Comparative Study of Capillary Filtration Coefficient (Kfc) Determination by a Manual and Automatic Perfusion System. Step by Step Technique Review.

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Received July 4, 2018
Accepted February 14, 2019
Epub Ahead of Print November 7, 2019

Summary

The purpose of calculating the capillary filtration coefficient is to experimentally evaluate edema formation in models of pulmonary ischemia-reperfusion injury. For many years, the obtaining of this coefficient implies a series of manual maneuvers during ex-vivo reperfusion of pulmonary arterial pressure, venous pressure and weight, as well as the calculation of the Kfc formula. Through automation, the calculation of capillary filtration coefficient could be easier and more efficient. To describe an automatic method designed in our laboratory to calculating the capillary filtration coefficient and compare with traditional determination of capillary filtration coefficient as gold standard method. An automatic three valve perfusion system was constructed, commanded by a mastery module connected to a graphical user interface. To test its accuracy, cardiopulmonary blocks of Wistar rats were harvested and distributed in manual (n=8) and automated (n=8) capillary filtration coefficient determination groups. Physiological parameters as pulmonary arterial pressure, pulmonary venous pressure, weight and capillary filtration coefficient were obtained. Results: Capillary filtration coefficient, pulmonary arterial pressure, venous arterial pressure shown no statistical significance difference between the groups. The automated perfusion system for obtaining Kfc was standardized and validated, giving reliable results without biases and making the process more efficient in terms of time and personal staff.

Key words

ex-vivo perfusion • Isolated organ • Lung edema • Capillary filtration coefficient • Automatic method

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Introduction

There are several experimental methods to evaluate pulmonary tissue edema, for example: imaging techniques, thermodilution and gravimetric methods; among which stand out, determination of wet lung, weight / weight ratio of wet lung, weight / dry lung weight ratio and capillary filtration coefficient (Kfc), the latter being more specific for the assessment of capillary permeability (Parker and Townsley 2004), and has been the method of choice for more than 40 years (Gaar *et al.* 1967). Kfc is a product of hydraulic conductivity (Lp) and filtration surface area (SA) that evaluates convective transport through the endothelial barrier (Parker and Townsley 2008). To perform the Kfc calculation it is necessary to have a perfusion system that allows the acquisition of lung weight in real time, pulmonary arterial pressure (PAP), pulmonary venous pressure (PVP) and capillary pressure (CP) (Drake, Gaar *et al.* 1978), which is obtained by the double occlusion method (Townsley *et al.* 1986, Kongstad and Grande 1998) from a isogravimetric state.

When the experiments are performed using the manual method, the researcher needs to know the time and all the maneuvers it requires, because in the phases that correspond to obtaining CP, it is important to clamp both the arterial side as well as the venous side of the

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circuit, and during the phase of the weight gain, the PVP must be increased manually. Therefore, the researcher must have a great experience and also rely on a staff member to perform these maneuvers with great precision and in a coordinated manner.

After a review of the literature, the use or development of some automated perfusion systems was not found in research. Therefore, it is hypothesized that an automated system of ex vivo lung perfusion could reduce biases, facilitate the researcher to register the parameters automatically in the conventional office package and finally calculate the Kfc.

To achieve these objectives, a domain module was built to automatically open and close the valves, turn on and off the rodent ventilator and the peristaltic pump, controlled through a graphical interface that was easily manipulated, and at the end of the experiment the Kfc was obtained automatically in an Excel sheet.

Finally, the validation of this automatic perfusion system was carried out by comparing the Kfc in the pulmonary blocks perfused by both methods, manual and automatic.

Materials and Methods

The protocol was approved by the Animal Research Committee of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México (protocol number, CEX-56-11/13-1), in compliance with the official Mexican guidelines for laboratory animals (NOM 062-ZOO-1999) and in accordance with the Guide for the Care and Use of Laboratory Animals (1985).

