large animal and combined six of these devices to a photo-ECMO system. We tested the device and the photo-ECMO system in an *in vitro* circuit of CO poisoning. We studied the effect of extracorporeal blood phototherapy in a nonlethal model of CO poisoning in 45-kg pigs. All *in vitro* experiments were at least repeated four times. All animal studies were performed using protocols approved by the Institutional Animal Care and Use Committee at our institution and in accordance with National Institutes of Health guidelines. The sample size was decided on the basis of pilot experiments to ensure a power of 80% and a significance of 5%. The investigators were not blinded to experiments. All data were included (no outlier values were excluded).

Construction of a “maxi” photo-ECMO device

To construct a “maxi” photo-ECMO device, 15 polypropylene hollow fiber membrane mats, each 160 mm by 160 mm, with a fiber density of 20 fibers/10 mm (Oxyphan 50/280; 3M), were placed on top of each other, with the hollow fibers of each layer rotated 90° relative to the layer below to maximize gas exchange area. Silicone rubber adhesive (Elastosil RT625; Wacker) was used to hold the membrane bundle together and the membranes were enclosed in a clear plexiglass case with four openings for blood and gas inlets and outlets. The device consists of three sealed compartments: One compartment with blood flowing around the membrane tubules and two individual compartments (separated by a silicone barrier) for gas inflow and outflow (Figure 1A and S1).

Assembling the photo-ECMO system

The photo-ECMO system consists of six “maxi” photo-ECMO devices arranged in parallel (Figures 6A and S4). A blood inflow port with six openings for blood entering the photo-ECMO system and a blood outflow port with six openings for blood exiting the photo-ECMO system was built to ensure equal blood flow distribution to all six

photo-ECMO devices. The blood in- and outflow branches consist of seven Y-pieces connected to silicone tubing. Compared to a single photo-ECMO device, the gas exchange and total surface area for incident light increased from 0.6 to 3.6 m² and from 450 to 2700 cm², respectively. The blood compartment volume increased from 60 to 360 ml. We used 240 LEDs in the photo-ECMO system with a total light power of 578 Watts.

In vitro assessment of COHb- $t_{1/2}$ using the photo-ECMO device and the photo-ECMO system

The first in vitro circuit consisted of one open reservoir, a peristaltic pump (NE-9004; New Era Pump Systems Inc.), silicone rubber tubing, the maxi photo-ECMO device, or the photo-ECMO system (Figure 1B). 300 ml (for preliminary testing) or 1 L of human whole blood from different, healthy volunteers (Research Blood Components), at a Hb concentration of 9.6 ± 0.3 g/dl (with 300 ml of blood) and 10.0 ± 1.4 g/dl (with 1 L of blood), anticoagulated with CPDA-1, was saturated with CO by circulating the blood through the device while it was ventilated with 2% CO diluted in air. A Level 1 hotline (Smiths Medical) was used to maintain the blood

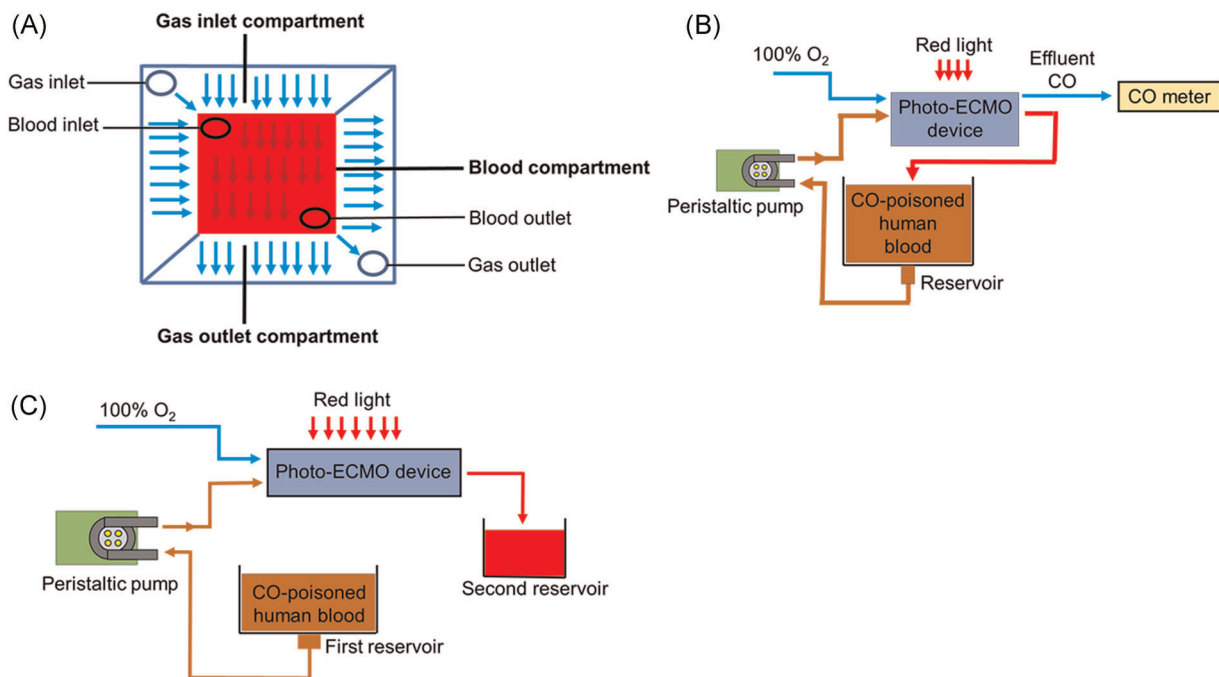


FIGURE 1 Depiction of the photo-ECMO device and in vitro models to test the ability of the photo-ECMO device to eliminate CO from blood. (A) Design of the photo-ECMO device. The photo-ECMO device consists of a central blood compartment and two peripheral gas compartments (gas inlet and outlet). Oxygen entered the gas inlet compartment to pass *through* the hollow membrane fibers to reach the gas outlet compartment. Meanwhile, CO-poisoned blood crossed the blood compartment and flows *around* the membrane fibers, while being illuminated by red light at 623 nm from both sides of the device. (B) Human whole blood circulated from a reservoir through a peristaltic pump to the photo-ECMO device and returned to the reservoir (assessment of COHb half-life). The COHb half-life is the most relevant, clinical parameter as it provides information on the time needed to remove CO from blood. (C) A peristaltic pump was used to transfer human blood from the first reservoir through the photo-ECMO device into a second reservoir (assessment of percent COHb reduction). COHb, carboxyhemoglobin; ECMO, extracorporeal membrane oxygenation

