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DEVELOPMENT OF A NEW LOW-VOLUME OXYGENATOR AND CREATION OF A HYDRODYNAMIC TEST BENCH FOR *EX VIVO* LUNG PERFUSION IN SMALL ANIMALS

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Small animal models are widely used in basic research. However, experimental hydrodynamic test benches, which include extracorporeal circuits, often have limitations associated with the size and filling volume of equipment. Thus, we aimed at developing and validating a miniature oxygenator as well as a low-volume hydrodynamic system for *ex vivo* perfusion of small animal lungs. A series of low-volume membrane oxygenators (n = 10) with 90–100 aligned microporous polypropylene hollow fibers, placed inside a sheath that is sealed at both ends to isolate the perfusing solution, was designed and manufactured. This design makes gas to flow through the hollow fibers and perfusate to circulate around the fibers. A low-volume hydrodynamic test bench was designed and assembled for isolated *ex vivo* lung perfusion and for evaluation of the performance characteristics of the oxygenators: gas and perfusate flow, perfusion pressure and temperature at 5–70 ml/min flow range.

Keywords: low-volume membrane oxygenator, oxygenator, isolated perfused organ, lung perfusion, hydrodynamic test bench, blood oxygenation.

INTRODUCTION

The development of extracorporeal membrane oxygenators for cardiac surgery has reached a high level of quality and reliability [1–4]. Currently, there are several systems available for adult patients, children, and newborns. However, even the most miniaturized systems are not suitable for basic protocols for experimental studies in small animal models with extracorporeal blood circulation or for perfusion of their isolated organs. Reducing the volume of oxygenators used in such experimental studies is critical to the usability of the system, since the filling volume of the oxygenator usually accounts for the bulk of the total filling volume of the system.

Currently, some "homemade" low-fill oxygenators have been described in the literature (see Table 1). However, most studies still require a device with a primary fill volume of a few milliliters, which requires additional volumes of donated animal blood [5–10].

In addition to technical difficulties, miniaturization of the oxygenator is a challenge in terms of its functionality and efficiency to guarantee the reproducibility and accuracy of the experiment [11–13].

So, the development of a low-volume oxygenator will allow to develop of standard perfusion systems for experimental studies involving small animals or their isolated organs. In this paper, we present a description of our own development of a low-volume membrane oxygenator, its characteristics that prove the functionality and reliability of the oxygenator, as well as a new hydrodynamic test bench for perfusion of small animal lungs using the oxygenator of our own design.

Table 1

Brief properties and characteristics of previously developed low-volume oxygenators

Author	Fill volume (mL)	Perfusate flow rate (mL/min)	Oxygenator size (mm)	Perfusion time (min)
Kim W.G.	29	21.2	–	30
Gunzinger R.	4	54	40 × 40 × 15	60
Jungwirth B.	4	57–64	128 × 27	45–105
Ordodi V.L.	8	17–42	–	180
Dong G.H.	4	50–75	–	60
Shang H.W.	10	14–40	–	60

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MATERIALS AND METHODS

Design features for the development of a low-volume oxygenator

An ideal 3D design of a new mini-oxygenator with the following given parameters was designed: total length of the oxygenator, 10 cm; inner diameter, 5 mm; outer diameter, 7 mm; dry weight of the oxygenator, up to 15 g; average number of fibers, 90–95 units; total effective working surface area, up to 90 cm^2; perfusate flow rate, up to 80 mL/min. The block diagram of operation and 3D design of the low-volume oxygenator is presented in Fig. 1.

The physical model of the oxygenator is a housing consisting of two $^1/_4 \times {}^1/_4$ inch disposable polystyrene connectors, each with one Luer port (Maquet Cardiopulmonary AG, Hirlingen, Germany), connected by silicone tubing (Raumedic AG, Helmbrechts, Germany) and symmetrically oriented (see Fig. 2).