Manual determination of Kfc

Perfusion system

The perfusion system used was composed of (Figs 1A, B): isolated organ perfusion glass (Kent Scientific, Inc., USA.), bubbles trap glass (Kent Scientific, Inc., USA.), 3 metal holders (Kent Scientific, Inc., USA.), weight sensor (Kent Scientific, inc., USA.), 2 pressure sensors to register PAP y PVP (Medex LogiCal, USA.) which are connected to pre-amplification modules (Kent Scientific, Inc. TRN006) in order to transform analog signals of PAP, PAP and W to digital signals. A peristaltic pump (Masterflex, 7523-30), thermostat (Polyscience, model 8005), connected to perfusion circuit for maintaining a steady temperature, rodent ventilator (Kent Scientific, RSP 1002), computer with a data acquisition card (DT 300, data translations) and data acquisition software (Matlab 2007, Matworks).

Graphical user interface

A graphical interface was performed and programed in Matlab software in order to visualizing: PAP, PVP, CP, W and in numerical and graphical way in real time. After recording acquired data, they were exported to Excel (Office 2011, Microsoft) where the means of PAP, PVP, CP and finally Kfc were manually calculated.

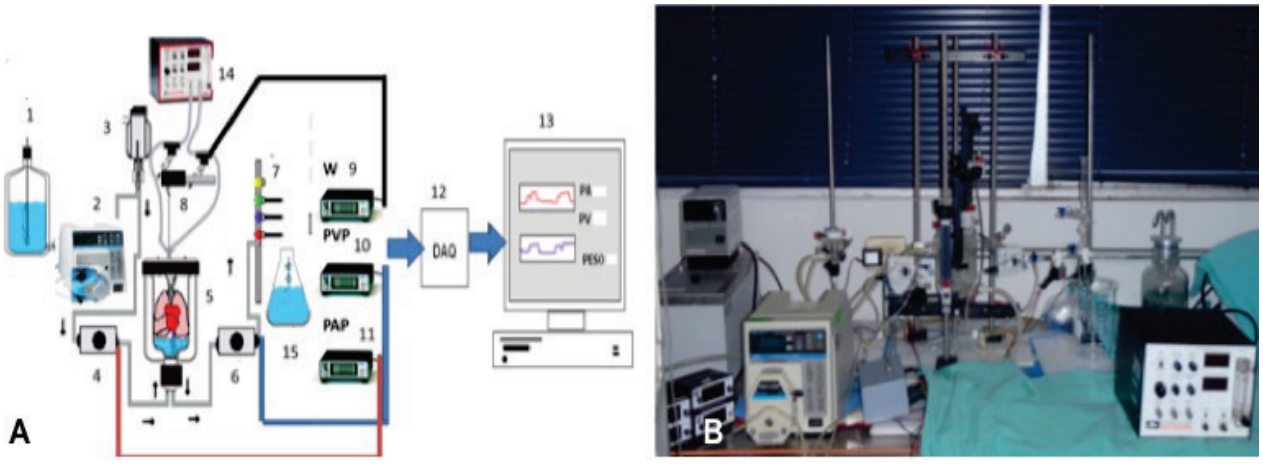


Fig. 1. A) Scheme illustrating the Perfusion System unit. The system consists of a perfusion solution (1), peristaltic pump (2), bubble trap (3), PAP sensor (4), isolated organ perfusion glass (5), PVP sensor (6), water column (7), W sensor (8), W pre-amplifier (9), PVP pre-amplifier (10), PAP pre-amplifier (11), acquisition data card (12), computer (13), rodent ventilator (14), outlet recipient (15). In thin arrows shows the flux direction, the thick arrows show the direction of the acquired data, the connection between the PAP sensor and the PAP pre-amplifier are show in red line, Finally, the blue line shows the connection between the PVP sensor and PVP pre-amplifier. **B**) Perfusion system photography.