temperature at 37°C. For experimental simplicity, blood was poisoned to a COHb concentration of 90%. After Hb was 90% saturated with CO, which was confirmed by blood gas analysis (ABL800 FLEX; Radiometer), 300 ml of reservoir blood passed through the photo-ECMO device at various blood flows (80, 160, and 240 ml/min) while the device was ventilated with 100% O₂ at various gas flow rates (1, 1.6, and 2.4 L/min) ($N=4$ per group). When the blood reservoir contained 1 L of blood, blood passed the photo-ECMO device at a blood flow rate of 92 ml/min and the photo-ECMO system at a blood flow rate of 550 ml/min. The photo-ECMO device or -system was either exposed to LEDs emitting light at a wavelength of 623 nm and an irradiance of 214 mW/cm² (power per unit area of light reaching the surface of the membrane oxygenator), or no light (control) ($N=4$ per group). The COHb concentration changes over time were used to calculate the COHb- $t_{1/2}$ of the blood volume in the reservoir. During the treatment period, the concentration of CO removed by the photo-ECMO device (effluent CO) was measured using a CO gas analyzer (MSA Altair 2X; MSA Safety Inc.). COHb- $t_{1/2}$ was assessed to determine the ability of the photo-ECMO device to remove CO from reservoir blood.

Assessment of percent COHb reduction and temperature increase with a single pass

The second in vitro circuit consisted of two open reservoirs, a peristaltic pump, silicone rubber tubing, and the photo-ECMO device (Figure 1C). CO-poisoned human blood at a Hb concentration of 10.6 ± 0.5 g/dl (effect of irradiance on CO removal) and 10.1 ± 0.3 g/dl (effect of different gas flow rates on CO removal) was pumped at a blood flow rate of 80 ml/min from the first reservoir through the peristaltic pump, to the photo-ECMO device, and to the second reservoir. While blood passed through the photo-ECMO device, it was either ventilated with 100% O₂ (control) at a gas flow rate of 1 L/min or ventilated with 100% O₂ at the same gas flow rate while being illuminated with red light at a wavelength of 623 nm. Blood samples were taken from the first reservoir and, after the blood passed through the photo-ECMO device, from the second reservoir. Four different irradiances (36, 101, 160, and 214 mW/cm²) and four different gas flow rates were tested (1, 1.6, 2.4, and 3.2 L/min). To assess the temperature increase on a single pass through the photo-ECMO device, the blood temperature was measured as blood entered and exited the device.

Animals

This study was approved by Institutional Animal Care and Use Committee (IACUC) at Massachusetts General Hospital, Boston, MA. Four 3 months old, female

Yorkshire pigs with an average weight of 45.3 ± 1.3 kg were studied. The Hb concentration in these pigs was 9.1 ± 0.3 g/dl. Animals were acclimatized 12 h before the experiment. Premedication with atropine (0.04 mg/kg), xylazine (2.2 mg/kg), and telazol (4.4 mg/kg) was used. Anesthesia was maintained with a continuous infusion of propofol (10 mg/kg/h) and fentanyl (3 µg/kg/h). Pigs were intubated with a 7.0-mm cuffed endotracheal tube and connected to a ventilator (Ohmeda 7000). Respiratory rate was adjusted to maintain CO₂ levels (35–45 mmHg). The FiO₂ during CO poisoning was 0.21 and during treatment 1.0. PEEP was fixed at 5 cm H₂O. Muscle relaxation was provided with a continuous infusion of pancuronium (0.06 mg/kg/h). For continuous anesthesia, a 24 Gauge catheter (BBraun) was placed in an ear vein. An 18 Gauge catheter (Teleflex) was inserted into the right carotid artery for blood sampling and pressure measurement. Body temperature was continuously measured with a rectal temperature probe.

Experimental protocol

After surgical preparation, pigs were poisoned while breathing 3000 ppm CO through the endotracheal tube until a COHb level of 50% was reached. All animals were first treated by breathing 100% O₂ through the endotracheal tube (control). Thereafter, pigs were re-poisoned with 3000 ppm CO until the previous COHb level was reached (COHb of 50%, CO poisoning 2). During that phase, animals were connected to the photo-ECMO system. 50% of all pigs were first treated with the photo-ECMO system ventilated with 100% O₂ *without* additional exposure of the device to light (Treatment 2). After the last CO-poisoning phase (CO poisoning 3), these pigs were then treated with the photo-ECMO system ventilated with 100% O₂ and additional exposure of the devices to light (Treatment 3). The other 50% of pigs were first treated with the photo-ECMO device ventilated with 100% O₂ with additional light exposure. After the third CO-poisoning phase, they were then treated with the photo-ECMO system which was ventilated with 100% O₂ *without* additional exposure of the devices to light.

Veno-venous extracorporeal circuit in pigs

CO-poisoned blood was drained from the animal through a 12 French cannula (Medtronic) placed in the right external jugular vein. The blood circulated through tubing with an internal diameter of 1/8 inches (Masterflex) and at a blood flow rate of 550 ml/min through a peristaltic pump (NE-9004; New Era Pump Systems Inc.) to the photo-ECMO system and returned to the animal through a 12 French cannula (Medtronic) inserted in the left external jugular vein. The extracorporeal circuit (photo-ECMO system and tubing) was primed with a sterile ringer's lactate solution. To avoid blood clotting,

three units of heparin per ml ringer lactate were added to the priming solution. All pigs received 300 U/kg of heparin after cannulation. ACT (activated clotting time) samples were drawn periodically. Additional heparin was given to maintain an ACT of 400–450 s.