Microporous polypropylene hollow fibers (Oxyphan PP50/200, Membrana GmgH, Wuppertal, Germany) placed in our housing are sealed together with connectors at each end of the shell using epoxy resin (bisphenol A/F-based epoxy resin and modified cycloaliphatic amine hardener, Epoxy Master, Russia). This design isolates the

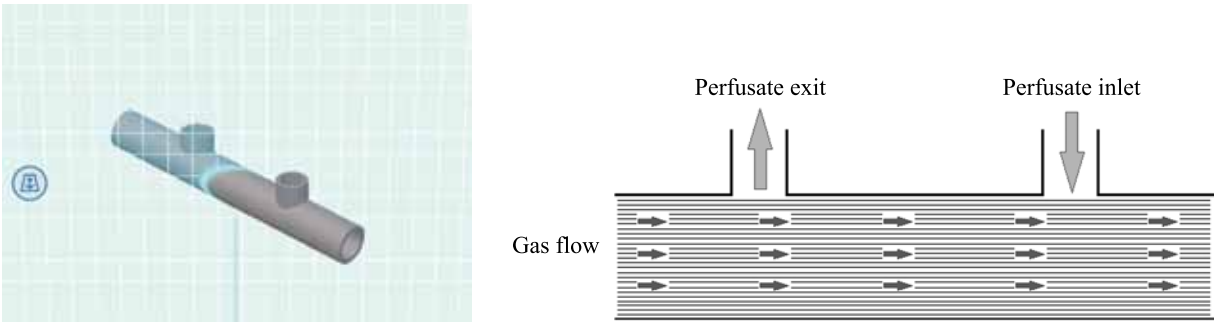

Fig. 1. On the left is an ideal model of the designed low-volume oxygenator. On the right is a block diagram of how the low-volume oxygenator operates

Fig. 2. A developed experimental model of the low-volume oxygenator

gas compartment, where gas passes through the interior of the hollow fibers, from the perfusate compartment, where the solution circulates around the hollow fibers from the outside. The perfusate flows through the low-volume oxygenator countercurrent to the gas flow.

A 3D cross-sectional model of the sealed ends of the oxygenator was designed to estimate the required effective working surface area of the fibers (see Fig. 3), and a cross-section of the designed oxygenator was taken to compare theoretical calculations and actual results obtained (see Fig. 4).

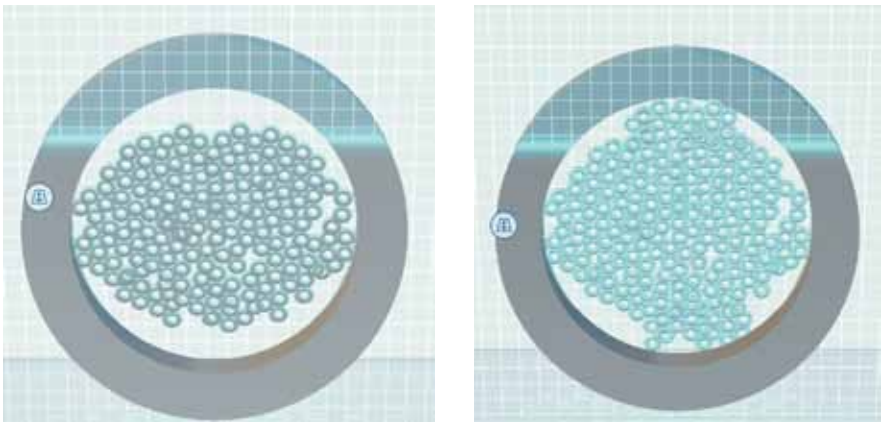

Fig. 3. A 3D model of the cross section of the ends of the oxygenator

The theoretical calculations were 98% consistent with the practical calculations (at 0.05% confidence interval with the general population parameter at the estimated confidence level), i.e., more than 95% of the polypropylene hollow fibers were open at both ends.

In vitro performance assessment of the developed low-volume oxygenators

The oxygenation capacity of the developed low-volume oxygenators was tested using modified Krebs–Henseleit (KH) buffer in vitro on a hydrodynamic perfusion recirculation bench. A Diagram of the test bench is shown in Fig. 5.

