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Evaluation of Peritoneal Microbubble Oxygenation Therapy in a Rabbit Model of Hypoxemia

Nathan D. Legband¹, Jameel A. Feshitan¹, Mark A. Borden*, and Benjamin S. Terry*

Abstract—Alternative extrapulmonary oxygenation technologies are needed to treat patients suffering from severe hypoxemia refractory to mechanical ventilation. We previously demonstrated that peritoneal microbubble oxygenation (PMO), in which phospholipid-coated oxygen microbubbles (OMBs) are delivered into the peritoneal cavity, can successfully oxygenate rats suffering from a right pneumothorax. This study addressed the need to scale up the procedure to a larger animal with a splanchnic cardiac output similar to humans. Our results show that PMO therapy can double the survival time of rabbits experiencing complete tracheal occlusion from 6.6 ± 0.6 min for the saline controls to 12.2 ± 3.0 min for the bolus PMO-treated cohort. Additionally, we designed and tested a new peritoneal delivery system to circulate OMBs through the peritoneal cavity. Circulation achieved a similar survival benefit to bolus delivery under these conditions. Overall, these results support the feasibility of the PMO technology to provide extrapulmonary ventilation for rescue of severely hypoxic patients.

Index Terms—Acute lung injury (ALI), acute respiratory distress syndrome (ARDS), airway obstruction, extrapulmonary ventilation, oxygen delivery.

I. INTRODUCTION

PATIENTS with airway failure or lung damage from disease or trauma may develop life-threatening hypoxemia, caused by the inability of the lungs to transfer oxygen to the blood. Even with modern medical treatment, recent studies have shown that patients suffering from respiratory failure experience a mortality rate of 31–45% [1]–[4], with some reports of mortality rates as high as 75% [3], [5]. Mechanical ventilation may be inadequate due to limited mass transfer in the damaged lung, and may even exacerbate the condition by causing overinflation, barotrauma, and cyclic closing and reopening of the alveoli, which can trigger an inflammatory response and multiple system organ failure [6]. Even if the lungs are healthy, treating hypoxemic patients with inspired oxygen or intubation may not be possible due to an airway obstruction or complex anatomy. Clinicians have

therefore sought a safe and effective method for extrapulmonary oxygenation to treat hypoxemic patients.

If mechanical ventilation is inadequate, the last resort for treating respiratory failure is extracorporeal membrane oxygenation (ECMO), a temporary support of the respiratory and cardiac systems requiring removal of blood from the body to an external oxygenation device comprising artificial membranes, and reintroduction of the treated blood back into the body [7]. ECMO has been used since 1972 [8], and its use for treating respiratory failure has significantly increased in the last decade owing to its effectiveness during the H1N1 flu pandemic [7] and a multicenter study showing improved survival in adults with acute respiratory distress syndrome (ARDS) [9]. Additionally, ECMO is now being used on patients undergoing surgical correction to airway obstruction [10]. Recent advances in ECMO include introduction of nonthrombogenic heparin-coated polymethylpentene hollow fibers, second-generation centrifugal pumps, and improved cannulae [11].

Despite these improvements and its proven clinical utility, ECMO presents significant technical complexity and cost, which restrict its use in ambulatory situations. Additionally, the invasiveness and risk of potentially lethal complications arising from the mechanically powered high-flow extracorporeal circuit limits the clinical use of ECMO. In particular, powerful anticoagulants (e.g., heparin) must be carefully administered to patients and regulated to mitigate thrombogenic effects of ECMO. These anticoagulants can cause unpredictable and lethal intracranial brain hemorrhage among other complications [7], [12]–[14].

Alternative oxygenation therapies are therefore necessary to reduce the risk of hemorrhagic complications. The ideal therapy would be simple, portable, cost effective, and able to provide extrapulmonary ventilation (i.e., both O₂ delivery and CO₂ removal) on demand without the need for anticoagulants. One exciting approach is to use the peritoneal cavity for extrapulmonary ventilation, which avoids removing blood from the body [15]. Peritoneal ventilation uses the large surface area of the peritoneum, a serous membrane that lines the abdominal cavity, as a gas exchanger. The peritoneum is a highly vascularized and absorptive tissue receiving a significant fraction of the cardiac output, and for this reason, the peritoneal cavity is often used for systemic drug delivery [16] or as an alternative to hemodialysis [17]. Solutes can easily transport between blood and fluid in the peritoneal cavity via diffusion through the mesothelium. Peritoneal ventilation would rely on the transport of the rapidly diffusing species oxygen and carbon dioxide into the blood of the splanchnic capillary beds. Thus, when implementing peritoneal ventilation, care must be taken to avoid high intraperitoneal pressure or other factors that could limit blood flow to the region.

