Mechanical properties of decellularized lungs.

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Abstract: The matrix of decellularized organ retains the three-dimensional architecture and biochemical composition, as well as the original tissue microvasculature. This propertie make the decellularized lung very promising for the generation of functional bioartificial organs. This study aims to describe an experimental animal model that allows to analyze the behavior of the mechanical properties of decellularized lungs.

Keywords: Respiratory Mechanics, Lung, Bioengineering, Decellularization, Biomedical Engineering.

INTRODUCTION

The interpretation of ventilatory variables volume, flow and pressure of the respiratory system under both physiological and pathological conditions, allows evaluate the mechanical behavior of the system and its isolated components [1]. Several studies with experimental models of animal showed that the mechanical properties can be checked separately in the lungs or respiratory system, comprising lungs and the chest wall.

The study of mechanical properties of lung tissue assumes evaluation of unicompartmental linear model (Figure 1) proposed by Otis (1956), in which it does not consider the slow pressure drop after occlusion of the airways at the end of inspiration, whereas elastance and resistance are dependent on the respiratory rate and hysteresis [2].

Bates et al. (1985) have proposed two-compartmental model where the viscoelastic mechanical properties of the tissue are represented by three elements: a resistor (Rt, or air damper), and two springs (E1 and E2) (Figure 2). The three components the R, R1, R2 together form what is known as Kelvin body. The spring stiffness E1 represents the static elastic behavior of the lung and the series combination of RT and E2 (which together form the Maxwell body) are responsible for the viscoelastic behavior [3].

According Kochi et al. (1988), during an inspiratory pause, the accumulated potential energy in the elastic components can be dissipated as heat in the resistive components. Since the dissipation of energy by lung tissue does not end at the same time that it stops the flow [4].

Compared to other organs, the lung structure is particularly complex, hindering the process of bioengineering. Despite this complexity, it has been shown that with the use of suitable protocols, the lung may be completely intact to obtain a decellularized extracellular structure [5]. The present study describes an experimental animal model that can be used in the evaluation of the behavior of the mechanical properties of decellularized lungs.

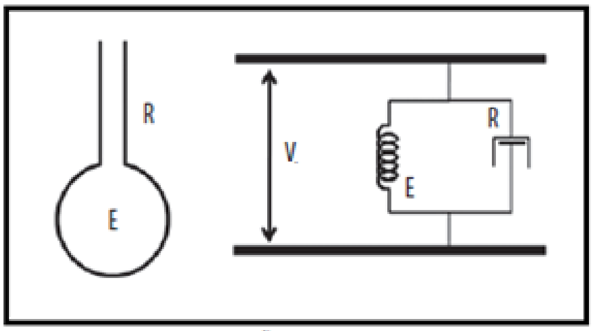

Figure 1. Anatomical and mechanical representation of linear Unicompartmental lung model.

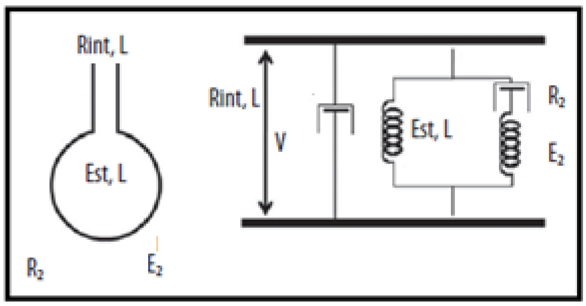

Figure 2. Anatomical and mechanical representation of a rheologic bicompartimental lung model.

MATERIALS AND METHODS

Experimental protocol – Small rodent animals such as mice or rats, can be used in this experimental model. Initially, the animals are sedated and anesthetized for being tracheostomized.

This procedure consists of a small longitudinal incision in the anterior neck, where the adjacent tissues are removed until the trachea is exposed.

Then, a longitudinal incision made between two fiber rings for introducing a cannula length and diameter appropriate for the individual animal (e.g. 0.5mm id for mice and 2.1 mm diameter for rats). After fixation, the endotracheal tube is connected to a pneumotachograph which is connected to a mechanical ventilator (Samay MVR17, Montevideo, Uruguay or Harvard Apparatus, model 683, South Natick, MA, USA) [7].

A pressure transducer is used to measure tracheal pressure (Ptr) (P23 Db, Gould-Statham, Oxnard, CA, USA) and differential pressure transducer (PT5A, grass, Quincy, MA, USA) used to verify the flow air airway (V'). The transducers are attached to the pneumotachograph, as illustrated in Figure 3.

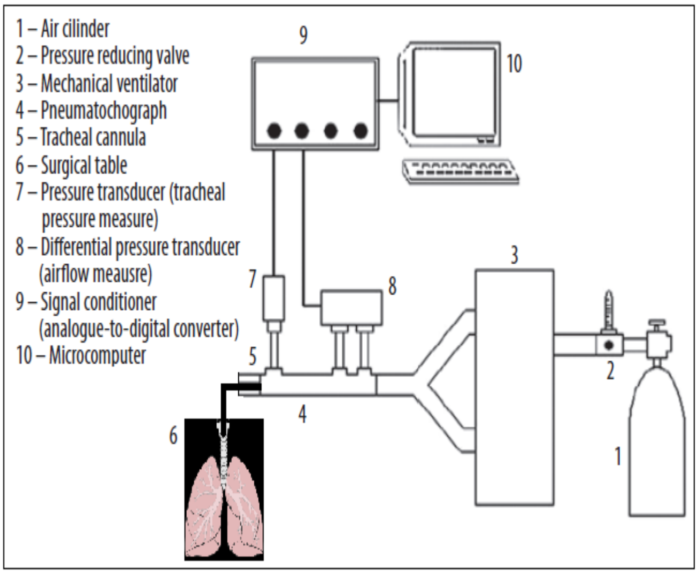

Figure 3. Set up of respiratory mechanics study.

