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Comparative decellularization and recellularization of normal versus emphysematous human lungs

Darcy E. Wagner [a,1,2], Nicholas R. Bonenfant [a,1,2], Charles S. Parsons [b,2], Dino Sokocevic [a,2], Elice M. Brooks [a,2], Zachary D. Borg [a,2], Melissa J. Lathrop [a,2], John D. Wallis [a,2], Amanda B. Daly [a,2], Ying Wai Lam [c,3], Bin Deng [c,3], Michael J. DeSarno [d,4], Takamaru Ashikaga [d,4], Roberto Loi [e,5], Daniel J. Weiss [a,*]

[a] Department of Medicine, University of Vermont, College of Medicine, 226 Health Science Research Facility, Burlington, VT 05405, USA
[b] Department of Surgery, University of Vermont, College of Medicine, 226 Health Science Research Facility, Burlington, VT 05405, USA
[c] Department of Biology, University of Vermont, 311 Marsh Life Sciences, Burlington, VT 05405, USA
[d] Department of Biostatistics, University of Vermont, 27 Hills Building, Burlington, VT 05405, USA
[e] Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy

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ABSTRACT

Acellular whole human lung scaffolds represent a unique opportunity for *ex vivo* tissue engineering. However, it remains unclear whether lungs from individuals with chronic lung diseases such as chronic obstructive pulmonary disease (COPD) can be appropriately decellularized and recellularized. To assess this, cadaveric human lungs from normal (non-smoking) patients and from patients with COPD (smoking history) were decellularized and found by histochemical and immunohistochemical staining, electron microscopy, and mass spectrometry to retain characteristic histological architecture and extracellular matrix components (ECM) reflecting either normal or COPD, particularly emphysematous, origin. Inoculation of human bronchial epithelial cells, endothelial progenitor cells, bone marrow-derived mesenchymal stem cells, and lung fibroblasts via airway or vascular routes into small, excised segments of the decellularized lungs demonstrated that normal lung scaffolds robustly supported initial engraftment and growth of each cell type for up to one month. In contrast, despite initial binding, all cell types inoculated into decellularized emphysematous lungs did not survive beyond one week. However, cell attachment and proliferation on solubilized ECM homogenates of decellularized normal and emphysematous lungs coated onto tissue culture plates was comparable and not impaired, suggesting that the 3-dimensional decellularized emphysematous scaffolds may lack the necessary ECM architecture to support sustained cell growth.

1. Introduction

Devastating lung diseases, such as pulmonary fibrosis and chronic obstructive pulmonary diseases (COPD), are increasing in prevalence and remain without a cure except for lung transplantation. However, there are not enough donor lungs to match the current clinical need and transplantation efficacy is further inhibited by acute and chronic rejection and by complications from immunosuppressive drugs. Alternative options need to be explored to increase the potential supply of donor lungs and the subsequent efficacy of transplantation. A rapidly growing body of literature suggests that decellularized (acellular) whole lung scaffolds recellularized with autologous stem or progenitor cells obtained from the intended transplant recipient may provide a potential means of utilizing failed donor or even cadaveric lungs in clinical transplantation approaches [1–22]. However, lungs available for producing acellular lung scaffolds may come from older individuals with a history of pre-existing pulmonary disorders, such as emphysema or pulmonary fibrosis. It is currently unknown whether these lungs could be used to produce suitable acellular scaffolds for *ex vivo* tissue regeneration. In previous studies, we found that murine alveolar epithelial cells (C10) had limited

* Corresponding author. Tel.: +1 802 656 8925; fax: +1 802 656 892.
E-mail address: dweiss@uvm.edu (D.J. Weiss).
[1] Dr. Wagner and Mr. Bonenfant are co-first authors.
[2] Tel.: +1 802 656 8110; fax: +1 802 656 8926.
[3] Tel.: +1 802 656 9722; fax: +1 802 656 2914.
[4] Tel.: +1 802 656 2526; fax: +1 802 656 3632.
[5] Tel.: +39 70 675 8638; fax: +39 70 666 062.

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Table 1
Clinical characteristics of lungs obtained from autopsy by smoking status.

	Smoker/former smoker ($n = 11$)	Non-smoker ($n = 7$)	Total ($n = 18$)	P value
Patient age at time of death (years)	71.1 ± 13.8	61.3 ± 14.6	65.7 (Range 38–89)	0.170
Sex (M:F)	9:2 (82%/18%)	3:4 (43%/57%)	12:6 (67%/33%)	0.141
Mean time from death to autopsy (hours)	32.8 ± 23.9	35.0 ± 22.6	33.7 (Range 4–89)	0.850
Cause of death				
Pulmonary[a]	3 (27%)	3 (43%)	6 (33%)	0.627
Cardiac	5 (46%)	2 (28.5%)	7 (39%)	0.637
Neurological	1 (9%)	2 (28.5%)	3 (17%)	0.528
Other[b]	2 (18%)	0 (0%)	2 (11%)	0.497
Pulmonary history				
COPD	5 (46%)	0 (0%)	5 (28%)	0.101
Emphysema[c]	3 (27%)	0 (0%)	3 (17%)	0.245
Emphysema + COPD	2 (18%)	0 (0%)	2 (11%)	0.497
Additional findings at autopsy				
Gastric aspiration	3 (27%)	2 (28.5%)	5 (28%)	1.000
Bronchopneumonia	4 (36%)	3 (43%)	7 (39%)	1.000
Pleural effusions	4 (36%)	2 (28.5%)	6 (33%)	1.000
Pulmonary congestion	5 (46%)	3 (43%)	8 (44%)	1.000