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Safe and rapid transabdominal catheterization techniques from laparoscopic and indwelling catheter surgery could be adapted for peritoneal ventilation; thus, making it suitable for long term, short term, and possibly ambulatory care [18]–[21]. The central purpose of peritoneal microbubble oxygenation (PMO) therapy is to provide a versatile and safe treatment method to supplement systemic oxygenation, either in tandem with current ventilation practices, or by itself.

We recently demonstrated the first successful application of peritoneal oxygenation using phospholipid-coated oxygen microbubbles (OMBs) [15]. OMBs consist of oxygen gas microbubbles encapsulated by a phospholipid shell and polyethylene glycol brush layer. They were originally used with inert gas as ultrasound contrast agents [22], [23], and have been rigorously characterized in prior work [24], [25]. OMBs provide hydration to the peritoneum, as well as high gas carrying capacity and rapid mass transfer for the delivery of oxygen and possible removal of carbon dioxide [26]. OMBs are designed with a pure oxygen core, they have different composition, structure, and properties than fluorinated blood substitutes, such as nanobubbles or nanodroplets [27], [28]. In our prior study, we demonstrated that PMO by a single bolus of OMBs can eliminate mortality and maintain normal levels of hemoglobin saturation and heart rate in rats suffering from an otherwise lethal right pneumothorax. This study addresses three critical goals for the further development of the PMO technology: 1) to determine whether PMO can extend life following complete loss of lung function (rather than partial loss as in the right pneumothorax) without resuscitation; 2) to scale up the procedure to a larger animal; and 3) to design and test a new delivery system to circulate OMBs through the peritoneal cavity.

II. MATERIALS AND METHODS

A. Oxygen Microbubble Generation and Characterization

All solutions were prepared using 0.2- μm filtered 18 M Ω -cm deionized water (Direct-Q, Millipore; Billerica, MA, USA). Glassware was cleaned with 70 vol% ethyl alcohol (Sigma-Aldrich, St. Louis, MO, USA) and rinsed with deionized water. Phospholipid 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) was purchased from NOF (Tokyo, Japan), and polyoxyethylene-40 stearate (PEG40S) was purchased from Sigma-Aldrich. DSPC and PEG40S were weighed and combined in a 9:1 molar ratio and, then, mixed with a filtered solution of 0.1-M NaCl phosphate buffered saline to create a final lipid concentration of 12 mg/mL. The mixture was then heated to 10 °C above the main phase-transition temperature (T_m) of DSPC using a digital stirring hotplate (Thermo Scientific; Asheville, NC, USA). The mixture was further dispersed using a Branson 450 sonifier (Danbury, CT, USA) with an output power of 5/10 until the solution was translucent. The lipid suspension was then stored in a refrigerator for OMB preparation. OMBs were prepared by adapting the reactor design developed by Swanson *et al.* [22] for synthesizing large volumes of OMBs. The reactor consisted of an ultrasonic horn enclosed in a water-cooled continuous flow chamber (Branson, Danbury, CT, USA). The lipid suspension was cooled to 5 °C, and combined with

oxygen gas in the flow chamber at roughly equal flow rates, where they were emulsified at full sonifier power output into a mixture of OMBs and macroscopic foam. OMBs were isolated from the macroscopic foam in a flotation column. OMBs were then concentrated to ~ 70 vol% by centrifuging at 110 relative centrifugal force units for 4 min, and stored in sealed 9-L serum bottles under oxygen atmosphere. The lipid suspension obtained by centrifugation was collected and recycled into the reactor system to produce more OMBs. The process was repeated until ~ 6 L volume of 70 vol% OMB was produced per bottle. The gas headspace purity in the serum bottle was confirmed using a model 6600 precision headspace gas analyzer (Illinois Instruments Inc.; Johnsburg, IL, USA).