Measurement and calculations

The hemoglobin concentration (Hb), oxygen saturation (SatO₂), and the fraction of COHb and partial pressure of oxygen (PO₂) in blood samples were measured using a blood gas analyzer. The oxygen transfer of the photo-ECMO device was calculated as follows:

Oxygen transfer normalized to

1L/min blood flow (mlO₂/L_{blood})

$$= [(c_{\text{post}}(\text{mlO}_2/\text{dl}_{\text{blood}}) - c_{\text{pre}}(\text{mlO}_2/\text{dl}_{\text{blood}})) \times 10 \text{ dl/L}] \\ \times Q_{\text{blood}} (\text{L}_{\text{blood}}/\text{min}) / 1 \text{ L}_{\text{blood}}/\text{min}, \text{ whereas}$$

$$c_{\text{pre}}(\text{mlO}_2/\text{dl}_{\text{blood}}) = \left[(\text{SatO}_{2\text{pre}}(\%)/100) \right. \\ \left. \times \text{Hb} (\text{g}/\text{dl}_{\text{blood}}) \times 1.34 \text{ mlO}_2/\text{g}_{\text{Hb}} \right] \\ + \left[0.003 \text{ mlO}_2/\text{mmHg} \right. \\ \left. / \text{dl}_{\text{blood}} \times \text{PO}_{2\text{pre}}(\text{mmHg}) \right]$$

$$c_{\text{post}}(\text{mlO}_2/\text{dl}_{\text{blood}}) = \left[(\text{SatO}_{2\text{post}}(\%)/100) \right. \\ \left. \times \text{Hb} (\text{g}/\text{dl}_{\text{blood}}) \times 1.34 \text{ mlO}_2/\text{g}_{\text{Hb}} \right] \\ + \left[0.003 \text{ mlO}_2/\text{mmHg} / \text{dl}_{\text{blood}} \right. \\ \left. \times \text{PO}_{2\text{post}}(\text{mmHg}) \right].$$

Q_{blood} is the blood flow rate, c is the oxygen content, whereas c_{pre} is the oxygen content of blood entering the device and c_{post} is the oxygen content of blood exiting the device. $0.003 \text{ mlO}_2/\text{mmHg}/\text{dl}_{\text{blood}}$ is the solubility coefficient of oxygen in blood and $1.34 \text{ mlO}_2/\text{g}_{\text{Hb}}$ is the oxygen-binding capacity of Hb. The Hb concentration was $10.5 \pm 0.3 \text{ g/dl}$.

The ability of the photo-EMCO device to remove CO from blood was determined by measuring the level of blood COHb before and after a single transit through the device using the following equation:

$$\text{COHb reduction} = \frac{\text{COHb}_{\text{pre}} - \text{COHb}_{\text{post}}}{\text{COHb}_{\text{pre}}} \times 100.$$

The Hb concentration in these studies was $10.5 \pm 0.3 \text{ g/dl}$ (effect of irradiance on CO removal) and $10.1 \pm 0.3 \text{ g/dl}$ (effect of different gas flow rates on CO removal).

To determine the COHb- $t_{1/2}$, the percentage of Hb that was in the form of COHb was measured at four different time points ($t = 0, 5, 10,$ and 15 min for the in vitro experiments and $t = 0, 10, 20,$ and 30 min for the in vivo experiments) after the beginning of treatment. An

exponential decay equation ($y = e^{-kx}$) was generated. The half-life of COHb was calculated as $\text{COHb-}t_{1/2} = \ln(2) k^{-1}$. During the treatment period, the concentration of exhaled CO from the animal and the CO eliminated from the photo-EMCO device or the photo-ECMO system (effluent) were measured using a CO gas analyzer (MSA Altair 2X; MSA Safety Inc.). The quantity of exhaled and effluent CO was calculated as the product between the concentration of CO and the sweep gas flow. The Hb concentration in these studies was $9.6 \pm 0.3 \text{ g/dl}$ (experiments with a blood volume of 300 ml) and $10.0 \pm 1.4 \text{ g/dl}$ (experiments with a blood volume of 1 L).

Assessment of hemolysis

To determine the amount of free Hb (mg/dl) in the porcine blood plasma, blood samples were taken every 30 min from the initiation of the ECMO circuit. Plasma Hb levels were measured using the Hemoglobin Assay Kit (Sigma-Aldrich) according to the manufacturer's instructions. Using a 365-well plate, 25 μl of plasma was added and mixed with 100 μl of Triton/NaOH reagent. Samples were measured at 400 nm with a spectrophotometer (SpectraMax M5; Molecular Devices).

Phototherapy

High power LEDs (LZ4-00R108; Mouser Electronics) with a maximum emitted optical power of 750 mW at a wavelength of 623 nm were used (Figure S5). The photo-ECMO device was illuminated using a total of 40 LEDs, with 20 on each side of the device. The photo-ECMO system was illuminated with a total number of 240 LEDs. The LEDs were attached to heat sinks (Mouser electronics). Fans (Dayton) were used to cool the LEDs. A power meter (Thorlabs) was used to measure the irradiance.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software (v.8.3). Normal distribution was tested by using the Shapiro–Wilk test. Data were analyzed using a two-tailed, paired t test, or one-way analysis of variance (ANOVA) with Sidak's multiple comparison test. A paired t test was used to compare oxygen saturation and partial pressure of oxygen in the studies to assess the oxygenating performance of the photo-ECMO device. A one-way ANOVA test was used to assess the effect of different oxygen flow rates on CO removal. A two-way ANOVA with Tukey's multiple comparison test, for repeated measurements, was used to compare variables over time between groups. Statistical significance was defined as a $p < 0.05$. All data are expressed as mean \pm standard deviation (SD) unless specified otherwise.

RESULTS

Development of a “maxi” photo-ECMO device

To construct a “maxi” photo-ECMO device, 15 polypropylene hollow fiber membrane mats (20 fibers/10 mm), each 160/160 mm, were placed on top of each other, with the hollow fibers of each layer rotated 90° relative to the layer below to maximize gas exchange area. Silicone rubber adhesive was used to hold the membrane bundle together and the membranes were enclosed in a clear plexiglass case with four openings for blood and gas inlets and outlets. The device consists of three sealed compartments: One compartment with blood flowing around the membrane hollow fibers and two individual compartments (separated by a silicone barrier) for gas inflow and outflow (Figures 1A and S1). The photo-ECMO device was illuminated with 40 high-power LEDs at a wavelength of 623 nm (Figure S2). In a previous study, we demonstrated that, compared to the illumination of the photo-ECMO device with light at shorter wavelengths, red light at 623 nm removed more CO from blood.²³ Compared to the previously-described mini photo-ECMO device,²³ the dimensions of the blood compartment (length × width) and therefore the surface area for light exposure was increased from 60 × 60 mm to 150 × 150 mm (6-fold). The blood compartment volume was increased from 4 to 60 ml (15-fold). The gas exchange and illumination areas were increased from 0.05 to 0.6 m² (12-fold) and from 0.0072 to 0.0450 m² (6-fold), respectively.

