Decellularization of Human and Porcine Lung Tissues for Pulmonary Tissue Engineering

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Background. The only definitive treatment for end-stage organ failure is orthotopic transplantation. Lung extracellular matrix (LECM) holds great potential as a scaffold for lung tissue engineering because it retains the complex architecture, biomechanics, and topologic specificity of the lung. Decellularization of human lungs rejected from transplantation could provide “ideal” biologic scaffolds for lung tissue engineering, but the availability of such lungs remains limited. The present study was designed to determine whether porcine lung could serve as a suitable substitute for human lung to study tissue engineering therapies.

Methods. Human and porcine lungs were procured, sliced into sheets, and decellularized by three different methods. Compositional, ultrastructural, and biomechanical changes to the LECM were characterized. The suitability of LECM for cellular repopulation was evaluated by assessing the viability, growth, and metabolic activity of human lung fibroblasts, human small airway epithelial cells, and human adipose-derived mesenchymal stem cells over a period of 7 days.

Results. Decellularization with 3-(3-Cholamidopropyl)dimethylammonio)-1-propanesulfonate (CHAPS) showed the best maintenance of both human and porcine LECM, with similar retention of LECM proteins except for elastin. Human and porcine LECM supported the cultivation of pulmonary cells in a similar way, except that the human LECM was stiffer and resulted in higher metabolic activity of the cells than porcine LECM.

Conclusions. Porcine lungs can be decellularized with CHAPS to produce LECM scaffolds with properties resembling those of human lungs, for pulmonary tissue engineering. We propose that porcine LECM can be an excellent screening platform for the envisioned human tissue engineering applications of decellularized lungs.

Lung transplantation is currently the only definitive treatment for nearly 25 million patients with end-stage lung disease [1]. The supply of donor lungs is limited, and long-term outcomes of transplantation remain hampered by immunosuppressive regimens [1]. To address these challenges, tissue engineering approaches are now being developed that use scaffolds and cells to create functional lung substitutes.

Owing to the complex hierarchical structure of the lung, successful strategies will require a highly specialized matrix that can support the engraftment, growth, and function of a diverse population of cells. Only limited success in engineering lung tissue has been achieved until recently, when 2 parallel landmark studies introduced a new paradigm by using native extracellular matrix (ECM) [2–4] that has been shown to provide the cells with topologically specific signals and attachment sites inherent to native tissues [5–10]. The plausibility of bioengineering lungs was shown by generating a three-dimensional scaffold by decellularization of rat lungs, reseeding pulmonary cells onto the endothelial and epithelial surfaces of the scaffold, and achieving functional gas exchange of the resulting graft for a period of several hours, both in vitro and in vivo [11].

If scaled up to human lungs, tissue engineering could potentially expand the pool of donor organs, particularly if lungs marginally unsuitable for transplantation could be improved upon by processing and seeding with the recipient patient’s autologous stem cells (provided the patient has no inherent relevant genetic defect such as cystic fibrosis). Additionally, there would be improved immunocompatibility because of the presence of autologous cells and gradual remodeling of the lung parenchyma. We propose that the “conditioning” of donor lungs by a combination of perfusion treatments and cell seeding could improve the quality of marginal lungs without structural defects to a level acceptable for transplantation.
Because healthy donor lungs are always used for transplantation, the availability of human lungs for tissue engineering studies is limited to those rejected for transplantation, wherein rejection is based on standard functional criteria such as inferior PaO2/FiO2, low compliance, or infection. For this reason, we considered porcine lungs, which are readily available, as a xenogeneic alternative to human lungs for research purposes. Porcine tissues have been useful for developing human tissue engineering strategies in a variety of applications [12]. The goal of the present study was to determine whether porcine lung could be used as a tissue engineering platform representative of human lungs.

Although decellularization by perfusion has been described for murine and rat lungs [11, 13, 14], very little is known about the perfusion of lungs from larger mammals. To facilitate our comparison of human and porcine tissue, we assessed the decellularization of human and porcine lungs by using three different methods: (1) sodium dodecyl sulfate (SDS), (2) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), and (3) a three-step method consisting of Tween-20, sodium deoxycholate, and peracetic acid. The structural, compositional, and biomechanical changes to human and porcine lung extracellular matrix (LECM) were assessed to determine the outcomes of decellularization and to select the optimal method. Decellularized human and porcine LECM was then seeded with human lung fibroblasts (hMRC-5s), human small airway epithelial cells (hSAECs), and human adipose-derived mesenchymal stem cells (hMSCs) to evaluate their capacity to support the growth of seeded human cells (Fig 1). The collected data support the suitability of porcine lungs as a source of LECM for pulmonary tissue engineering applications.