B. In Vivo Rabbit Tracheal Occlusion Model for Hypoxemia

Our previous work showed successful bolus delivery of OMBs to the peritoneal cavity and oxygenation in a rat right pneumothorax model for hypoxemia [15]. In this study, a larger animal model was chosen in order to take the next step toward clinical relevance. The rabbit (male New Zealand white rabbit, $n = 19$, 2.23 ± 0.18 kg) was selected as the animal model for this study because the ratio of the intraperitoneal cavity volume to its body mass is more similar to humans than that of the rat [29]. Furthermore, the resting oxygen consumption rate of the rabbit is lower than that of the rat owing to larger body mass, which allows for a more relevant analysis to human therapy [30], [31]. The larger body mass also increases the total oxygen demand and, thus, requires scale up of the manufacture of OMBs.

All animal studies were performed in accordance with the University of Nebraska-Lincoln, Institutional Animal Care and Use Committee. To evaluate the PMO therapy, rabbits were treated with either OMBs or saline as the control. Two infusion methods (bolus or circulation) were tested, resulting in four groups: 1) saline bolus [(SB), $n = 2$]; 2) saline perfusion [(SP), $n = 2$]; 3) OMB bolus [(OB), $n = 9$]; and 4) OMB perfusion [(OP), $n = 6$]. The SB and SP groups were limited to two animals each (four animals total) as the use of more animals was deemed excessive considering these four trials matched the well-documented time of death for an untreated asphyxiation rabbit model [32–34].

To model complete respiratory failure, the rabbit was intubated with a cuffed endotracheal tube (ETT), which could be hermetically sealed by occluding the end of the ETT with a rubber membrane and plastic cap. Thus, all gas exchange in the lungs was eliminated, causing the animal's oxygen intake, as well as carbon dioxide removal, to occur through OMBs, and any extension of life was entirely due to PMO therapy. The experimental endpoint was cardiac arrest determined by cessation of heartbeat and pulse. To prevent the disruption of hemodynamics, intraabdominal pressure (IAP) was monitored and kept below 8 mmHg [35], [36]. The criterion for exclusion was the rabbit being able to breathe around the sealed ETT; however, no animals were observed to breathe past the ETT once it was sealed. During the experiment, body temperature, pulse rate, and SpO_2 were monitored in 8 s averages and recorded every

30 s. The IAP and fluid infusion temperature were monitored and recorded every second. Survival time was calculated as the time between the ETT sealing (time = 0) and cardiac arrest (the experimental endpoint).

Each rabbit was weighed before the procedure and, then, sedated with 5% isoflurane to effect through a nosecone. The throat was then sprayed with cetacaine and intubated with the cuffed ETT (3 mm, JorVet). The ETT cuff was inflated and, then, secured by cotton gauze tied around the head and front incisors. The rabbit was placed in the supine position on a warming pad (T/pump Classic, Gaymar), and the ETT was attached to the anesthetic machine with the rabbit maintained on 2% isoflurane. The abdomen was shaved and sterilized with betadine scrub and alcohol. A veterinary monitor (SurgiVet Advisor, Smith's Medical) was used to monitor vitals. A pulse oximetry sensor (V1700, Smith's Medical) was used to measure the pulse rate and arterial oxygen hemoglobin saturation (SpO_2). A temperature probe (WWV3418, Smith's Medical) monitored body temperature. Both sensors were placed rectally. An intramuscular injection of ketamine-xylazine solution (35–5 mg/kg dose, 35 mL/kg solution) was then given to anesthetize the rabbit for the remainder of the procedure, while the rabbit was weaned off of the isoflurane. Once completely weaned off isoflurane, the ETT was disconnected from the anesthetic machine and the rabbit was allowed to breathe room air. Once the rabbit was confirmed unresponsive to pain by paw pinches, a small incision was made in the upper left quadrant of the abdomen for insertion of the fluid infusion tubing (3.2 mm inner diameter, Tygon) into the peritoneal cavity. The incision was then sutured closed around the tubing. A 12-gauge indwelling catheter was then inserted into the peritoneal cavity at the upper right quadrant of the abdomen and connected to two pressure transducers (4426-005G, Measurement Specialties Inc.) for measuring IAP via a custom data acquisition system (myDAQ, LabVIEW, National Instruments).

