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DEVELOPMENT OF A LOW PRIMING VOLUME HYDRODYNAMIC TEST BENCH FOR ISOLATED *EX VIVO* PERFUSION OF SMALL ANIMAL LUNGS

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Objective: to develop and validate a hydrodynamic test bench (HTB) with a small filling volume for *ex vivo* normothermic machine perfusion (NMP) of donor lungs of small experimental animals (rats) using the open-loop technique. **Materials and methods.** An HTB was developed for *ex vivo* NMP of donor lungs of rats. It is a prefabricated structure with stands that hold the following equipment: a ventilator for small laboratory animals, a heating element, a low priming volume membrane oxygenator and a dome for donor lung storage, as well as roller peristaltic pump, sensors and device for invasive pressure measurement in the circuit, bubble filter and a line kit. Wistar rats (n = 6) were used to investigate the effectiveness of the HTB. Following the removal of donor lungs, the graft was positioned on the HTB and *ex vivo* lung perfusion (EVLP) was initiated with selected parameters. During the rat donor lung perfusion procedure, *ex vivo* PaO_2/FiO_2 ratio, oxygenation index (OI), pulmonary artery pressure (PAP) and peripheral pulmonary vascular resistance (pPVR) were measured. **Results.** High OI values were obtained at the end of the procedure (460 ± 32 at p = 0.028); constant PAP values were recorded in all cases throughout the EVLP procedure – from 9.13 to 7.93 mmHg at p > 0.05. The criterion for HTB functionality was pPVR, which tended to decrease in all cases – from 603.3 ± 56 to 89.1 ± 15 dynes/sec/cm^{-5} at p = 0.000. No design flaws impacting the donor lungs' functional condition during *ex vivo* NMP procedure were found in the circuit of the hydrodynamic low priming volume bench during experimental studies. **Conclusion.** The efficiency and technical functionality of the HTB were demonstrated by the results of the experimental study conducted on the laboratory animals, rats. The observed dynamics of decrease in pPVR and the high OI values at stable PAP allowed for the conclusion that both the *ex vivo* perfusion itself and the technical design of the HTB are efficient.

Keywords: EVLP, ex vivo normothermic lung perfusion, hydrodynamic bench, low priming volume oxygenator, donor lung chamber.

INTRODUCTION

Introduction of *ex vivo* perfusion into clinical practice has made it possible to evaluate and rehabilitate initially compromised donor lungs and increase the number of transplants using suboptimal donor organs [1, 2]. Modern development of normothermic machine perfusion of donor lungs outside the donor body is extremely intensive and is aimed at improving lung transplant outcomes. According to world statistics, more than 30% of lung transplants initially deemed unsuitable for transplantation are successfully implanted in recipients after an *ex vivo* lung perfusion (EVLP) procedure, and 3-year patient survival is about 70% in the absence of chronic organ rejection [3, 4].

However, creation and introduction of new drugs and molecules, modified perfusion solutions and agents for cold cardioplegia of donor lungs, as well as active research on the use of mesenchymal stromal cells and gene products to reduce ischemia-reperfusion injury to the lung graft and the risk of severe primary dysfunction after implantation dictate the need to search for an optimal animal model for research [5, 6]. Although sheep and pig experimental models are the most common, economic limitations are a major obstacle to creating a large research sample [7–12]. In turn, today the rat model, which is characterized by a high degree of validity and reproducibility, has been actively introduced into biomedical research on the pathophysiology of EVLP and lung transplantation [13, 14]. Special attention should be paid specifically to a model for *ex vivo* normothermic perfusion of donor lungs of rats, since it is the perfusion procedure that allows a detailed comprehensive assessment of the graft and objectification of research results [15], but the lack of an established EVLP technique in small laboratory animals and the scientific search for an optimal perfusion protocol dictate the need to improve laboratory technologies for scientific research [16]. The use of modern 3D printing techniques, optimization of available equipment for laboratory animals and develop-

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ment of a universal EVLP protocol for rats will increase the efficiency of scientific research, and achieving a perfusion volume close to the volume of the animal's circulating blood will ensure the reliability of results when assessing the concentrations of biochemical markers and drugs [17].