The photo-ECMO device efficiently transfers oxygen to blood

To determine the ability of the photo-ECMO device to oxygenate blood in vitro, the device was first ventilated with pure nitrogen to decrease the blood oxygen sa-

turation to 28.6% ± 4.0%. The device was then ventilated with 100% O₂ while blood at a Hb concentration of 10.5 ± 0.3 g/dl passed through the device at blood flow rates ranging from 80 to 400 ml/min, at a blood temperature of 37°C. Blood oxygen transfer was calculated from samples taken before and after one passage through the device. At a blood flow rate of 80, 160, 240, 320, and 400 ml/min, the oxygen transfer rate (in ml_{O₂}/L_{blood flow}) was 108.3 ± 4.1, 117.8 ± 2.8, 107.7 ± 4.2, 112.6 ± 4.7, and 114.2 ± 5.5 ml/L, respectively (Table S1). The exiting oxygen saturation increased from 28.6% ± 4.0% (blood inlet) to 96.1% ± 0.8% (blood outlet) (average oxygen saturation over all blood flow rates tested, *p* < 0.001). The PO₂ concentration increased from 30.0 ± 7.5 mmHg (blood inlet) to 512.9 ± 47.0 mmHg (blood outlet) (average PO₂ over all blood flow rates tested, *p* < 0.001). These results show that the photo-ECMO device successfully oxygenates blood in a single pass through the device at blood flow rates ranging from 80 to 400 ml/min.

Elimination of CO from human blood

An in vitro circuit was used to assess the ability of the maxi photo-ECMO device to remove CO from 300 ml human whole blood (Figure 1B). When CO-poisoned human blood (COHb level of 90%) at a blood temperature of 37°C circulated through the device at 80 ml/min, while the device was ventilated with 100% O₂ but not exposed to light (control), the COHb-*t*_{1/2} was 42.6 ± 1.5 min (Figure 2A). Illumination of the device with red light reduced the COHb-*t*_{1/2} by 50% (red light absent vs. present, 42.6 ± 1.5 min vs. 21.4 ± 1.4 min, *p* < 0.001). The quantity of CO eliminated by the photo-ECMO device (effluent CO) was greater when the device was exposed to red light and ventilated with 100% O₂, compared to when the device was ventilated with 100% O₂ without light (Figure 2B). These results demonstrate

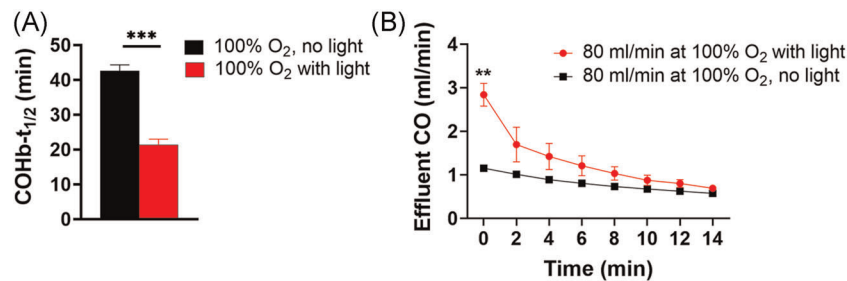


FIGURE 2 In vitro CO elimination from 300 ml of human whole blood with the photo-ECMO device. (A) There was a 50% decrease in COHb-*t*_{1/2} during in vitro treatment of 300 ml of CO-poisoned blood with 100% O₂ and red phototherapy, compared to oxygen without light (*N* = 4 per group). Hb concentration was 9.6 ± 0.3 g/dl; blood flow was 80 ml/min; gas flow rate was 1 L/min. Blood temperature was 37°C. ****p* < 0.001, two-tailed paired *t* test. All data represent mean ± SD. (B) Compared to 100% O₂ alone (control), the combination of 100% O₂ and red light increased the quantity of CO eliminated from the photo-ECMO device (effluent CO). All data represent mean ± SD. ***p* = 0.006 versus control, two-way analysis of variance. COHb-*t*_{1/2}, half-life of carboxyhemoglobin; ECMO, extracorporeal membrane oxygenation

that the maxi photo-ECMO device increases the rate of CO elimination from blood *in vitro*.

Effect of oxygen flow rate on CO removal

To determine whether higher gas flow rates increase CO elimination during a single pass through the device, the percent decrease in COHb concentration at different oxygen flow rates, but a constant blood flow rate was measured (Figure 1C). CO-poisoned human whole blood (COHb 90%) at a Hb concentration of 10.1 ± 0.3 g/dl and at a blood temperature of 37°C , passed through the photo-ECMO device at a rate of 80 ml/min, while the device was ventilated with 100% O₂ and exposed to red light at 623 nm. Changing the gas flow rate from 1 to 1.6, 2.4, or to 3.2 L/min did not alter the percent reduction in COHb in a single blood pass through the device ($31.5\% \pm 0.2\%$, $32.3\% \pm 0.6\%$, $32.1\% \pm 0.2\%$, and $31.7\% \pm 0.2\%$, $p =$ not significant; Table S2).

Effect of irradiance on CO removal rate and blood temperature

The irradiance is the power of light reaching the surface of the device, measured in Watts/m². To investigate whether an increase in irradiance increases CO elimination, we measured the change in the level of COHb after a single pass through the photo-ECMO device (Figure 1C) at irradiances ranging from 36 to 214 mW/cm² (Figure 3 and Table S3). Compared to control (no light), illumination with red light at an irradiance of 36 mW/cm² increased the percent COHb reduction in a single pass through the device from $13.6\% \pm 0.1\%$ to $18.7\% \pm 0.2\%$. Further increases in irradiance to 101 or 160 mW/cm², increased the percent CO elimination to $22.6\% \pm 0.5\%$ and $26.9\% \pm 0.4\%$, respectively, ($p < 0.001$ for each comparison). Illumination with an irradiance of 214 mW/cm² increased CO elimination to $31.5\% \pm 0.2\%$