C. Bolus Delivery of OMBs

Fig. 1 shows a schematic of the experimental setup for the bolus technique. A single static volume of OMBs or saline is injected into the cavity. The OMB or SB was pumped through an inline fluid warmer (iWarm, Midmark) set at 40.4 °C and, then, into the peritoneal cavity with a peristaltic pump (Thermo Scientific, FH100M) at 80 mL/min for 4 min, and then at 12.6 mL $\text{min}^{-1} \cdot \text{kg}^{-1}$ thereafter. The ETT was then hermetically sealed upon reduction of the pump. Fluid infusion lasted until the IAP reached 5 mmHg, and the total infused volume of fluid was recorded.

D. Circulation of OMBs

Fig. 1 shows a schematic of the peritoneal circulation technique, where fresh OMBs were continually perfused through the peritoneal cavity to continuously deliver oxygen. To remove OMBs from the peritoneal cavity, a medial incision was made into the peritoneal cavity, and a customized OMB scavenging port was inserted. A suture was then used to close the incision around the port collar. A scavenging tube (7.9 mm inner

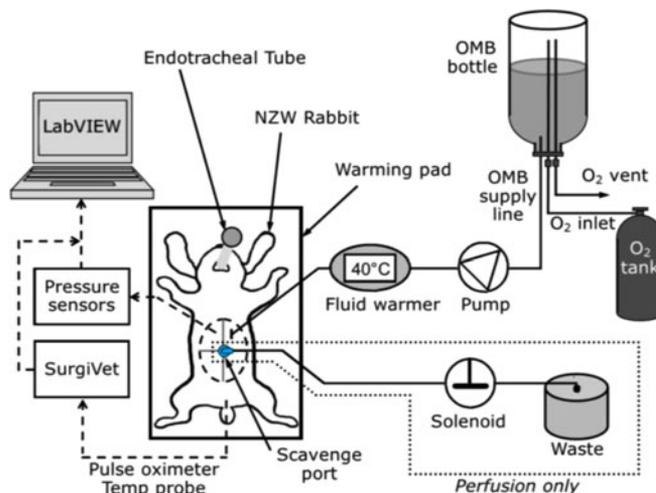


Fig. 1. Experimental setup of the circulating OMB delivery to the peritoneum of rabbits. The perfusion method has an additional incision for the scavenger port and tubing line into the peritoneal cavity for removal of expended OMBs.

diameter, Tygon) was then attached to the port. The OMB or saline perfusate was then pumped through an inline fluid warmer (iWarm, Midmark) set at 40.4 °C and, then, into the peritoneal cavity with a peristaltic pump (Thermo Scientific, FH100M) at 80 mL/min for 4 min, and then at 12.6 mL $\text{min}^{-1} \cdot \text{kg}^{-1}$ thereafter. The IAP was maintained between 3 and 4 mmHg using a 36-V solenoid (ROB-10391, Sparkfun) driven valve to control the fluid flow. Once circulation of OMBs or saline was attained, the ETT was hermetically sealed. Infusion of fluid continued until the time of death, after which the total infused volume of was recorded. Fig. 2 shows the experimental process flow diagram for the procedure.

E. Statistical Analysis

Kaplan–Meier survival curves (see Fig. 3) were created to analyze the survival benefit of OMBs versus saline control. A log-rank (Mandel Cox) test was performed for significant differences between the groups ($\alpha = 0.01$). Analysis of covariance (ANCOVA) with separate means was performed to test the overall differences among groups between the rate change of the heart rate and SpO_2 after the ETT was sealed. Multiple comparison tests of the means were performed to determine which groups were significantly different ($\alpha = 0.05$).

III. RESULTS

A. Bolus Delivery of OMBs

PMO resulted in a significant increase in the survival time of rabbits following sealing of the ETT (see Table I). Rabbits treated with a bolus infusion of saline control (SB group) experienced cardiac arrest within 7 min. The rabbits receiving a bolus injection of OMBs (OB group) had a mean survival time of 12.2 ± 3.0 min, which was found to be significantly different than for the SB group ($X^2 = 12.36, p = 0.0004$).