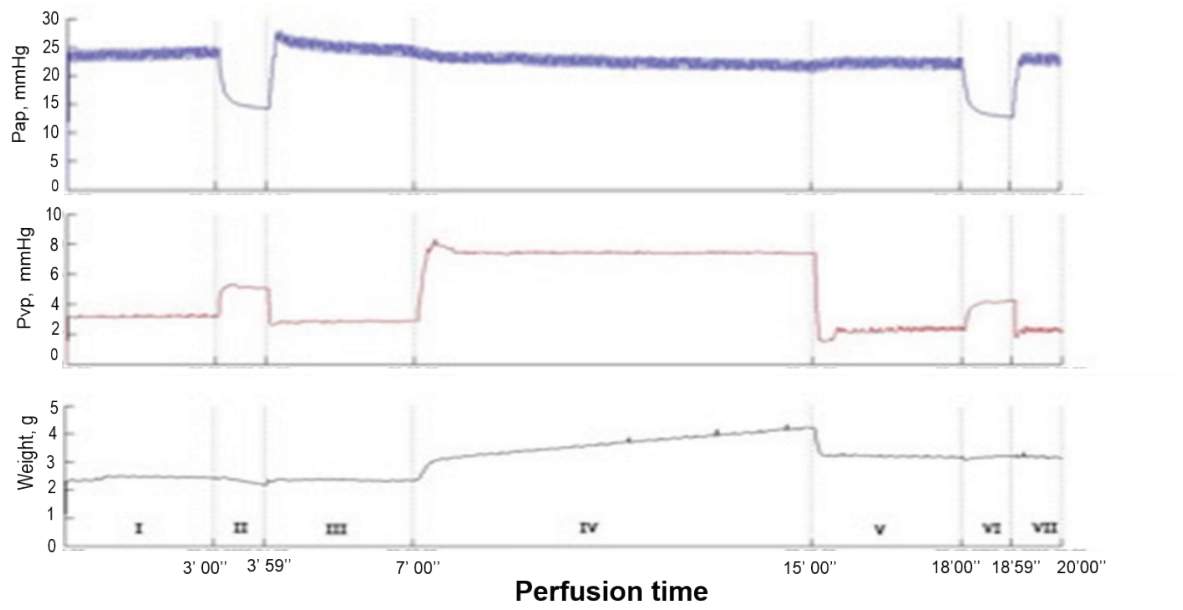


Fig. 2. Experimental phases. I. Isogravimetric state, II. CP1, III. basal line 1 IV. PVP elevation V. basal line 2 VI. CP2 VII. basal line 3.

Kfc calculation

The Kfc was calculated by Drake modified method (Equation 1). Each experiment was carried out for 20 min, which was composed of VII phases (Fig. 2): I. Isogravimetric state (0-3 min). II. Capillary pressure 1 (CP1) (3-4 min). III. Basal state 1 (4-7 min). IV. PVP elevation (7-15 min); achieved by rising abruptly and manually from 2 to 7-8 mmHg through three-way connector, V. Basal state 2 (15-18 min). VI. Capillary pressure 2 (CP2) (18-19 min). VII. Basal state 3 (19-20 min). The slow weight gain (8-15 min) was taken from phase IV for Kfc calculation, because in this phase intravascular flow flux to interstitial space. For CP acquisition, double occlusion maneuver was made by manual clamping. The PAP and PVP means were calculate from the phases I and V. PVP elevation was made manually.

$$(\Delta W/\Delta t)t_0/\Delta P_C$$

Equation 1. ΔW = Lung weight difference, Δt = Time difference, t_0 = extrapolated to zero time, ΔP_C = Capillary pressure difference.

Automatic determination of Kfc

Perfusion system in automatic method

The same perfusion system was used and 3 valves were placed (USM826074.ASCO) for automatic clamping to sense CP and to rise the PVP whenever it was required. (Fig. 1)

Master module

By biomedical engineering, an electrical module was designed and built. It opens and close the valves, and turn on or off the peristaltic pump and the rodent fan by supplying electrical power, and a graphical user interface previously designed and connected to the module to control it from a personal computer. This module was developed in the experimental surgery department.

Graphical User Interface for automatic method

Another interface was designed from the previous one with the aim of showing PAP, PVP, W and CP in numerical and graphic form in real time, but also to automatically manage the perfusion system. Depending on the needs, the interface was powered by specific commands, showing two main windows. The first, to calibrate the amplifiers before each experiment, was performed with the lungs placed in the perfusion system (Fig. 3A). The second plotted the acquired data in real time in numerical and graphic form (Fig. 3B).