The KH buffer was freshly prepared for each experiment (mmol): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2 \cdot 2H_2O$ 1.25, $NaHCO_3$ 25 and glucose 11. The hydrodynamic test bench contained a deoxygenation tank in which the perfusate was bubbled with a 95% N_2 / 5% CO_2 gas mixture and a low-volume oxygenator through which 95% O_2 / 5% CO_2 was passed.

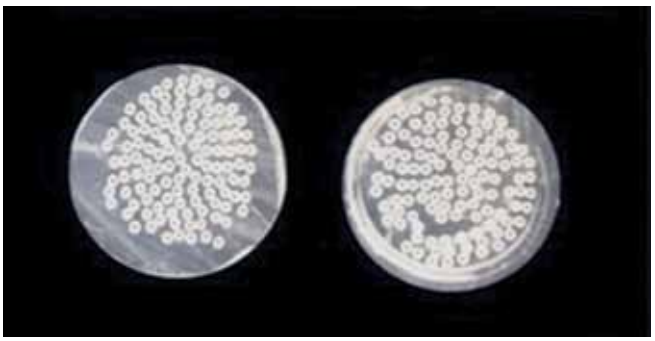

Fig. 4. Visualization of slices of the sealed ends of the oxygenator using a digital microscope

N_2/CO_2 was maintained at 0.4 L/min, and O_2/CO_2 at 0.8–1.2 L/min. A peristaltic pump was used to circulate perfusate from the tank through the oxygenator and back to the tank. Ports were also installed in the circuit to draw perfusate before and after passing through the low-volume oxygenator.

Perfusate pressure was recorded using pressure sensors (Edwards Lifesciences, USA). Flow sensors (Transonic Systems, USA) and temperature sensors were included in the circuit immediately before and after the oxygenator. Flow and pressure measurements were continuously recorded using an Angioton multichannel module (Biosoft-M, Russia) on a Pumpax high-performance data acquisition system (Biosoft-M, Russia). The partial pressure of oxygen (pO_2) of oxygenated and deoxygenated buffer, gas flow rate, pre- and post-oxygenator perfusate pressure, and temperature were measured every 10 minutes at perfusate flow rates ranging from 5 to 65 mL/min. For the duration of the experiment (90 minutes), the gas mixture was heated to 37.0 °C using a thermostat and water bath (XMTE-205, China).

Design and development of a new isolated ex *vivo* system for small animal lung perfusion using the new oxygenator

A schematic diagram of a hydrodynamic test bench for *ex vivo* perfusion of small animal lungs using a new low-volume oxygenator was developed (see Fig. 6).

An analytical review of the assembly technique, positive pressure ventilation settings, perfusate composition, flow rate conditions and lung cannulation, was carried out. Based on its results and on the block diagram, the hydrodynamic test bench, presented in Fig. 7, was assembled.