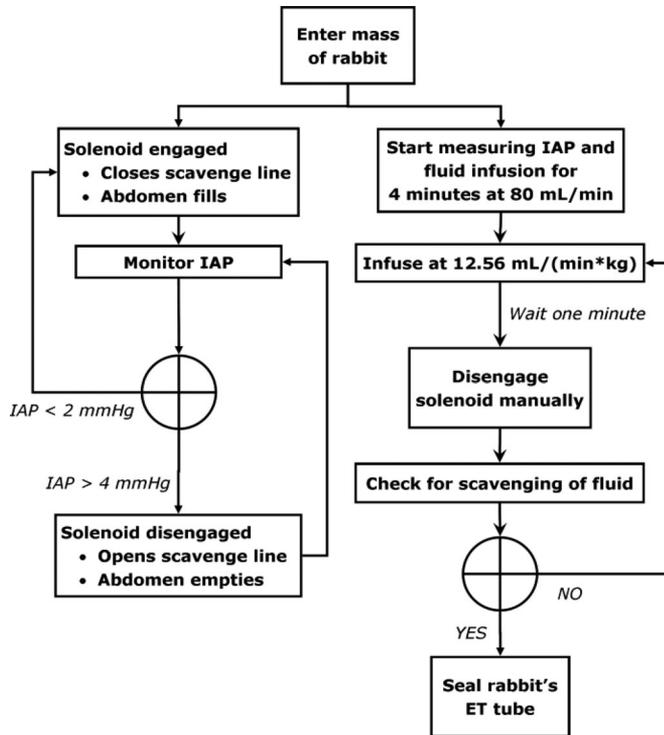


Fig. 2. Control scheme implemented for the perfusion experiments. IAP was monitored by a custom LabVIEW program and used to regulate the fluid flow by clamping the scavange tubing with a solenoid. The left and right paths of this process are performed simultaneously.

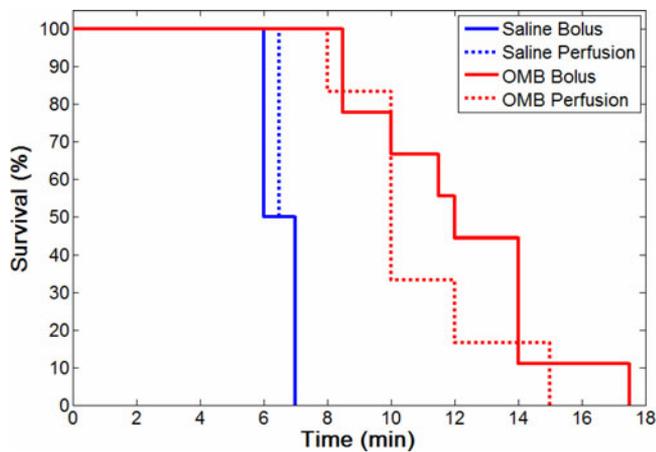


Fig. 3. Kaplan–Meier survival curves of the SB, SP, OB, and OP treatment groups.

TABLE I
MASS, INFUSED VOLUME, AND SURVIVAL TIME DATA OF ALL EXPERIMENTAL GROUPS

Treatment	<i>n</i>	Mass (kg)	Infused volume (mL)	Survival time (min)
SB	2	2.53 ± .02	336 ± 9	6.5 ± 0.7
OB	9	2.25 ± .16	558 ± 66	12.2 ± 3.0
SP	2	2.03 ± .01	431 ± 37	6.8 ± 0.4
OP	6	2.18 ± .12	646 ± 72	10.8 ± 2.4

All data is presented as average ± SD over the number of samples, *n*, in the treatment group.