In phases I, III, V, VII, the 3 valves remained open, the peristaltic pump and the ventilator remain on. Phases II and VI, valves 1 and 2 were closed simultaneously, which led to double occlusion, at this stage the third window was deployed and showed the CP in real time (Fig. 3C). In phase IV, the PVP was increased, valves 1 and 2 remained open while the third was closed. Therefore, PVP increased due to the flow of a column from 0 to 10 cmH_20. At the end of each of the experiments, the program automatically stopped. After

that, all the data was sent to the Excel package and the values of Kfc, PAP, PVP, CP, ΔW / Δt and finally ΔPC were plotted both numerically and graphically (Fig. 4). In addition, a window was displayed to review the data or video recording. Finally, the interface included a digital filter (low pass 0.00001 Hz) to reduce external noise, in order to obtain accurate data (Fig. 5). The calculations of Kfc, PAP, PVP, CP were made with the same formula used to calculate the Kfc using the manual method.

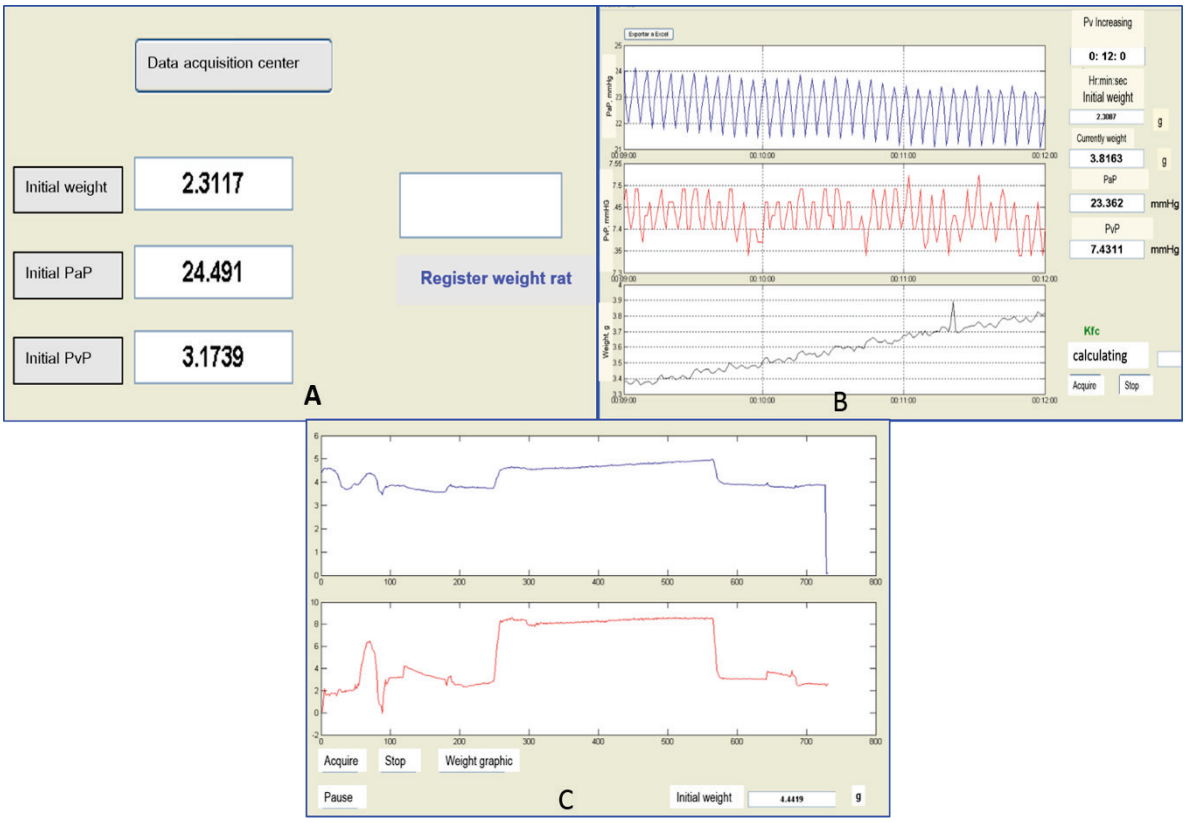
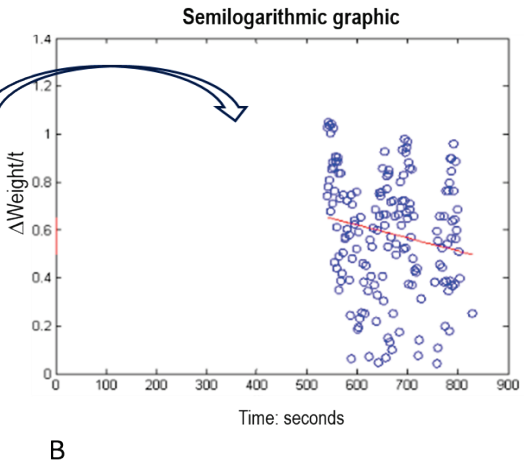