Material and Methods

Organ Removal

Three human lungs and three porcine lungs were removed, and similar regions of the lower left lobes were used for decellularization and characterization. Human lungs rejected for transplantation were procured from the New York Organ Donor Network under a protocol approved by the Institutional Review Board at Columbia University. The porcine lungs were obtained from Yorkshire pigs weighing 40 to 50 kg after the animals were used in another research study not affecting lungs and were killed under a protocol approved by the Columbia University Institutional Animal Care and Use Committee.

Decellularization

Upon removal, the lungs were cleared of blood and immediately frozen at −80°C until use. The lungs were partially thawed and sectioned to 2-mm-thick sheets that were washed with 2X phosphate buffered saline (PBS) for 15 minutes and placed in a series of decellularization solutions on an orbital shaker, using 1 of the following three protocols:

1. SDS: Four 2-hour washes with 1.8 mM SDS, each followed by dH2O (5 min) and 2X PBS (15 minutes).
2. CHAPS: Four 2-hour washes with 8 mM CHAPS, each followed by dH2O (5 minutes) and 2X PBS (15 minutes).
3. Three-step method: 2-hour wash with 3% Tween-20, 2-hour wash with 4% sodium deoxycholate, 1-hour wash with 0.1% peracetic acid.

All slices were then subjected to alternating 1X PBS and dH2O washes (2 of each). Then, 7-mm-diameter discs of

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**Fig 1.** Overall approach. Decellularized slices from human and porcine lungs were compared with respect to their histomorphology, biochemical composition, mechanical properties, and ability to support the growth and metabolism of cultured cells. Three different methods of decellularization and three different types of human cells (lung fibroblasts, small airway epithelial cells, mesenchymal cells) were evaluated. (ECM = extracellular matrix.)
decellularized tissue were punched with a biopsy punch under sterile conditions and used in experiments. Pen/strep (5% each) was added to all solutions to eliminate native and pathologic bacteria from the LECM.

For all assays, three random samples were collected from three different lungs (n = 9 for each human and porcine lung) before and after decellularization.

DNA
The DNA content of decellularized tissues was quantified by use of the Quant-iT PicoGreen dsDNA assay kit (Invitrogen) according to the manufacturer’s instructions. Tissue samples were weighed and digested in 125 µg/mL papain (Sigma) overnight at 60°C. Fluorescence was measured at 520 nm with excitation at 480 nm. DNA was quantified by use of a standard curve prepared with λ-phage DNA and normalized to the tissue wet weight.

Histologic Analysis
Tissue samples were fixed in 3.7% formalin, embedded in paraffin, and sectioned to 5-µm thickness. General evaluation was performed with hematoxylin and eosin, and distributions of matrix proteins were evaluated by use of Masson’s trichrome (collagen), Alcian blue (proteoglycans), and Van Gieson’s (elastic fibers) stains. All samples were stained at the same time and imaged under the same exposure and imaging conditions.

Collagen
Tissue samples were initially weighed and digested in 0.1 mg/mL pepsin overnight at 4°C (15 mg tissue per 1 mL digest solution). Collagen was then quantified with the Sircol collagen assay kit (Biocolor) according to the manufacturer’s instructions.

Sulfated Glycosaminoglycans
Sulfated glycosaminoglycans (sGAG) were quantified using the dimethylene blue dye assay. Tissue samples were weighed and digested in 125 µg/mL papain (Sigma) overnight at 60°C (45 mg tissue per mL digest solution), mixed with dimethylene blue dye (1:5 ratio), and absorbance was immediately measured at 595 nm and normalized to the tissue wet weight.

Elastin
Elastin was quantified by use of the Fastin elastin assay kit (Biocolor) according to the manufacturer’s instructions. Tissue samples were weighed, and water-soluble z-elastin was extracted via three hot 0.25-M oxalic acid extractions, which were combined for each sample (35 mg tissue per 1 mL solution).

Scanning Electron Microscopy
Tissue samples were frozen, lyophilized, coated with a thin layer of gold under vacuum, and mounted onto a cylindrical stage for scanning electron microscopic imaging with a JEOL JSM 5600LV scanning electron microscope at ×350.

Immunohistochemistry
Samples for immunohistochemistry were fixed in formalin for 30 minutes, embedded in paraffin, cut to 8 µm, and mounted on slides. Sections were deparaffinized, subjected to boiling citrate buffer for 16 minutes for antigen retrieval, and blocked with 10% normal goat serum in PBS for 2 hours at room temperature. Primary antibody staining was performed for 2 hours at 4°C with the following primary antibodies: collagen IV (Rb pAb to coll IV (ab6586) diluted 1:200), laminin (Rb pAb to laminin...
(ab11575) diluted 1:100), elastin (Rb pAb to elastin (ab21610) diluted 1:200), and fibronectin (Rb pAb to fibronectin (ab23750) diluted 1:200). The secondary antibody (goat pAb to Rb IgG (ab98464) diluted 1:200) was incubated for 1 hour at room temperature. Completed sections were mounted in Vectashield mounting medium with 4',6-diamidino-2-phenylindole, cover-slipped, and imaged with an Olympus IX81 microscope at ×10.