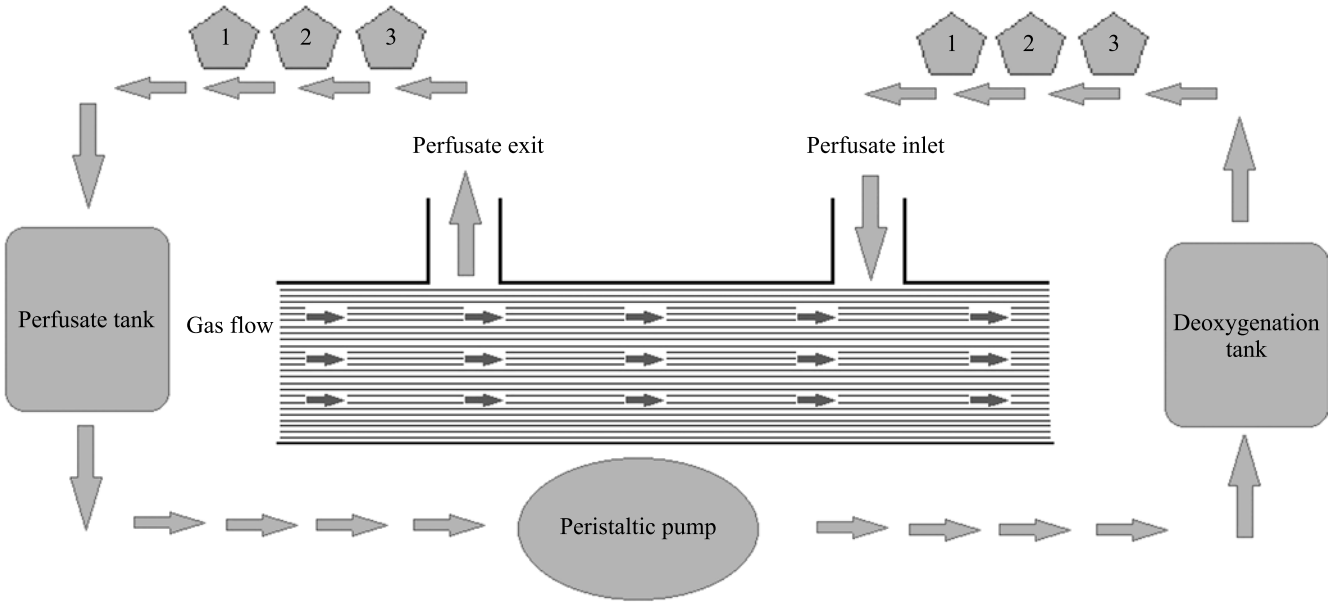

Fig. 5. Diagram of the hydrodynamic test bench for the study of the developed oxygenators in vitro (1, 2, 3 – temperature and pressure sensors and a sampling port)

Once all cannulas were inserted and connected to the circuit, we made sure that the lungs were ventilated and there were no perfusate leaks along the entire line. Oxygen exchange increased as soon as the ventilator was turned on and inflated the lungs to engage more alveoli for gas exchange. We have further research ahead of us to optimize the hydrodynamic test bench and further develop an *ex vivo* lung perfusion technique.