Fig. 3. Graphical user interface used in automatic method. **A**) Calibration window for automatic perfusion system. This window is displayed in order to calibrate the pre-amplificators a-priori of each experiment. It shows: initial weight, PAP and PVP. The buttons placed below switch on or off the peristaltic pump, rodent ventilator and valves to purge the perfusion system. **B**) Window of acquisition data. It shows in real time, from up to down: PAP, PVP and W. In the right side shows PAP, PVP, W numerically and in real time. **C**) CP acquisition data window. The upper windows displayed arterial and venous CP1 in real time; inferior windows show arterial and venous CP2. It displays automatically in phases when PC have to be measured.

Pulmonary arterial Pressure	Pulmonary venous Pressu	Weight	Filtered out weight	ΔW	ΔW/Δt	norm(ΔW/Δt)	log10(norm(ΔW/Δt))
0	0	0	0.0000000	0.0009	0.055368	5.5367936	0.743258
27.450980	1.0528725	5.04005	0.0148843	0.0017	0.106965	10.696477	1.029291
23.697261	1.0223545	4.90578	0.0300474	0.0018	0.112613	11.261305	1.051589
25.497826	1.0223545	4.92409	0.0472612	0.0010	0.05997	5.9970096	0.777935
27.011528	1.0528725	4.99428	0.0679531	0.0010	0.064192	6.4192484	0.807484
26.081010	1.1444266	5.04005	0.0893286	0.0017	0.109146	10.91458	1.038007
23.483635	1.1139086	4.85695	0.1237624	0.0016	0.101098	10.109777	1.004742
25.436790	0.9918364	4.91188	0.1611574	0.0008	0.047798	4.7797714	0.679407
27.145800	1.0223545	4.99733	0.2065085	0.0014	0.071525	7.1524957	0.854458
25.879301	1.0528725	5.00649	0.2604354	0.0018	0.110347	11.034733	1.042762

A



B

Fig. 4. Data sent from graphical interface to Excel. When the experiment has been finished, the results are automatically displayed in numerical and graphical way in Excel. **A**) Excel data sheet shows all data (PAP, PVP, W, filtered W, ΔW, ΔW/Δt, logΔW/Δt) and the means values of PAP, PVP, PCP, CP. **B**) Simultaneously Kfc is plotted both numerical value and the semilogarithmic graphic.

