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Short communication

Behavior of vascular resistance undergoing various pressure insufflation and perfusion on decellularized lungs

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ABSTRACT

Bioengineering of functional lung tissue by using whole lung scaffolds has been proposed as a potential alternative for patients awaiting lung transplant. Previous studies have demonstrated that vascular resistance (Rv) could be altered to optimize the process of obtaining suitable lung scaffolds. Therefore, this work was aimed at determining how lung inflation (tracheal pressure) and perfusion (pulmonary arterial pressure) affect vascular resistance. This study was carried out using the lungs excised from 5 healthy male Sprague-Dawley rats. The trachea was cannulated and connected to a continuous positive airway pressure (CPAP) device to provide a tracheal pressure ranging from 0 to 15 cmH_2O. The pulmonary artery was cannulated and connected to a controlled perfusion system with continuous pressure (gravimetric level) ranging from 5 to 30 cmH_2O. Effective Rv was calculated by ratio of pulmonary artery pressure (P_{PA}) by pulmonary artery flow (V'_{PA}). Rv in the decellularized lungs scaffolds decreased at increasing $V'_{PA,}$ stabilizing at a pulmonary arterial pressure greater than 20 cmH_2O. On the other hand, CPAP had no influence on vascular resistance in the lung scaffolds after being subjected to pulmonary artery pressure of 5 cmH_2O. In conclusion, compared to positive airway pressure, arterial lung pressure markedly influences the mechanics of vascular resistance in decellularized lungs.

1. Introduction

In 2012, more than 1300 patients were awaiting lung transplantation in the United States of America (Organ Procurement and Transplantation Network–OPTN) owing to the limited supply of donor lungs. Since lung transplantation is often complicated by chronic rejection and adverse effects associated with immunosuppressive treatment (Barberà et al., 1994; Lopez et al., 2006), novel alternatives are required.

Recently, the engineering of bioartificial organs by using scaffolds with an aim to regenerate functional lung tissue has been proposed as a potential alternative for lung transplantation (Daly et al., 2012). However, for the proper functioning of the bioartificial organs, it is imperative that these scaffolds preserve the lung's structure and composition to present an ideal macro- and micro-environment facilitating cell attachment and engraftment for effective repopulation (Badylak et al., 2012; Ren et al., 2015).

Given that lung cells are exposed to different physical stimuli during breathing, the lung scaffold should be exposed to ventilation and perfusion stimuli mimicking the ones during normal breathing to provide a physiologically appropriate environment for seeding of stem cells in the decellularized lung. Previous study from our group demonstrated that effective vascular resistance varies considerably during the process of decellularization (da Palma et al., 2015). However, data on circulatory resistance of scaffolds as a function of airway and vascular pressures are unavailable.

This information is of considerable interest since adequate distribution of cells during scaffold seeding and subsequent cell homing could be modulated by vascular resistance. Monitoring vascular resistance could also be a useful quality control tool for future high-throughput production (da Palma et al., 2015).

Accordingly, this work aimed at determining the mechanism by which lung inflation (tracheal pressure) and perfusion (pulmonary artery) pressure affect vascular resistance.

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2. Methods

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health by using the lungs excised from 5 healthy male Sprague-Dawley rats (250–300 g). The experimental procedures were approved by the Ethical Committee For Animal Research of the University of Barcelona. The rats were anesthetized with intraperitoneal urethane (1 mg/kg, heparin 250 U/kg) and sacrificed by exsanguination through the abdominal aorta. Immediately after euthanasia, the diaphragm was punctured and the rib cage was cut open to reveal the lungs.

The lungs were perfused via the right ventricle with phosphate-buffered saline (PBS) containing 50 U/ml heparin (Sigma-Aldrich Co. LLC, St. Louis, MO, USA) and 1 μg/ml sodium nitroprusside – SNP (Fluka Analytical, Sigma-Aldrich Co. LLC, St. Louis, MO, USA) to prevent the formation of blood clots in the lungs. After perfusion was complete, the heart, lungs, and trachea were dissected and removed in bloc and stored in a –80 °C freezer until the decellularization process was carried out.

2.1. Lung decellularization

As described in a previous study (Nonaka et al., 2014), pulmonary decellularization was carried out by a combination of freezing/thawing methods, and SDS removed cellular debris while preserving the mechanical properties of the structure. No significant changes in the resistance values and elastance of the lungs were observed during conventional mechanical ventilation.

The first step in lung decellularization involves thawing the lungs in a water bath at 37 °C and freezing them again at −80 °C; this cycle was repeated four times. Once the trachea and pulmonary artery were cannulated and placed into the experimental system, the trachea was connected to a continuous positive airway pressure (CPAP) device that was set to provide a tracheal (i.e., transpulmonary) pressure of 10 cmH_2O to inflate the lung close to total lung capacity in an attempt to avoid atelectasis (da Palma et al., 2015). The following sequence of decellularizing process, the lungs were perfused through the pulmonary artery: 1) PBS 1 × for 30 min, 2) deionized water for 15 min, 3) 1% sodium dodecyl sulfate (SDS) for 150 min and 4) PBS for 30 min, at a pressure of 20 cmH_2O.