The signal transducers are connected to a signal conditioner (*EMG System of Brazil*, SJCampos, Brazil) with eight analog input channels, 1000 × amplification, sampled at 250 Hz with a 12-bit digital analog, used in signal processing with the aid of a microcomputer and Windaq/Pro (DATAQ Instruments, Akron, OH, USA) software. The ventilator flow is generated by a source of compressed air using a pressure reducing valve. The flow resistance produced by the system (req), including the endotracheal tube should be considered. The pressure resistance of equipment is subtracted from the resistive pulmonary pressures in order to make the intrinsic values close to the real [7].

After tracheotomy procedure, the animals are paralyzed with curare for inducing muscle relaxation and then the tracheal tube is connected to the pneumotachograph and the ventilator controlling tidal volume (VT), airflow (V') and positive end expiratory pressure (PEEP) .

With the stabilization of ventilatory parameters, its possible initiates the maneuvers to verify the behavior of the mechanical properties of the lung or respiratory system. The techniques of inspiratory pause or equation of motion can be used [8].

Equation of motion analysis – Pulmonary resistance and compliance are calculated from signals recorded during mechanical ventilation. First the signal of V is calculated by digital integration of the V'. After recording, the sign of tracheal pressure (Ptr) is corrected by subtracting the pressure drop (PCAN) caused by non-linear resistance of the intubation tube, which had been previously calibrated and characterized (PCAN • K1 = V '+ K2 • |V'| • V, where K1 and K2 are linear and nonlinear parameters of a model Rohrer). Further, the resistance and elastance of the lungs decellularized are calculated by linear regression of the appropriate signals recorded Ptr, V 'and V with conventional mechanical breathing model Ptr = EL + E • • V RL + V' where E is a parameter PEEP to measure externally by the ventilator. For each lung resistance and elastance are calculated through five breaths [9].

End-inspiratory airway occlusion analysis – The respiratory system, with intact chest wall, tracheal pressure (Ptr) is dissipated by system pressure and esophageal pressure (Pes) is the pressure generated by the chest wall. When the analyzes are performed with open chest (chest wall removed), the Ptr is the pressure of the lung parenchyma. Following occlusion of the airway at the end of inhalation (Figure 4), there is a sudden drop in Ptr after the maximum value (Pmax) to a point of inflection (PI), from which the pressure drop becomes slower, reaching a plateau.

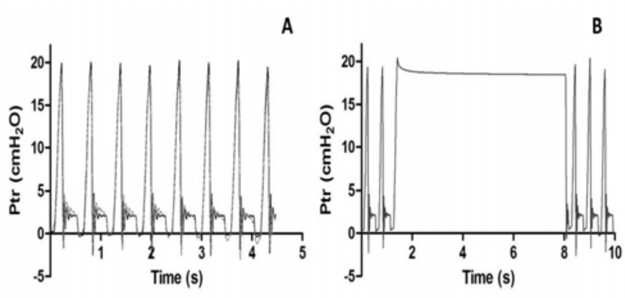

Figure 5. A: tracheal pressure (Ptr) during conventional mechanical ventilation. B: Representative example of the pressure recorded during an end-inspiratory occlusion.

The plateau phase corresponds to the elastic recoil pressure of the lungs (Pel). The pressure difference (ΔP1) featuring a rapid initial fall (as represented by the difference between Pmax and Pi) corresponds to the viscous component. The second variation of pressure (ΔP2), represented by the slow fall, which is verified from Pi to the plateau (Pel), reflects the pressure dissipated to overcome the viscoelastic component. The sum of ΔP1 and ΔP2 is the total change in pulmonary pressure (ΔPtot). The static elastance (Est) and dynamic compliance (Cdyn) are determined by dividing Pel and Pi, respectively.

The Est and Edyn be obtained by means of five end-inspiratory occlusions, each performed after a normal minute ventilation mechanical [10].

For obtaining Pi is used, a non-linear fit to two-exponential decay curves, determining time of fast and slow down, and thus from this, the pressure value at the time of passage for $\Delta P1$ and $\Delta P2$.

RESULTS AND DISCUSSION

The evaluation of the mechanical properties of the respiratory system is specifically carried out by the oscillation of flow through the trachea into the lungs, by measuring the resulting pressure generated in the trachea and in accordance with a mathematical model relating the two signals with each other [11].

To evaluate lung mechanics, has already been reported that acellular organs are undergoing conventional mechanical ventilation to measure resistance and elastance, as is usually done in humans [12].

An study of our group show the mechanical changes throughout the different steps of lungs decellularization process. Lungs resistance (RL) and elastance (EL) were measured along decellularization steps and were computed by linear regression fitting of tracheal pressure, flow, and volume during mechanical ventilation. Transients differences found were more distinct in an intermediate step after the lungs were rinsed with deionized water and treated with 1% SDS, where upon the percentage of variation reached approximately 80% for resistance values and 30% for elastance values. In conclusion, although a variation in extracellular matrix stiffness observed during the decellularization process, this variation can be considered negligible overall because the resistance and elastance returned to basal values at the final decellularization step [13].

These studies of mechanical properties of decellularized lungs contributes to the successful bioengineering of organs.

CONCLUSION

The equation of motion and the occlusion method at the end of inspiration are effective for the evaluation of behavior of mechanical properties of decellularized and recellularized lungs.

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