A work was carried out to create a laboratory test bench with optimal functional characteristics for *ex vivo* normothermic perfusion of donor lungs in rats with a low priming volume circuit filling and integrated oxygenator [18, 19] to recreate optimal conditions for graft functioning outside the living organism.

In order to apply this innovation as a platform for preclinical research of new medications, the **objective** of this work is to perform EVLP in a rat model using a laboratory low priming volume bench to assess the performance of donor lungs during *ex vivo* perfusion.

MATERIALS AND METHODS

In this study, a complex hydrodynamic test bench (HTB) with a small circuit filling volume was developed for *ex vivo* normothermic machine perfusion of donor lungs of small laboratory animals (rats). Many components of the extracorporeal circuit were modeled, calculated and created based on the given medical and technical requirements corresponding to the size of the small laboratory animals. The basic scheme of the perfusion circuit device for EVLP, which took into account the existence of the main components for *ex vivo* procedure, served as a basis (Fig. 1).

The developed HTB is a prefabricated structure with racks on which the main components of the extracorporeal circuit are fixed (Fig. 2).

Prior to the first series of experimental investigations, perfusate was poured into the circuit, which circulated through the system and was heated to 37.0 °C. After the lungs were procured, they were placed in a specially designed container.

Computer-aided design software system SolidWorks (SolidWorks Corporation, Dassault Systèmes) was used to create this element of the system. The 3D mathematical model of the dome is shown in Fig. 3, a. The optimal

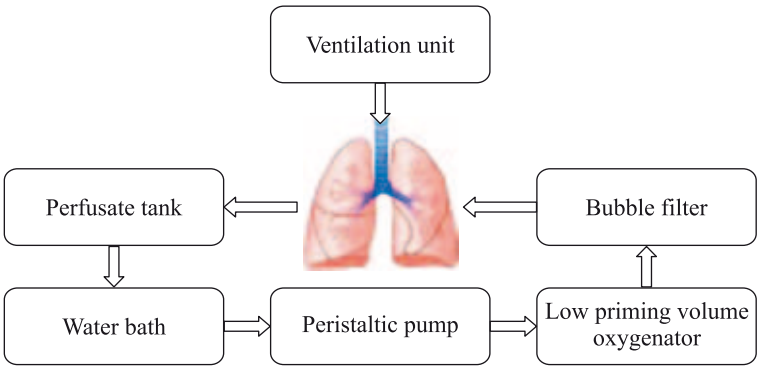

Fig. 1. Block diagram of the hydrodynamic test bench with low priming volume

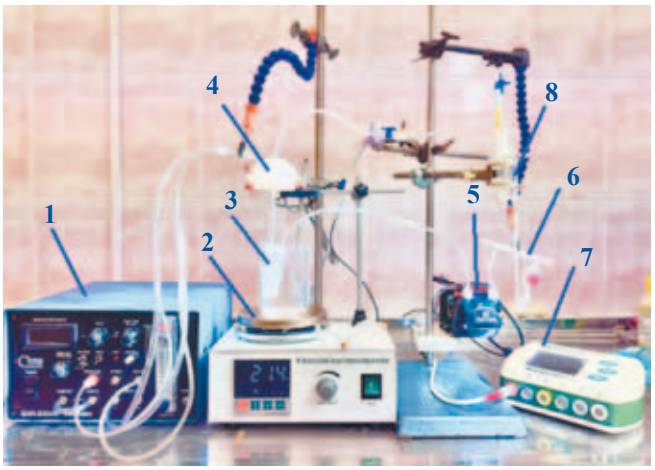

Fig. 2. The developed bench for normothermic machine perfusion of lungs in small animals (1, ventilator; 2, heating device; 3, sealed container; 4, developed container for donor lungs; 5, roller (peristaltic) pump; 6, developed low priming volume membrane oxygenator; 7, pressure measuring apparatus; 8, bubble filter)

dimensions of the base with an opening for perfusate evacuation, the plate for placing the donor lungs of the animal and the container lid itself were all designed in this software environment and on the basis of topographic-anatomical studies on the rats (Fig. 3, b).

The container parts were created based on mathematical models (Fig. 4). Despite the small size (assembled: depth 30 mm, height 45 mm, diameter 65 mm), the design provided full functionality and efficiency.

