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Research Paper

Pressure- and flow-controlled media perfusion differently modify vascular mechanics in lung decellularization

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ABSTRACT

Organ biofabrication is a potential future alternative for obtaining viable organs for transplantation. Achieving intact scaffolds to be recellularized is a key step in lung bioengineering. Perfusion of decellularizing media through the pulmonary artery has shown to be effective. How vascular perfusion pressure and flow vary throughout lung decellularization, which is not well known, is important for optimizing the process (minimizing time) while ensuring scaffold integrity (no barotrauma). This work was aimed at characterizing the pressure/flow relationship at the pulmonary vasculature and at how effective vascular resistance depends on pressure- and flow-controlled variables when applying different methods of media perfusion for lung decellularization. Lungs from 43 healthy mice (C57BL/6; 7–8 weeks old) were investigated. After excision and tracheal cannulation, lungs were inflated at 10 cmH_2O airway pressure and subjected to conventional decellularization with a solution of 1% sodium dodecyl sulfate (SDS). Pressure (P_{PA}) and flow (V'_{PA}) at the pulmonary artery were continuously measured. Decellularization media was perfused through the pulmonary artery: (a) at constant $P_{PA}=20$ cmH_2O or (b) at constant $V'_{PA}=0.5$ and 0.2 ml/min. Effective vascular resistance was computed as $R_v=P_{PA}/V'_{PA}$. R_v (in cmH_2O/(ml/min)); mean±SE) considerably varied throughout lung decellularization, particularly for pressure-controlled perfusion (from 29.1±3.0 in baseline to a maximum of 664.1±164.3 ($p<0.05$), as compared with flow-controlled perfusion (from 49.9±3.3 and 79.5±5.1 in baseline to a maximum of 114.4±13.9 and 211.7±70.5 ($p<0.05$, both), for V'_{PA} of 0.5 and 0.2 ml/min respectively. Most of the media infused to the pulmonary artery throughout decellularization circulated to the airways compartment across the alveolar-capillary membrane. This

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study shows that monitoring perfusion mechanics throughout decellularization provides information relevant for optimizing the process time while ensuring that vascular pressure is kept within a safety range to preserve the organ scaffold integrity.

1. Introduction

Lung bioengineering has recently emerged as a potential alternative for obtaining available organs for transplantation in the forthcoming years (Ott et al., 2010; Song et al., 2011; Nichols et al., 2012; Garcia et al., 2012). According to this approach, biofabricated lungs would help in reducing the current long waiting lists for transplantation, and also the increased expected demands caused by the progressive aging of the population in both developed and developing countries (Yusen et al., 2010). Although experimental studies have already established convincing proof of concept on the feasibility of lung bioengineering (Ott et al., 2010; Song et al., 2011), it should be mentioned that the field is still at its scientific infancy and thus requires considerable research before reaching evidence to plan the first clinical tests.

Given the structural complexity of the lung (typically having 300 millions alveoli, each with a diameter of 300 μm, separated from capillaries with a diameter of 10 μm by a membrane with a thickness of 3 μm and surface of 70 m^2; all packed in a volume of 5 l) and the inability of current technologies to construct such a micron-scale 3D structure, the approach for lung bioengineering is to use lung scaffolds obtained from natural organs (Price et al., 2010; Cortiella et al., 2010). Indeed, the concept for biofabricating a lung is to first decellularize an organ that is not available for transplantation and then to use its acellular scaffold as a platform for seeding cells that, after proliferating and differentiating, regenerate the lung (Ott et al., 2010; Song et al., 2011; Weiss, 2014).

The procedure for decellularizing a lung – or any other organ – should strike a difficult balance: it must be sufficiently aggressive to eliminate all the cell material from the donor but at the same time must be smooth enough to preserve the structure and composition of its extracellular matrix (Badylak et al., 2012). Although how to optimally obtain acellular lungs has not been so far clearly established, different published protocols have shown to provide lung scaffolds exhibiting reasonable quality in terms of low DNA donor load and high preserved extracellular matrix structure and composition (Ott et al., 2010; Daly et al., 2012; Bonenfant et al., 2013; Gilpin et al., 2014a).

Perfusion of different decellularizing media (e.g. deoinized water, detergents, enzymes) through the vascular circuit of the organ is a conventional procedure for eliminating donor cell material since the widespread distribution of the capillary network within the organ allows both to distribute the media to short diffusion distance from any cell and to washout the resulting debris. In the specific case of the lung, different experimental studies in animal and human organs have shown that perfusing media through the pulmonary artery allows to adequately decellularize not only the vessel walls but also to eliminate the cells in the lung parenchyma and in the airway walls (Girard et al., 2013; Price et al., 2014; Gilpin et al., 2014a, 2014b; Wagner et al., 2014a, 2014b).

Perfusing media through the pulmonary artery is a mechanical process that could be carried out manually (Jensen et al., 2012; Wallis et al., 2012; Daly et al., 2012; Bonenfant et al., 2013). However, this procedure does not allow to easily control the rate of infusion, thereby risking lung damage. Alternatively, media perfusion can be automatically controlled by regulating pressure or flow at the entrance of the pulmonary artery. The limited knowledge currently available on these procedures suggests that both pressure- and flow-control could result in well decellularized and preserved lung scaffolds. Indeed, recent studies have compared perfusion methods (e.g. pressure- vs flow-controlled and manual vs automated) in terms of elimination of donor cell material in the scaffold, preservation of the extracellular matrix composition and structure, and suitability of the acellular scaffold as substrate for culturing cells (Girard et al., 2013; Wagner et al., 2014a, 2014b; Guyette et al., 2014). Nevertheless, none of these studies has investigated lung vascular resistance in detail. Remarkably, each perfusion procedure has its own advantages and drawbacks from a fluid mechanics viewpoint. On the one hand, controlling perfusion pressure at the pulmonary artery ensures that no barotrauma is induced within the scaffold, but accumulation of debris caused by disrupted cells could strongly reduce flow or even interrupt it in case of insufficient pressure. On the other hand, controlling the flow infused at the pulmonary artery ensures a rate of media circulation but – also owing to debris accumulation – may induce high pressure inside the lung vessels, thereby risking to damage the alveolar-capillary membrane. However, no detailed study has investigated this issue in detail.

Furthering our understanding of the fluid dynamics in media perfusion during lung decellularization may provide insights to optimize the process, both for research purposes and also to facilitate automatization for high-throughput production of lung scaffolds. Accordingly, this work was aimed at characterizing the pressure/flow relationship at the pulmonary vasculature and at studying how effective vascular resistance depends on pressure- and flow-controlled variables when applying different conventional ways of perfusing media for lung decellularization.

2. Methods

2.1. *Animals and lung excision*

This study was carried out on lungs excised from 43 C57BL/6 male healthy mice, 7–8 weeks old (17–18 g), following experimental procedures approved by the Ethical Committee for