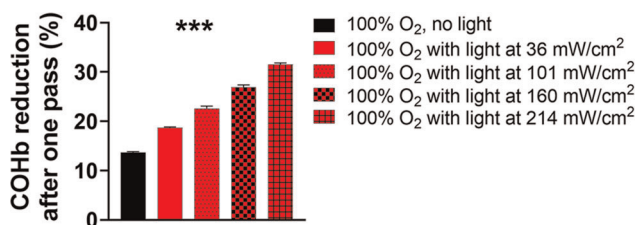


FIGURE 3 The effect of increasing irradiance on COHb reduction during treatment of human whole blood with the photo-ECMO device with 100% O₂ (control), and red light at 623 nm. Blood flow rate was 80 ml/min; gas flow rate was 1 L/min and Hb concentration was 10.6 ± 0.5 g/dl. Blood temperature was 37°C . All data represent mean \pm SD. $R^2 = 0.9914$. $***p < 0.001$ all groups versus control and between groups ($N = 4$ per group), using one-way analysis of variance. COHb, carboxyhemoglobin; ECMO, extracorporeal membrane oxygenation

($p < 0.001$). The changes in temperature of blood passing through the device at blood flow rates of 80, 160, or 240 ml/min, with light at 214 mW/cm² were $2.2^\circ\text{C} \pm 0.6^\circ\text{C}$, $1.4^\circ\text{C} \pm 0.4^\circ\text{C}$, and $0.9^\circ\text{C} \pm 0.1^\circ\text{C}$, respectively (Table S4). These results show that increasing irradiance results in increased CO elimination during a single pass through the device and that the blood temperature increase caused by the highest level of irradiance (at each blood flow rate tested) was below that which might cause adverse effects, such as protein denaturation.²⁴

Effect of changes in blood flow rate on COHb- $t_{1/2}$

To assess whether the efficacy of CO removal is altered by changes in the blood flow rate, the COHb- $t_{1/2}$ was determined at blood flows ranging from 80 to 240 ml/min (Figure 1B). When treating 300 ml of human whole blood at a Hb concentration of 9.9 ± 0.7 g/dl with 100% O₂ and phototherapy, an increase in blood flow rate from 80 ml/min to either 160 or 240 ml/min reduced the COHb- $t_{1/2}$ from 21.4 ± 1.4 min to 19.4 ± 0.8 min and from 21.4 ± 1.4 min to 14.6 ± 0.7 min ($p < 0.001$) respectively (Figure 4A and Table S5). A further increase in blood flow rate from 160 to 240 ml/min reduced the COHb- $t_{1/2}$ from 19.4 ± 0.8 min to 14.6 ± 0.7 min ($p < 0.001$). The quantity of CO eliminated by the photo-ECMO device (effluent CO) during phototherapy was greater at a blood flow rate of 240 ml/min compared to either 80 ml/min or 160 ml/min (Figure 4B). The quantity of CO eliminated by the photo-ECMO device (effluent CO) was also greater during ventilation with 100% O₂ combined with phototherapy at each blood flow rate when compared to oxygen only (without phototherapy) at the same blood flow rate (Figure S3). These results show that the maxi photo-ECMO device is effective over a range of blood flow rates and, that when used with higher blood flow rates, more CO is eliminated.

In vitro studies to assess the ability of the photo-ECMO system to remove CO from human blood

We hypothesized that assembling multiple maxi photo-ECMO devices in parallel, to create a “photo-ECMO system” (Figure S4) would be required to further increase the CO elimination rate. By increasing the number of devices, the total volume of blood that could be treated in a given amount of time would increase. To investigate whether the photo-ECMO system, consisting of six maxi photo-ECMO devices in parallel, further increases the CO elimination rate, the COHb- $t_{1/2}$ was determined in an *in vitro* circuit containing 1 L of CO-poisoned human blood (COHb level of 90%) at a Hb concentration of 10.0 ± 1.4 g/dl (Figures 1B and 5A). Compared to a single maxi photo-ECMO device with a blood flow rate of 92 ml/min, the photo-ECMO system (blood flow rate of 550 ml/min) reduced the

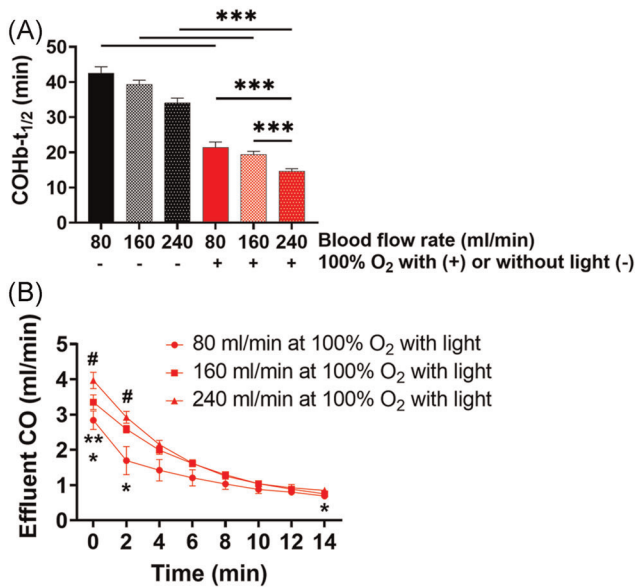


FIGURE 4 Effect of different blood flow rates on COHb- $t_{1/2}$ and effluent CO using the photo-ECMO device. (A) The effect of different blood flow rates on the COHb- $t_{1/2}$ during treatment of blood with 100% O₂ alone (black bars) or with 100% O₂ and red phototherapy (red bars) are shown ($N = 4$ per group). *** $p < 0.001$, one-way ANOVA. Blood volume was 300 ml; Hb concentration was 9.9 ± 0.7 g/dl; blood flow rate was 80, 160, and 240 ml/min; gas flow rate was 1, 1.6, and 2.4 L/min. Blood temperature was 37°C. All data represent mean \pm SD. (B) The effect of different blood flow rates on the quantity of CO eliminated by the photo-ECMO device (effluent CO) during treatment of blood with 100% O₂ and red light at 623 nm ($N = 4$ per group). ** $p < 0.01$ at 80 versus 160 ml/min and * $p < 0.05$ at 80 versus 240 ml/min; # $p < 0.05$ at 160 versus 240 ml/min, two-way ANOVA. All data represent mean \pm SD. ANOVA, analysis of variance; COHb- $t_{1/2}$, half-life of carboxyhemoglobin; ECMO, extracorporeal membrane oxygenation

COHb- $t_{1/2}$ from 69.0 ± 2.1 min to 19.2 ± 4.7 min during ventilation with 100% O₂. When red light was applied to each device, under the same oxygen and blood flow rate conditions, the COHb- $t_{1/2}$ decreased to 34.8 ± 2.4 min (single maxi photo-ECMO device) and 6.3 ± 1.2 min (photo-ECMO system) (Figure 5B and Table S6). The quantity of CO eliminated by the photo-ECMO system (effluent CO) was greater compared to a single maxi photo-ECMO device with or without light (Figure 5C). These results show that in this in vitro system, the photo-ECMO system removes CO faster from CO-poisoned blood than a single maxi photo-ECMO device.