2.2. Vascular mechanics

To analyze vascular mechanics in the decellularized lungs, the cannulated trachea was connected to a CPAP device that was set to provide tracheal (i.e., transpulmonary) pressure ranging from 0 to 15 cmH_2O. The cannulated pulmonary artery was connected to a controlled perfusion system with continuous pressure (gravimetric level) ranging from 5 to 30 cmH_2O. A pressure transducer (011-OP229-01; ICU Medical, USA) and a differential pressure transducer (5100J0005H2Y5000; American Sensor Technologies, USA) allowed the measurement of pulmonary artery pressure (P_{PA}) and pulmonary artery flow (V'_{PA}), respectively, at the entrance of the pulmonary artery. These transducer signals were analogically low-pass filtered, sampled, and stored for subsequent analysis. Hence, continuous measurement of V'_{PA} and P_{PA} allowed for the assessment of effective vascular resistance (Rv) as $Rv = P_{PA}/V'_{PA}$.

2.3. Statistical analysis

All values are expressed as mean ± SE. Values of vascular resistance (Rv) and flow (V'_{PA}) at each pulmonary arterial (5–30 cmH_2O) and tracheal pressure (0–15 cmH_2O) value were compared by means of paired *t*-tests.

3. Results

As shown in Fig. 1, vascular resistance in the decellularized lungs decreased from ~6–7 $cmH_2O\ min\ ml^{-1}$ to ~3 $cmH_2O\ min\ ml^{-1}$ in response to an increase in pulmonary arterial pressure from 5 to 20 cmH_2O, remaining steady at up to 30 cmH_2O at the entrance of the pulmonary arterial system. Values of vascular resistance did not depend on CPAP.

4. Discussion

According to our knowledge, this is the first study reporting vascular resistance values in decellularized lungs as a function of variations in pulmonary artery and airway pressures. It is known that the absence of surfactant in decellularized lungs may cause

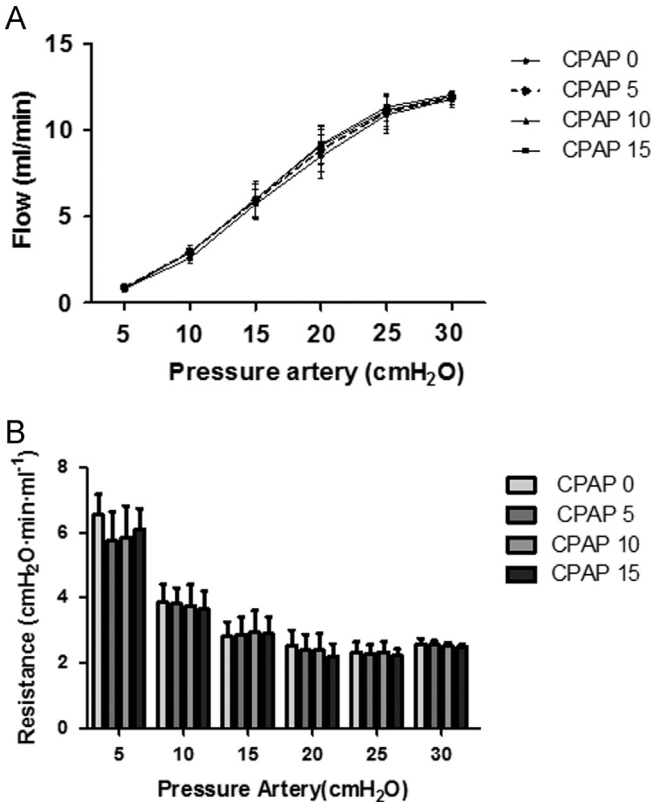

Fig. 1. Pressure (P_{PA}) and flow (V'_{PA}) at the pulmonary artery in the decellularized lung undergone diverse values of pressure perfusion and continuous positive airway pressure. Corresponding vascular resistance (Rv). Data are mean ± SE.

the alveolar walls to collapse; therefore, in a previous study, we used a CPAP of 10 cmH_2O to keep the lungs inflated during the decellularization process (da Palma et al., 2015). However, the influence of CPAP on vascular resistance in acellular lungs was not known. In this study, vascular resistance in the acellular lung was found to be almost constant at CPAP ranging from 0 to 15 cmH_2O, provided pulmonary arterial pressure > 15 cmH_2O.

Several studies have demonstrated successful transplantation after re-building of the lungs with stem cells; however, the lungs were capable of maintaining gas exchange for a maximum of 7 days (Petersen et al., 2010; Ott et al., 2010; Song et al., 2011). It seems clear, however, that the differentiation and maturation of cells in the reseeded graft need to be improved. According to Stabler et al. (2015), reconstitution of physiological pulmonary vasculature in its entirety will significantly improve the generation of whole lungs through bioengineering organs. Therefore, it could be expected that variations in arterial and alveolar pressures could influence cell adhesion, considering that a reduced flow through the lung circuit would decrease cell distribution. Hence, according to this study, we can suggest that the optimal value of flow and vascular resistance for optimal dynamics is achieved at physiological values of pulmonary arterial pressure (15–30 cmH_2O).

The decellularization process eliminates lung cells, i.e., type II alveolar epithelial cells, which secrete lung surfactant, thereby increasing the lung compliance as described previously (da Palma et al., 2015; Nonaka et al., 2014). Owing to low lung elastance, this decellularized lung can no longer increase the tension in the alveolar walls to alter vascular resistance, thereby explaining the slight influence of CPAP on vascular resistance.

In conclusion, we demonstrated that compared to positive airway pressure, arterial lung pressure markedly influences the mechanics of vascular resistance in decellularized lungs excised from healthy rats. This study provides information that could be

relevant for future stem cell repopulation by using vascular resistance as a facilitator of cell distribution throughout the pulmonary circuit.

Conflict of interest statement

The authors confirm that they have no financial affiliation or involvement with any commercial organization that has direct financial interest in any matter included in this manuscript.

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