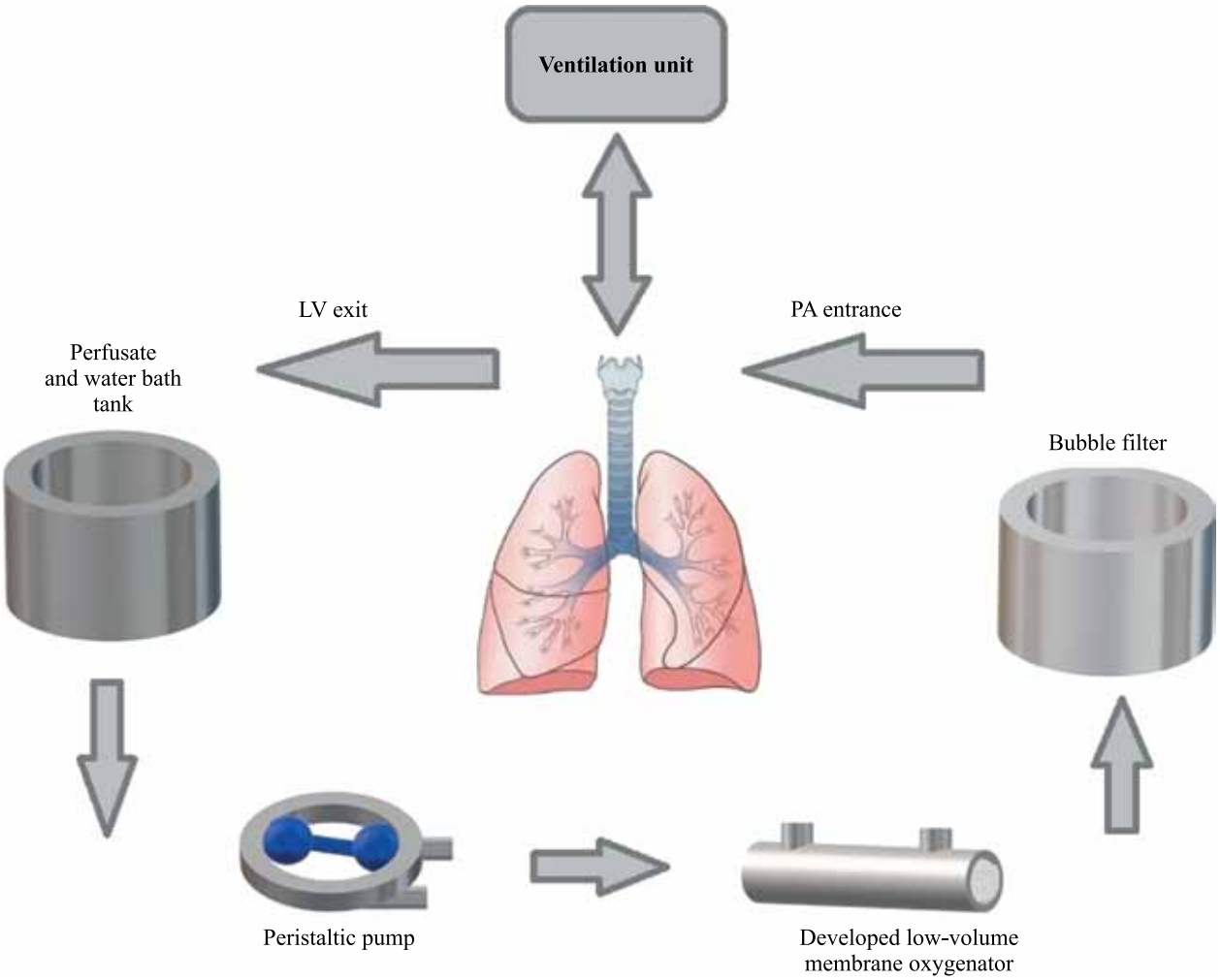

Fig. 6. Diagram of hydrodynamic test bench for isolated *ex vivo* perfusion of small animal lung

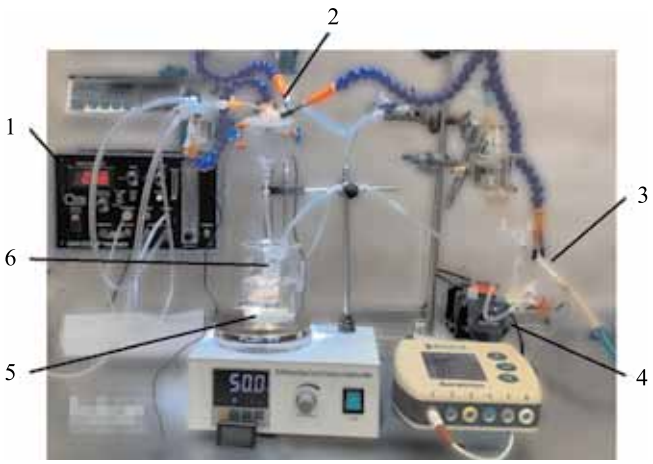

Fig. 7. Hydrodynamic test bench for isolated *ex vivo* perfusion of small animal lungs. 1, ventilation unit; 2, small animal donor organs; 3, new low-volume oxygenator; 4, peristaltic pump; 5, water bath; 6, perfusate tank

RESULTS

A series of low-volume membrane oxygenators (N = 10) was designed and developed. Table 2 summarizes the calculated and obtained physical characteristics of the oxygenators.

The average filling volume of the developed oxygenator is 1.5 ± 0.5 ml.