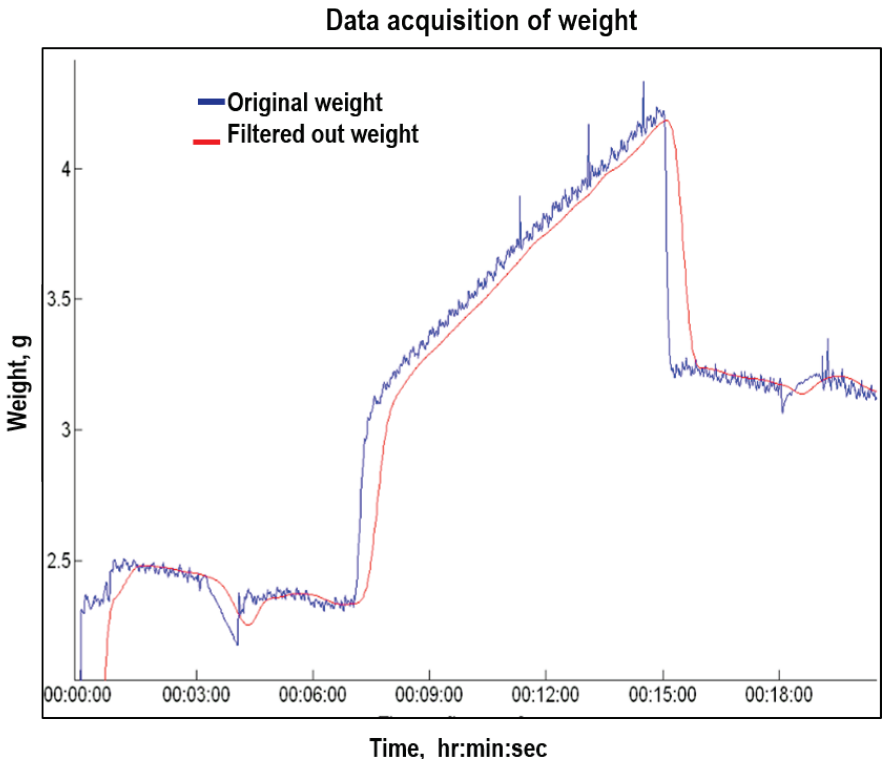


Fig. 5. Filtered weight. The blue line shows the acquired weight in real time. In contrast, the red line plots the filtered weight.

Automatic Method vs. Manual Method

To evaluate the function of the automatic method, Kfc was determinated in rat lung blocks by the two methods, automatic and manual.

Animals

Wistar rats (N=16) with body weight of 300-400g were divided in two groups: Manual group (n=8): Cardiopulmonary blocks were harvested and immediately perfused; the Kfc was calculated by manual method. Automatic group (n=8): Cardiopulmonary blocks were harvested and immediately perfused; Kfc was calculated by the automatic method. All animals were managed according to the NOM-ZOO-062-1999 and the protocol was approved by Animal Research Committee of "Instituto Nacional de Ciencias Medicas y Nutrición "Salvador Zubirán" (CEX-56-11/13-1).

Cardiopulmonary block harvesting

The rats were anesthetized with pentobarbital (50 mg/Kg, ip) and tracheostomy was realized for placing to rodent ventilator with 10 ml/Kg tidal volume and 60 strokes per minute. Medium sternotomy was realized, cranial and caudal cava veins, as well as, aorta pulmonary artery (Parker and Townsley) were referred with suture silk (3-0) and dissected. Heparin 1000 UI was administrated in the right ventricle and the cardiac apex was sectioned. Posteriorly, two cannulas were inserted; one in the right ventricle, specifically in PA, and the another in the left atrium through the left ventricle (LA). The lung block was flushed with Krebs-Henseleit solution through the PA cannula until it turned clear through the LA cannula exit. At last, the cardiopulmonary block was taken out.

Cardiopulmonary block perfusion

The cardiopulmonary block was wrapped with plastic membrane; furthermore, it was set and suspended at weight sensor transducer, and placed in to isolated organ glass. The PA and LA cannulas were connected to the perfusion system, besides that, the tracheal cannula was connected to rodent ventilator. The lungs were reperfunded with Krebs-Henseleit solution (0.013 ml/kg/min) and ventilated with room air (O_2 21 %), 60 strokes per minute and 2-3 cmH_20 of PEEP, maintaining the lungs in zone 3 (PAP>PVP> alveolar pressure (Pa)).

Statistical analysis

The results were expressed in mean ± standard error, Student *t* test was carried on to compare between groups (SPSS. V20. $p<0.05$).

Results

Parameters of PAP, PVP, and CP were obtained by both, manual and automatized methods. The means ±

SE in manual and automatic group were respectively (mmHg): PAP: 19.00±2.17; 20.31±1.23, p=0.13. PVP: 3.03±0.37; 2.23±0.29, p=0.35. CP1: 5.89±1.06; 7.56±0.86, p=0.59. CP2: 6.99±1.07; 5.10±0.73, p=0.45. ΔCP: 2.86±0.35; 2.55±0.30, p=0.89. There was no statistical significance difference between the groups. (Table 1)

Although in manual method the mean of CP1 determination time was of 1.30 min, and CP2 was 1.40 min in average, the automatized method did not give difference between CP1 and CP2 because the time of its recording was exactly 1 min. However, there was not significant difference between groups.

Kfc determination

Kfc values were expressed in ml/min/mmHg/100gr (mean ± SE): Manual group: 0.28±0.028. Automatic group: 0.21±0. Kfc was slightly higher in manual group than automatic group, but there was no significant difference between the groups, p=0.45, (Table 1).