Donor lungs were ventilated using a SAR-830/AP device (CWE, USA). The outlet of the organ container was connected to a flask. The sealed plastic container was placed in a heat-resistant laboratory beaker with distilled water and an immersed temperature sensor to control the temperature. The beaker was placed on a heating device (XMTE-205, China), which monitored the temperature of perfusate in the circuit.

The heat-exchange flask was connected in series with a Kamoer roller peristaltic pump (KCM-S403-ODMA, China). The speed range of this pump is from 0.1 to

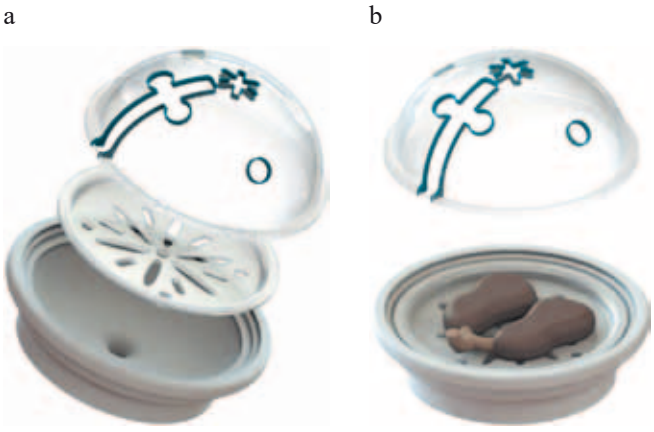

Fig. 3. The container for the donor lung of small animals: a, three-dimensional mathematical model of all components; b, topographic-anatomical location of the donor lungs in the chamber

350 rpm, which is suitable for the purposes of this study. Perfusate flow rate was reproduced within 1% at fixed rotational speed. After the pump, the perfused solution was passed through a specially designed membrane oxygenator with a low priming volume [11, 12]. This component was designed in a mathematical environment (Fig. 5, a). The inlet and outlet temperature perfusion streams at the selected design were analyzed in Ansys (ANSYS Inc., USA), a universal finite element analysis software system (Fig. 5, b).

The developed oxygenator was connected by a system of trunk lines to a bubble filter acting as an air trap in the system, and then the circuit was closed by a cannula entering the pulmonary artery of the donor lungs of the animals. The circuit also included ports for pressure measurement using a universal module based on the OEM IBP Angioton (Biosoft-M, Russia).

RESULTS

Male Wistar rats weighing 300–350 g (N = 6) were used for testing and evaluation of the functional characteristics of HTB. After heart-lung procurement and cold preservation of donor lungs, *ex vivo* normothermic perfusion was performed for 120 minutes, where the main parameters – oxygenation index (OI), pulmonary artery pressure (PAP) and peripheral vascular resistance in the lungs – were measured. During animal preparation and the donor lung procurement procedure, 20 ml dextran-40-based perfusion solution was poured into the circuit and circulation was switched on to complete a full circle and heat the perfusate. The system for monitoring *ex vivo* donor lung perfusion parameters continuously recorded the main parameters – PAP and volumetric flow rate – during two hours of the procedure. The heart-lung complex during the *ex vivo* normothermic perfusion procedure with dextran-40-based solution is presented in Fig. 6.

Fig. 4. Manufactured parts of the container for placing donor lungs of small animals