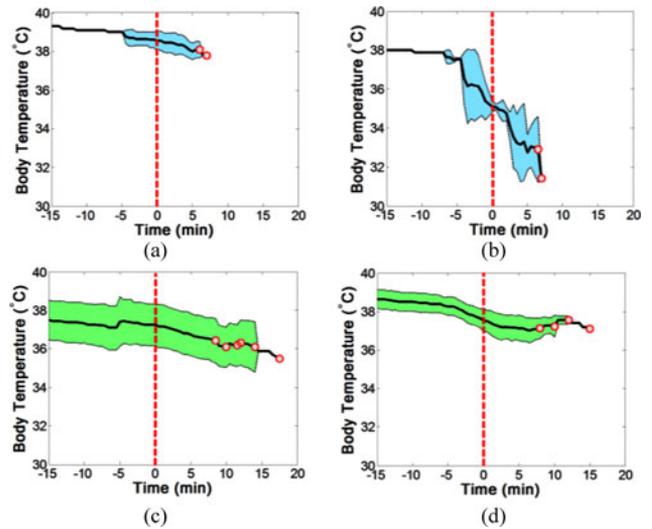


Fig. 4. Average body temperature of all treatment groups before and during experiment: (a) SB, (b) SP, (c) OB, and (d) OP. The shaded region indicates standard deviation, where there is no shaded region either there is only one sample or the samples values are coinciding at the time ($std = 0$). The dashed line at time zero represents when the ETT was sealed. The cardiac arrest times of each rabbit are indicated by circles.

The temperature of the fluid infused into the peritoneal cavity ranged from 20.5 to 28.0 °C and the IAP was maintained below 6 mmHg. The body temperature of the SB and OB groups dropped slightly below the normal body temperature range for rabbits of 38–40 °C [37], [38]; however, the body temperature of all treated rabbits were in the range of 35–40 °C during the period of fluid infusion (see Fig. 4). The slight decrease in body temperature of the SB and OB groups resulted from the combination of anesthesia, shaving of the abdomen and peritoneal infusion of fluid that was slightly cooler than body temperature. This slight hypothermic condition was not enough to endanger the rabbit or cause hypothermia based on the duration of the procedure [39], [40].

Fig. 5(a) and (c) shows the average pulse rate for the bolus treatment groups. The SB group had pulse rates of 130–325 beats/min, which are within the normal range for a sedated rabbit [37], [38], [41], while the OB group with the prolonged survival time eventually experienced pulse rates below 130 beats/min. Interestingly, the SB group experienced a rapid decline in pulse rate following sealing of the ETT, whereas the OB group appeared to experience a steadier and less drastic decline in pulse rate. Rabbits infused with OMBs showed a short period of stability in pulse rate 5–10 min after the ETT was sealed.

Fig. 6(a) and (c) shows the average blood oxygen saturation (SpO_2) for the bolus treatment groups. The SB group experienced a rapid decline in SpO_2 following sealing of the ETT, whereas the OB group experienced a steadier and less drastic decline in SpO_2 . While the oxygen saturation was below a healthy baseline of 90% in both groups, there appears to be an overall improvement in SpO_2 for the OB group compared to the SB group. From a clinical view, however, the rabbits were not well oxygenated as the SpO_2 was around 60% for most of the

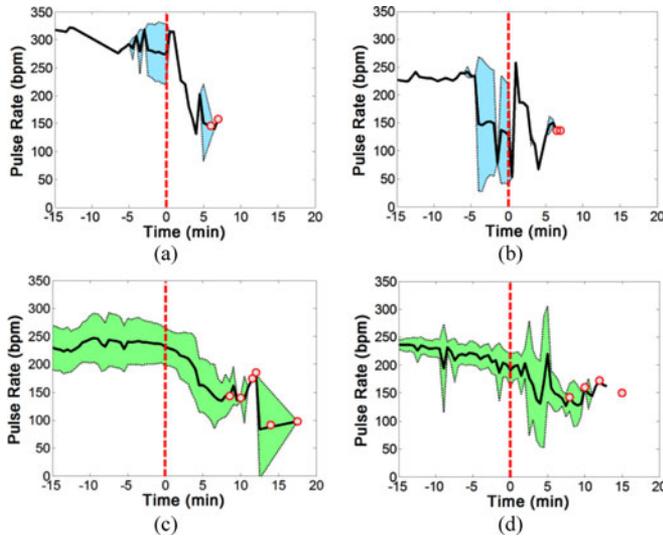


Fig. 5. Average pulse rate of all treatment groups before and during experiment: (a) SB, (b) SP, (c) OB, and (d) OP. The shaded region indicates standard deviation, where there is no shaded region either there is only one sample or the samples values are coinciding at the time ($\text{std} = 0$). The dashed line at time zero represents when the ETT was sealed. The cardiac arrest times of each rabbit are indicated by circles.