Elimination of CO from a 45-kg CO-poisoned pig using the photo-ECMO system

To investigate the ability of the photo-ECMO system to remove CO from blood in vivo, we tested the device in a porcine model of CO poisoning with an initial COHb level of $53.3\% \pm 2.5\%$. The COHb- $t_{1/2}$ was 21.6 ± 2.6 min when animals breathed 100% O₂ through the endotracheal tube,

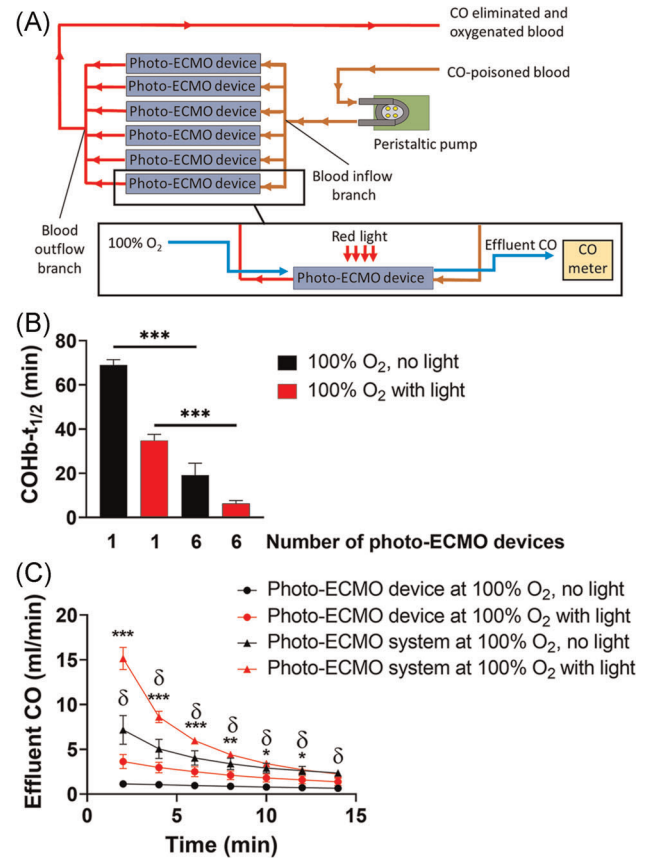


FIGURE 5 Photo-ECMO system and in vitro CO elimination including six identical photo-ECMO devices (photo-ECMO system). (A) Depiction of the photo-ECMO system. The photo-ECMO system consisted of six maxi photo-ECMO devices connected in parallel. A peristaltic pump was used to propel CO-poisoned blood into the photo-ECMO system. Blood exited the photo-ECMO system through a blood outflow branch. Each photo-ECMO device was either ventilated with 100% O₂ alone at a gas flow rate of 1 L/min or additionally exposed to red light at 623 nm while blood circulated through the photo-ECMO system. CO exiting the photo-ECMO system (effluent CO) was measured using a CO meter. (B) COHb- $t_{1/2}$ during treatment with one photo-ECMO device and the photo-ECMO system. Blood volume was 1 L; Hb concentration was 10.0 ± 1.4 g/dl; total blood flow rate of one photo-ECMO device was 92 ml/min; total blood flow rate of the photo-ECMO system was 550 ml/min, total gas flow rate for one photo-ECMO device was 1 L/min; total gas flow rate for the photo-ECMO system was 6 L/min (1 L/min per photo-ECMO device) ($N = 4$ per group). Blood temperature was 37°C. *** $p < 0.001$, one-way ANOVA. All data represent mean \pm SD. (C) The quantity of CO eliminated from the photo-ECMO device and photo-ECMO system (effluent CO) during treatment with 100% O₂ alone or ventilation with 100% O₂ and illumination with red light at 623 nm ($N = 4$ per group). *** $p < 0.001$, ** $p < 0.005$, and * $p < 0.05$ one photo-ECMO device versus photo-ECMO system during treatment of blood with 100% O₂ and red light. $\delta p < 0.05$ one photo-ECMO device versus photo-ECMO system during ventilation with 100% O₂ alone, two-way ANOVA. All data represent mean \pm SD. ANOVA, analysis of variance; COHb- $t_{1/2}$, half-life of carboxyhemoglobin; ECMO, extracorporeal membrane oxygenation

before being connected to the photo-ECMO system (control) (Figure 6A and Table S7). After the animals were connected to the photo-ECMO device system, the blood flow rate through the device was set 550 ml/min and the