Table 1. Kfc values are expressed in ml/min/mmHg/100g. PAP, PVP, PC1, PC2 and ΔCP are shown in mmHg. p<0.05

	Kfc	**PAP**	**PVP**	**CP1**	**CP2**	***Δ*CP**
Manual	0.28±0.02	19.00±2.17	3.03±0.37	5.89±1.06	6.99±1.07	2.86±0.35
Automatic	0.21±0.09	20.31±1.23	2.23±0.29	7.56±0.86	5.10±0.73	2.55±0.30
p value	0.45	0.13	0.35	0.59	0.45	0.89

Discussion

Design and creation of automatic perfusion system.

There are several reports in the literature about the Kfc calculation. Since 1969 many research groups around the world have been using perfusion systems widely with the aim of evaluate lung injury. Although this technique is realized in multiple laboratories, there are a little bit reports that pretend making easier the Kfc obtaining (Bernard *et al.* 1997, Guerra-Mora *et al.* 2017). In addition, there are not reports in medical literature about implementation of automatic system for reperfusion of isolated organs, even, nowadays some perfusion systems are successfully used to perfuse ex-vivo lung block (Noda, Shigemura *et al.* 2014, Tanaka, Noda *et al.* 2015). For that reason, it was designed and constructed an automatic perfusion system for Kfc calculation. Some advantages of this system are that it can be implemented for evaluating other variables in other organs; for instance; liver, bowel, kidney and heart. Besides that, it can be modified according the needs of researchers and under different experimental conditions. For their development and building was necessary the knowledge of a biomedical engineer in order to obtain the interface with specifications that researcher asks according to the behavior of the experiments. Also the interface designed included a digital filter, hence the external signals (noise generated by other devices) can be eliminated (Fig. 5). Finally, with some mathematical formulas, experiment data are exported in numerical and graphic way automatically to excel, accessible software for everyone that facilitates the use of the data in statistical software.

Perfusion system validation

The automatic perfusion system gives feasible data, because there was not significant difference between groups.

For one accurate of Kfc obtaining, researcher should take care of the following conditions (Bernard, Dahlby *et al.* 1997):

1) Increased microvascular filtration causes weight gain;

2) the surface area of filtration remains constant between different experimental conditions;

3) Starling forces are constant both during the weight increase, as well as in different conditions; and

4) barrier sensitive endothelial signaling is not related during the weight transient.

All conditions above mentioned were achieved in these experiments. Firstly, PVP elevation lasted 8 min, of which, the first minute was eliminated because it corresponds to fast weight gain and the other 7 min are from slow weight gain, which are used to calculate Kfc. Some authors suggest that the minimum time of PVP elevation to obtain reliable results, it is about 18 to

20 minutes; however, in experiments in which lung injury is induced, the time of PVP elevation can be less, because the reperfusion model can induce edema quickly by itself. All experiments were made in zone 3 ventilation (PAP>PVP>Pa), because in this mode, the microvascular bed does not change in number as long as the experiment last, due to the augmentation of the alveolar pressure. However, when PVP is increased these capillaries are recruited and distended. The lung blocks were perfused with Krebs-Henseleit solution, which contains albumin and maintains the coloidosmotic pressure in intravascular liquid. It is important to keep in mind if great edema formation exist, the interstitial pressure increase and this could lead us to underestimate the Kfc. Additionally, the researcher could realize this experiments easily and could made other activities while lung blocks are perfused with automatic device, whereas by manual method, the researcher was unable to realize another activity. By manual method, calculation time of the variables was too long and tedious, and the probability of making mistakes is high. Likewise, with the automatic method, the researcher was able to realize another surgery in the same time that the cardiopulmonary block was prefunded, being more efficient in the laboratory, the results were obtained immediately and 100 % feasible, showing the same results in a mathematical analysis that was made by manual method and the user interface was easy to use.

Conclusions

The automatic reperfusion system was standardized and validated, its building was relatively easy giving reliable results without biases and saving time to the researcher. The system is easy to reproduce in other laboratories and to work with it.

Conflict of Interest

There is no conflict of interest.

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