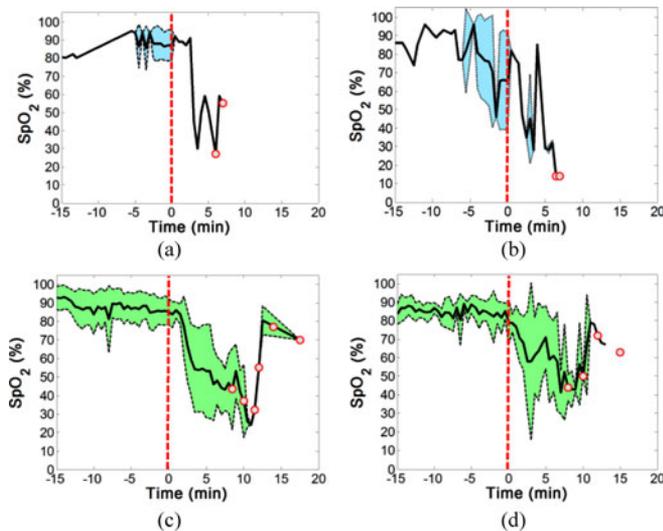


Fig. 6. Average oxygen saturation, measured as SpO_2 , of all treatment groups before and during experiment: (a) SB, (b) SP, (c) OB, and (d) OP. The shaded region indicates standard deviation, where there is no shaded region either there is only one sample or the samples values are coinciding at the time ($\text{std} = 0$). The dashed line at time zero represents when the ETT was sealed. The cardiac arrest times of each rabbit are indicated by circles.

experiments. Note that a couple OB trials did have higher O_2 levels ($\sim 80\%$). Table II shows that no significant difference in heart rate or SpO_2 was indicated by ANCOVA.

B. Circulation of OMBs

Table I also shows data for experiments involving circulation of OMBs. The rabbits in this study had a baseline pulse rate of 245 ± 40 beats/min and oxygen saturation of $87 \pm 4\%$

TABLE II
MULTIPLE COMPARISON TEST RESULTS USING ANCOVA

	Group 1	Group 2	C.I. ^a	Significant difference ^b	
Heart rate	SB	SP	[-42.01, -3.20]	YES	
	SB	OB	[-28.37, 0.59]	NO	
	SB	OP	[-34.20, -4.87]	YES	
	SP	OB	[-4.88, 22.31]	NO	
	SP	OP	[-10.73, 16.85]	NO	
	OB	OP	[-10.48, -0.83]	YES	
SpO_2	SB	SP	[-7.56, 7.82]	NO	
	SB	OB	[-9.42, 1.77]	NO	
	SB	OP	[-11.59, -0.25]	YES	
	SP	OB	[-9.50, 1.61]	NO	
	SP	OP	[-11.68, -0.41]	YES	
	OB	OP	[-4.07, -0.12]	YES	

when intubated and freely breathing room air. Rabbits receiving saline control (SP group) experienced hypoxic cardiac arrest within 7 min after complete asphyxiation by sealing the ETT. Rabbits receiving a circulation of OMBs (OP group) had a mean survival time of 10.8 ± 2.4 min, which was significantly longer than the SP group ($X^2 = 8.33$, $p = 0.004$). The survival time for the bolus and perfusion groups were not significantly different for either saline ($X^2 = 0.059$, $p = 0.808$) or OMBs ($X^2 = 0.705$, $p = 0.401$).

The temperature of the fluid infused into the peritoneal cavity ranged from 20.5 to 28.0 $^{\circ}\text{C}$, and the IAP was maintained in the range of 2 to 4 mmHg. The body temperature of the OP treatment group dropped slightly below the normal body temperature range for rabbits, but was in the range of 35 – 40 $^{\circ}\text{C}$ during the period of fluid infusion (see Fig. 4). The body temperature of the SP group, on the other hand, decreased from 38 to 31 $^{\circ}\text{C}$. The combination of saline inlet temperature that was below body temperature, high heat capacity of the saline, and circulation led to lower body temperatures in the SP group. This was not seen in the OP treatment group because OMBs are 70% O_2 gas by volume and, therefore, have a lower heat capacity than saline.