Experimental studies on the hydrodynamic test bench to evaluate the operation of an oxygenator with a KH buffer with a range of perfusate flow rate variation from 5 to 70 mL/min allowed us to obtain the following indicator values – pO_2 after passing through the oxygenator varied on average from 400 to 500 mmHg; the value averaged from 100 to 200 mmHg after the deoxygenation tank. Transport of oxygen and PO_2 in the oxygenated buffer gradually decreased with increasing perfusate flow rate;

Table 2

Theoretical and actual characteristics of low-volume membrane oxygenators

	Theoretical value	Actual value
Oxygenator total length (cm)	10	13
Oxygenator outer diameter (mm)	5	5–6
Oxygenator inner diameter (mm)	7	7–8
Oxygenator weight (g)	≤15	12.7 ± 1.1
Average number of open fibers (units)	90–95	100 ± 8
Effective working surface of fiber line (cm^2)	90	78 ± 6
Perfusate flow rate (mL/min)	≤ 80	≤70

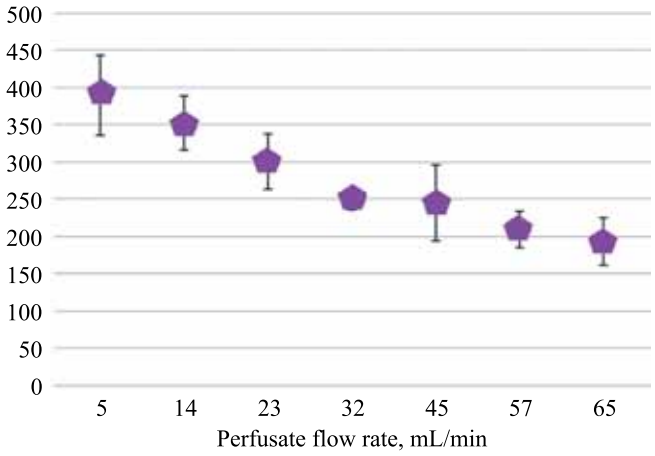

Fig. 8. Oxygen transport provided by the oxygenator

whereas PO_2 gradually increased in the deoxygenated buffer (see Fig. 8).

The developed hydrodynamic test bench for isolated *ex vivo* perfusion revealed no obvious problems. So, we plan to conduct a series of research studies with small animals.

DISCUSSION

We have developed an efficient mini-oxygenator with a very small filling volume of ≈1.2 mL, which is one of the lowest values currently available among membrane oxygenators. Our oxygenator efficiently oxygenates the KH buffer at perfusate flow rates of up to 70 mL/min, providing a pO_2 ≈400 mmHg for at least 90 minutes.

There is now a wealth of information in the public domain on small animal models (rats), which are of great value in terms of investigating many aspects of the cardiovascular and cardiopulmonary systems [11, 14–20]. Evaluation of inflammatory response, evaluation of solutions, activation of the coagulation system, biocompatibility of new materials – these and many other aspects of medical practice can be studied in experimental animal models. In these situations, a miniaturized hydrodynamic system is convenient and effective, especially if the experiment requires a blood-filled circuit. It is for such circuits with low perfusate filling volumes that an oxygenator such as the one that has been developed is required.

Although a series of several mini-oxygenators with filling volumes <2.0 mL were produced, some differences between the samples, influencing the initial evaluation of the developed mini-oxygenator, were observed. The results presented in this article should be considered as a starting step towards development of more advanced models and optimization of its manufacture.

The potential for these oxygenators is high. Of great research and practical interest are the data obtained when using the mini-oxygenator as part of a circuit on the developed hydrodynamic test bench for isolated *ex vivo* perfusion of lungs of small animals. Both the mini oxygenator and the miniature hydrodynamic bench open fundamentally new possibilities in the provision of technical support for experiments on *ex-vivo* perfusion of lungs on small laboratory animal models.

CONCLUSION

A mini-oxygenator has been developed and its functionality evaluated. The oxygenator is efficient and reliable for oxygenating physiological buffers over the range of flow rates commonly used in small laboratory animal models.

The performance parameters of the oxygenator remain stable for at least 90 minutes, which is a sufficient time for most experimental protocols.

The created oxygenator made it possible to develop experimental protocols that were impossible to implement due to perfusate volume and/or size of available oxygenators. For instance, the hydrodynamic test bench for isolated *ex vivo* perfusion of small animal lungs is relevant for the study and development of proprietary methods and approaches for *ex-vivo* perfusion of donor lungs. Further research in this area will be pursued.

The authors declare no conflict of interest.

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