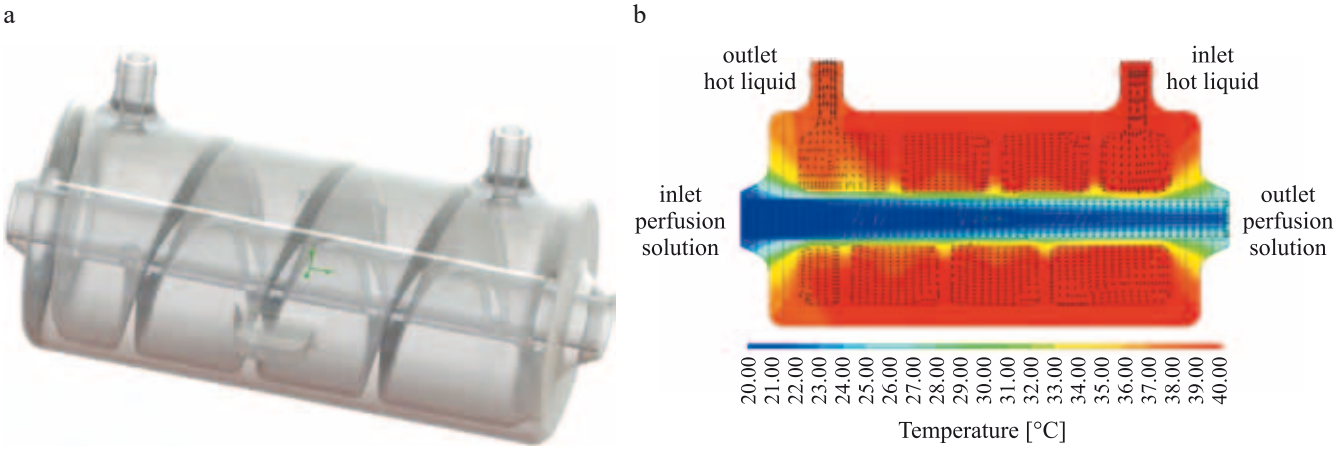

Fig. 5. Membrane low-volume oxygenator: a, three-dimensional model; b, analysis of temperature perfusion flows in the oxygenator design

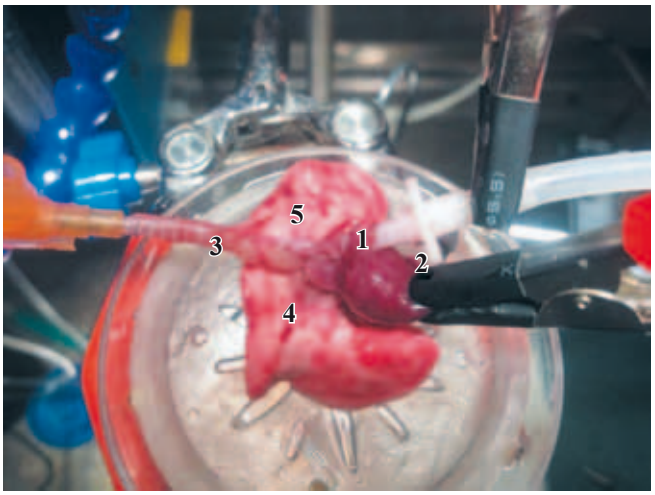

Fig. 6. Donor lungs and heart of an experimental animal (1, pulmonary artery; 2, left atrium; 3, trachea; 4, left lung; 5, right lung)

In a series of experiments to evaluate the functionality of the test bench during *ex vivo* normothermic perfusion of the lungs, the PaO_2/FiO_2 ratio, OI, PAP and peripheral pulmonary vascular resistance (pPVR) were measured. The measured results were entered into a data register, processed and summarized graphically (Fig. 7). All calculations were performed and compared by one-way analysis of variance (one-way ANOVA) using 95% confidence interval; the statistical significance of differences was considered reliable at $p < 0.05$.

OI was the main indicator of pulmonary function assessment and *ex vivo* perfusion efficiency that was investigated. The average OI at the beginning of *ex vivo* perfusion on the developed stand was 355 ± 20; in the course of the procedure, OI tended to increase, which by the end of the study averaged 460 ± 32 ($p = 0.028$). PAP constancy was the main criterion for the absence of technical problems during *ex vivo* donor lung perfusion. At the beginning of the procedure, PAP values averaged 9.07 ± 1.1 mmHg, and by the end of the study, where the mean value was 8.47 ± 0.4 mmHg, there was no statistically significant difference ($p > 0.05$). The pPVR was a calculated index demonstrating the compliance of the vascular bed, which suggests that both the *ex vivo* perfusion itself and the technical design of the bench are effective. Thus, pPVR decreased with time, where the initial mean value of the index was 603.3 ± 56 dynes·sec/cm^{-5}, and by the end of the procedure, mean pPVR decreased to 89.1 ± 15 dynes·sec/cm^{-5} at $p = 0.000$.