Data are given as mean ± SD or as number of patients (%).
P values calculated using Student's t-test or Fisher's Exact Test with 95% confidence interval.
[a] Pulmonary causes of death include: Acute bronchopneumonia, Aspiration pneumonia and Pulmonary embolism.
[b] Other causes of death include: Metastatic breast cancer and Rhabdomyolysis from trauma.
[c] Emphysema includes: Centrilobar, Paraseptal and Centriacinar.

survival when inoculated into acellular mouse lung scaffolds obtained from mice with experimentally-induced emphysema compared to normal mice [13]. To assess whether these results translated to repopulation of acellular emphysematous human lung tissue, acellular scaffolds were generated from cadaveric lungs obtained from emphysematous and healthy individuals. Lung architecture and residual protein content were assessed by histology, immunohistochemistry, and mass spectrometry as well as the ability of the acellular scaffolds to support short and long term recellularization with a variety of human cell types including human bronchial epithelial cells (HBEs), endothelial progenitor cells (CBFs), lung fibroblasts (HLFs), and bone marrow-derived MSCs

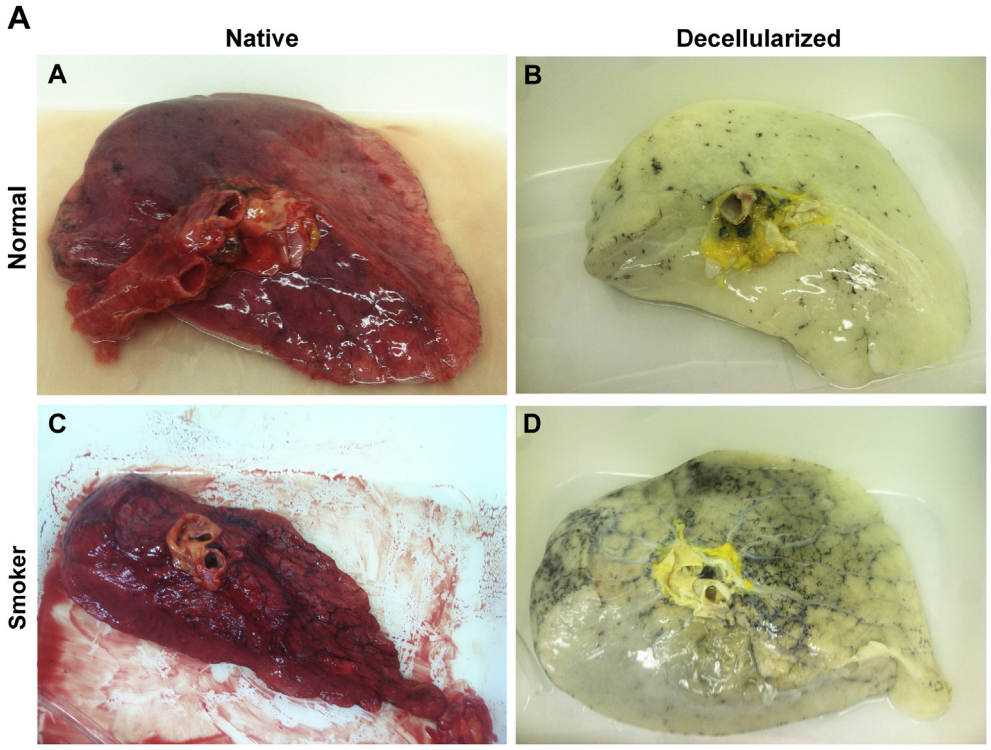

Fig. 1. Decellularized normal and emphysematous human lungs retain characteristic gross and histologic appearances. 1A) Representative images of naïve and decellularized normal and emphysematous human lungs. Anthracotic pigment (black, dense deposits) is grossly retained following decellularization (panels B,D). 1B,C) Representative photomicrographs demonstrate maintenance of characteristic histologic and collagen content but loss of elastin and glycosaminoglycans following decellularization of both normal (1B) and emphysematous human lungs (1C) a = airways, bv = blood vessels. Arrows highlight individual blood vessels. Original magnifications 100×. Inserts indicate higher power images (200×) of areas indicated by *. 1D) Electron micrographs demonstrate retention of characteristic normal and emphysematous alveolar septa following decellularization with alveolar wall thinning observed in emphysematous samples. Collagen (labeled c) and elastin (labeled e) are indicated with black and red arrows, respectively. All scale bars are 10 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)