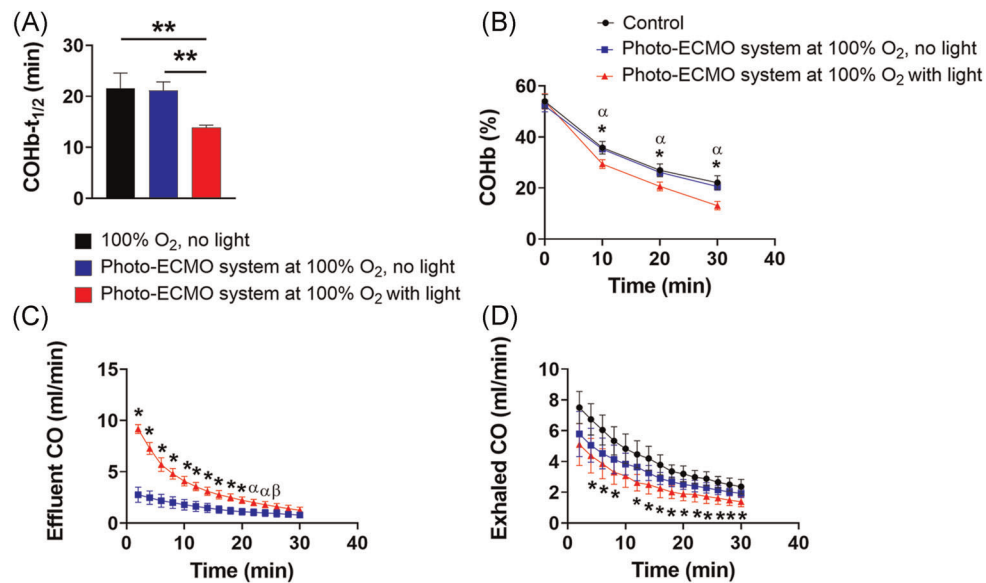


FIGURE 6 Effect of extracorporeal CO removal with the photo-ECMO system on the rate of CO elimination in CO-poisoned pigs. (A) Calculated COHb- $t_{1/2}$ during treatment. $**p = 0.001$ control and photo-ECMO system without light versus photo-ECMO system with light, one-way ANOVA. All data represent mean \pm SD. $N = 4$ animals in total with each group being tested once in each animal. (B) Variation in arterial COHb over time. $*p < 0.05$ control versus photo-ECMO system with light. $^{\alpha}p < 0.05$ photo-ECMO system without light versus photo-ECMO system with light, two-way ANOVA. All data represent mean \pm SD. (C) The quantity of CO eliminated from the photo-ECMO system (effluent CO). $*p < 0.001$; $^{\alpha}p < 0.01$, and $^{\beta}p < 0.05$ versus photo-ECMO system without light, two-way ANOVA. All data represent mean \pm SD. (D) Exhaled CO from the animal. $*p < 0.05$ control versus photo-ECMO system with light, two-way ANOVA. All data represent mean \pm SD. ANOVA, analysis of variance; COHb, carboxyhemoglobin; ECMO, extracorporeal membrane oxygenation

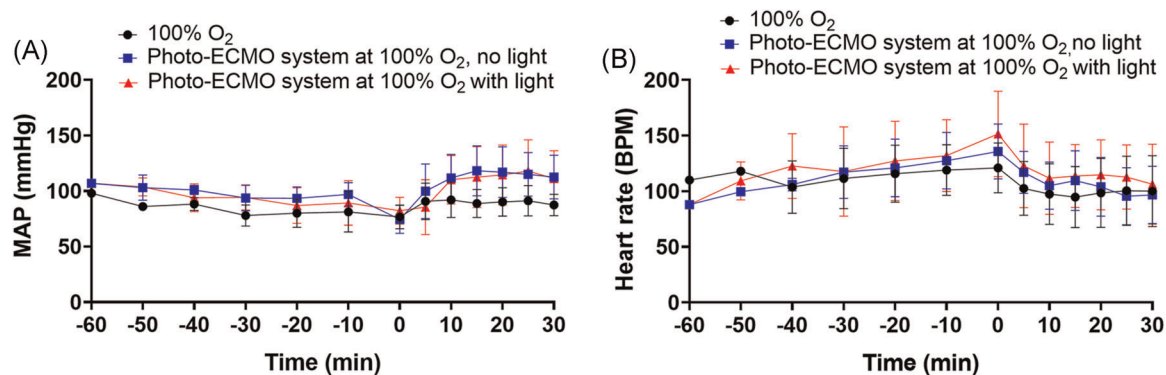


FIGURE 7 Hemodynamic parameters in a pig model of CO poisoning during CO poisoning and during treatment with 100% O₂ alone or after being connected to the photo-ECMO system with and without exposition to light. (A) Mean arterial pressure (MAP) and (B) Heart rate (HR). Two-way ANOVA was carried out from time point -30 to 30 min (missing values before time point -30 min due to variable duration of CO poisoning). p = not significant in between groups. All data represent mean \pm SD. $N = 4$ animals in total with each group being tested once in each animal. COHb, carboxyhemoglobin; ECMO, extracorporeal membrane oxygenation

device was either ventilated with 100% O₂ (without light) or with 100% O₂ while being exposed to red light (phototherapy). When the photo-ECMO system was ventilated with 100% O₂ but not exposed to red light, the COHb- $t_{1/2}$ was unchanged (21.1 ± 1.5 min vs. 21.6 ± 2.6 min). Addition of phototherapy to the photo-ECMO system decreased the COHb half-life by 36% compared to control (13.9 ± 0.4 min vs. 21.6 ± 2.6 min) ($p = 0.001$). During the 30-min treatment period, the arterial COHb decreased from $53.9\% \pm 2.6\%$ to $13.1\% \pm 1.4\%$ in animals treated with the photo-ECMO

system exposed to red light. In contrast, in animals treated with 100% O₂ through the endotracheal tube alone (control), the arterial COHb decreased from $54.0\% \pm 2.3\%$ to $22.1\% \pm 2.4\%$ (Figure 6B). The quantity of CO eliminated by the photo-ECMO system (effluent CO) was greater when the system was exposed to light compared to ventilation with 100% O₂ alone (Figure 6C). Pigs in the control group (breathing 100% O₂ through the endotracheal tube alone) exhaled more CO from the lungs compared to pigs treated with the photo-ECMO system with light. This result was

expected because the remaining COHb concentration in the blood of pigs in the control group was higher at each time point (Figure 6D). The body temperature of the pig increased by $1.2^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ when the photo-ECMO system was exposed to light. During treatment with the photo-ECMO system, the plasma-free Hb concentration increased but was steadily below 100 mg/dl (Figure S5). There was no difference in hemodynamics between the treatment groups (Figure 7). Taken together, the results show that the photo-ECMO system increases the rate of CO elimination in vivo, without significantly increasing the animal's body temperature or causing hemodynamic instability.

DISCUSSION

In this study, we developed and tested a photo-ECMO system designed to treat patients with CO poisoning. The system is composed of six "maxi" photo-ECMO devices, each consisting of 15 polypropylene membrane layers and four openings for blood and gas inlets and outlets. Compared with the previously described "mini" photo-ECMO device,²³ the illumination area and the blood compartment volume were increased 6- and 15-fold, respectively. The illumination and gas exchange areas were increased 6- and 12-fold, respectively. Each of the maxi photo-ECMO devices was able to completely oxygenate blood in a single pass through the device. Ventilation of the device with 100% O₂ and exposure to red light increased the rate of CO elimination compared to ventilation with 100% O₂ alone. The rate of CO elimination was constant over a broad range of gas flow rates while increased blood flow rates (over the range tested) enhanced CO elimination. Increases in the intensity of light also increased CO elimination, without significantly increasing blood temperature. In vitro, when the photo-ECMO system was ventilated with 100% O₂ and illuminated with red light, the COHb-*t*_{1/2} in blood decreased by almost 70%, from 19.2 to 6.3 min compared to ventilation with 100% O₂ alone. Treatment of CO-poisoned pigs with the photo-ECMO system (with additional light exposure) increased CO elimination by 36%.