Fig. 5(b) and (d) shows the average pulse rate for the perfusion treatment groups. Results between the OMB and saline control groups were similar to those found above for bolus injection. The OP group had heart rates within the normal range, while the SP group experienced pulse rates below 130 beats/min. As before, rabbits infused with OMBs showed a short period of stability in pulse rate 5–10 min after the ETT was sealed.

Fig. 6(b) and (d) shows the average blood oxygen saturation (SpO_2) for the perfusion treatment groups. The OP group showed a significant increase in SpO_2 compared to all other treatment groups. Interestingly, the decline in SpO_2 and heart rate for the SP group began about 4 min before the ETT was sealed, when infusion was initiated. The reason for this anomaly is unknown.

Overall, the measured vitals of heart rate and oxygen saturation in the OMB treated rabbits showed less drastic decline than that of the saline treatment groups, but only the OP method proved to significantly increase SpO₂. Also, as shown in Table II, the SpO₂ of the OP group was significantly different from all other groups.

IV. DISCUSSION

Our first goal was to determine whether PMO can extend life following complete loss of lung function. Based on these results, we conclude that PMO therapy in a complete asphyxiation model significantly increases time to cardiac arrest by a factor of 1.7. This increase was shorter than the at least 6.5-fold increase in survival time that we previously reported for PMO therapy in a rat lung injury model [15]. In the prior injury model, however, we estimate that lung function was limited to about 40% [42], possibly improving over the 2-h experimental timeframe as the underlying pneumothorax healed. In this study, the sealed ETT reduced lung function to 0%. The significant improvement in survival time and oxygenation (SpO₂) for PMO-treated animals over controls therefore demonstrates the potential promise of this technology, even in the extreme scenario of complete airway obstruction.

Our second goal was to scale up the procedure from rats to a larger animal. One of the potential limitations of PMO treatment is the limited amount of blood flow through the splanchnic circuit. In humans, only 20–30% of the total blood volume passes through the peritoneal region [43], which is similar to rabbits where total splanchnic circulation is 22% of the total blood flow [44]. This limits the amount of oxygen that can be transported from the peritoneum, which may explain the difficulty in maintaining normoxic levels in this complete asphyxiation model.

Our final goal was to design and test a new delivery system to circulate OMBs through the peritoneal cavity. While we were able to achieve circulation, we found that the method of infusion (bolus versus circulation) had no measured impact on survival time in the complete asphyxiation model. This was an unexpected outcome. In the circulation infusion method, OMBs fully loaded with oxygen were continuously delivered into the peritoneal cavity. However, no method was implemented to encourage complete mixing of OMBs in the peritoneal cavity before they exit circulation. It is possible that fresh OMBs exited the cavity through the scavenge tubing before releasing their oxygen load. Thus, transfer efficiency may have been negatively impacted for the continuous infusion method such that it was analogous to a single bolus injection. In the future, this will be confirmed by determination of OMB quality and gas concentrations (O₂, CO₂, N₂) directly before and after the treatment. Additional refinements of the circulation technique may be needed to increase mass transfer between the OMB perfusate and peritoneum.

The variance within the experimental groups merits future investigation. Several factors could have caused this variability. For example, rabbit weight, individual metabolism, and depth of anesthesia all affect O₂ consumption. The splanchnic blood flow and vascularization of the peritoneum vary between animals and

impact the diffusion rate of O₂ into the body. Some of these variables could possibly be controlled. For example, enhancing splanchnic blood flow could be achieved by administration of drugs such as cisapride [45]. Also, varying IAP levels could be investigated in order to determine the pressure threshold for vasculature collapse and its effect on OMB diffusion dynamics.

It is apparent from this study of total asphyxiation in rabbits that our current PMO system was inadequate to provide 100% of a sedated rabbit's oxygen demand because animals showed a gradual decrease in SaO₂ before death. Our previous study [15], however, showed that PMO could fulfill 60% of a rat's oxygen demand. If the therapy shows similar efficacy in humans as it does in rats and rabbits, PMO therapy may be able to provide supplemental oxygen for systemic oxygenation for a wide variety of respiratory conditions including lung injury, drowning, asthma, sepsis, pneumonia, and other causes of ARDS.

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Authors' photographs and biographies not available at the time of publication.