CONCLUSION

The design effort led to the development and testing of an experimental model of the HTB with a low priming volume for *ex vivo* normothermic machine perfusion of rat lungs. The obtained results demonstrate that functional and design features of the HTB not only maintain the initial functional status of the lung transplant, but also improve donor organ parameters. The presented data showed how effective and technically sound the developed HTB was.

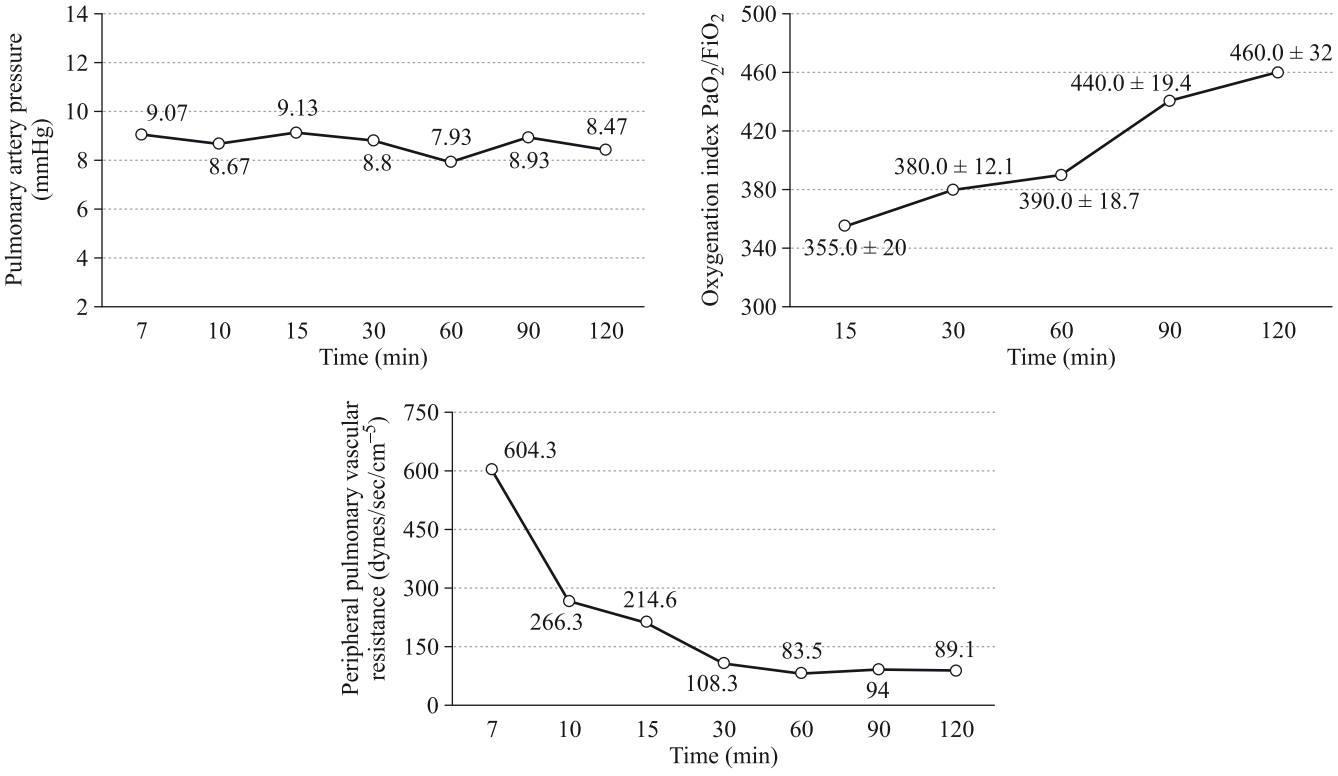

Fig. 7. Average values of parameters measured during experimental studies

The development of new techniques for examining the pathophysiological aspects of the impact of EVLP and lung transplantation on ischemia-reperfusion injury in donor organs is crucial for the development of new medications, perfusion, and preservative agents. One significant step in this process is the introduction and improvement of a HTB with a low priming volume for *ex vivo* normothermic perfusion of donor lungs on a rat model.

The authors declare no conflict of interest.

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