The most basic requirement for a photo-ECMO device for the treatment of CO poisoning is the ability to oxygenate circulating blood. The normal oxygen consumption in a resting individual is 250 ml/min at a cardiac output of 5 L/min.²⁵ Thus, the membrane oxygenator must provide at least 50 ml O₂ per L blood flow to meet the minimum oxygen requirement.²⁶ We showed that the photo-ECMO device fulfilled this requirement with an average oxygen delivery of 112 ml O₂ per L blood flow. In addition, a high blood PO₂ level accelerates CO elimination.¹⁹ In humans with healthy lungs, the arterial O₂ partial pressure increases from 100 to 600 mmHg when breathing 100% O₂ instead of 21% O₂.²⁷ Commercially available membrane oxygenators reach post-membrane PO₂ levels of 400–500 mmHg.²⁸ In comparison, our photo-ECMO device showed similar

post-membrane PO₂ levels, demonstrating an oxygenating performance equivalent to commercial devices.

The application of extracorporeal blood phototherapy to treat CO poisoning in a large animal requires additional, special design considerations. The blood compartment needs to be thin, so as to enable light penetration, and the illumination area has to be as large as possible to facilitate the exposure of blood to light. Commercially available membrane oxygenators are not suitable for phototherapy, because their blood compartment is too thick, thereby limiting light penetration. Compared to commercially available membrane oxygenators, the blood compartment of the photo-ECMO device is one-third as thick and the illumination area is almost three-fold greater.²⁹

We found that CO elimination is efficient at a blood flow rate of 550 ml/min, which corresponds to 5%–12% of a pig's cardiac output.³⁰ Because this relatively low blood flow rate is sufficient to achieve adequate CO removal, the photo-ECMO system can be used with relatively small catheters, which could be placed by paramedics into a patient at the site of CO poisoning, rather than requiring placement in the emergency room or ICU.

CO-poisoned patients often suffer from acute lung injury caused by the inhalation of different toxic components in smoke (e.g., NO₂, hydrogen chloride) as well as by thermal injuries from the inhalation of hot gases.^{20,21,31–34} Treatment of these patients with inhaled 100% O₂ has a relatively little benefit because of decreased gas exchange in the damaged lungs. We anticipate that the use of the photo-ECMO device will be especially beneficial for these patients.

Hemolysis is associated with increased morbidity and mortality due to impaired renal function.³⁵ While using the photo-ECMO device in pigs, the concentration of free Hb increased at the beginning of treatment but remained below 100 mg/dl until the end of the treatment. We anticipate that in CO-poisoned patients, the photo-ECMO system will only be required for a short time, thereby limiting the patient's exposure to free Hb.

This study has some limitations. The photo-ECMO device was not directly compared to other novel approaches to treat CO poisoning. These include neuroglobin³⁶ and hemoCD,³⁷ both high-affinity CO scavengers, as well as hydroxocobalamin,³⁸ which metabolizes CO to CO₂. Because these therapies increase CO elimination through different mechanisms, a combination of these treatments with photo-ECMO could possibly facilitate CO removal in CO-poisoned patients. Further studies are needed to assess and to compare combinations of these therapies. A second limitation of this study is that we did not directly compare treatment using the photo-ECMO device to HBO therapy. However, we anticipate that these two approaches will have different applications and advantages. In contrast to the proposed photo-ECMO therapy, treatment with HBO requires transporting the patient to specialized treatment. In addition, in patients with severe acute lung injury, HBO will be less effective because of decreased gas

exchange in the lung.¹⁸ Contrarily, extracorporeal blood phototherapy might be applied immediately after CO poisoning.

In summary, we developed a veno-venous extracorporeal photo-ECMO system and demonstrated its feasibility and effectiveness for the elimination of CO from 45 kg pigs with CO poisoning. Future modifications of the photo-ECMO system will be directed at making the device more compact and portable so as to permit treatment of CO-poisoned patients at the scene of their poisoning, during transit to a local emergency room, and in hospitals that lack HBO facilities.

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CONFLICT OF INTERESTS

Warren M. Zapol, R. Rox Anderson, Walfre Franco, William A. Farinelli, Luca Zazzeron, and Anna Fischbach are listed as inventors on a patent application submitted by the Massachusetts General Hospital on the technology described in this paper (System and method for extracorporeal carbon monoxide removal with phototherapy).

AUTHOR CONTRIBUTIONS

Conceptualization: Anna Fischbach, Aranya Bagchi, R. Rox Anderson, and Warren M. Zapol. *Methodology:* Anna Fischbach, Steffen B. Wiegand, Luca Zazzeron, and Lisa Traeger. *Investigation:* Anna Fischbach, Steffen B. Wiegand, Luca Zazzeron, Lisa Traeger, Raffaele di Fenza, Luigi Grassi, William A. Farinelli, Walfre Franco, and Sandeep Korupolu. *Funding acquisition and Project administration:* Anna Fischbach and Warren M. Zapol. *Supervision:* Warren M. Zapol and R. Rox Anderson. *Writing original draft:* Anna Fischbach, Donald B. Bloch, R. Rox Anderson, and Warren M. Zapol. *Writing—review and editing:* Anna Fischbach, Steffen B. Wiegand, Luca Zazzeron, Lisa Traeger, Raffaele di Fenza, Aranya Bagchi, William A. Farinelli, Walfre Franco, Sandeep Korupolu, Jutta Arens, Luigi Grassi, Francesco Zadek, Donald B. Bloch, R. Rox Anderson, and Warren M. Zapol.

DATA AVAILABILITY STATEMENT

The photo-ECMO device is not commercially available but is available at cost via material transfer agreement (contact Warren M. Zapol). All the data used for the manuscript are present in the main text and/or in the Supporting Information Materials.

ORCID

Anna Fischbach  <http://orcid.org/0000-0003-4997-428X>
Walfre Franco  <http://orcid.org/0000-0003-0905-4083>

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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