Mechanical Testing

Mechanical testing was conducted with an Instron testing machine Model 5848 with a 10-N load cell. With the use of a custom-made mold, samples were cut into 3-cm × 1-cm pieces from randomly selected transverse sections of the lower left lobe. This single orientation and the lower left lobe were selected and consistently maintained to minimize the effects of lung anisotropy on mechanical data. The 2 ends of the strips were secured with sandpaper to prevent slippage and mounted on the Instron, and a preload of 0.003 N was set. Samples were kept hydrated with 1X PBS at room temperature. Uniaxial stretch of 20% was applied at a rate of 1% strain/second and frequencies of 0.25, 0.50, or 0.75 Hz (all samples were tested at the same grip-to-grip distance for consistency).

Cell Culture

Human lung fibroblasts (hMRC-5s) were obtained from ATCC (www.atcc.org) and cultured in Dulbecco’s modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS) and 1% pen/strep under standard culture conditions. The hSAECs were kindly provided by Dr Gao and Dr Minna (Dallas, TX) and were cultured in small airway growth media from ATCC. The hMSCs were kindly provided by Dr Gimble (Baton Rouge, LA) and cultured in DMEM/F12 (1:1) with 10% FBS and 1% pen/strep.

Growth Study

Cells were passaged by trypsinization after 7 days, by which time 70% to 80% confluency had been reached, seeded onto decellularized lung punches at an initial density of 2.5 × 10^4 cells/mL, and cultured for 7 days. Cell growth was assessed by quantifying the DNA content of recellularized lung scaffolds with a Quant-iT PicoGreen dsDNA assay kit.

Metabolic Activity

Metabolic activity of the cells during the growth study was measured using Alamar Blue reagent according to
the manufacturer’s instructions (Life Technologies). The reagent was added at various time points to the cells in culture and incubated for 9 to 12 hours before samples were collected and absorbance was measured at 570 nm with reference wavelength of 600 nm.

Live/Dead Imaging

Cells were stained with a Live/Dead kit (Invitrogen). Calcein-AM was used to indicate live cells (green), and ethidium homodimer-1 was used to indicate dead cells (red). Samples were imaged with an Olympus Fluoview FV1000 confocal microscope at ×20.

Statistics

A 2-way analysis of variance was performed with a Bonferroni ad hoc test, and p values less than 0.05 were considered significant.

Results

Decellularized human and porcine lung tissue slices appeared translucent, with visible conduits throughout the matrix (Fig 2A). Decellularization consistently removed more than 95% of the nuclear material (Fig 2B). Hematoxylin and eosin staining showed no discernible nuclei (Fig 2C). Histologic analysis revealed retention of small anthracotic aggregations (black regions) in some human lung sections; these regions were generally avoided during imaging.

Several assays were used to compare the relative retention of major LECM components after decellularization. Both human and porcine LECM retained 80% of total collagen regardless of the decellularization method used (Figs 3A, 3D). However, all three methods substantially decreased the amount of sGAG (Figs 3B, 3D), with no apparent differences between the human and porcine LECM. There was a significant difference between human and porcine LECM in the amount of elastin retained after decellularization using CHAPS and the three-step method (Fig 3C). These quantitative results were consistent with histologic staining using Masson’s Trichrome (collagen, blue), Alcian Blue (sGAG, blue), and Van Gieson’s (elastic fibers, black) (Fig 4). Collagen IV was found in the basement membrane of both native and decellularized human and porcine tissue with no apparent differences between decellularization methods (Fig 5A). By contrast, laminin and fibronectin were not well retained in either porcine or human LECM, with fibronectin most depleted by the SDS method (Figs 5B, 5C). Finally, there was higher retention of elastin for CHAPS compared with the SDS and three-step methods (Fig 5D).

The ultrastructural morphologies of human and porcine LECM before and after decellularization were similar, as evidenced by electron microscopy (Fig 6). Native lung slices showed smooth surfaces that were disrupted after decellularization, resulting in a more fibrillar structure and a rougher topographic profile. Decellularization using SDS showed the most fibrillar ultrastructure of all the methods (Fig 6). Overall, there were no major differences in ultrastructural morphology between human and porcine LECM.

Mechanical testing of decellularized human and porcine LECM was performed as shown in Figure 7 for transverse sections of the lower left lobe, and significant
Fig 5. Distributions of extracellular matrix proteins. Representative immunohistochemical stains are shown for (A) collagen IV, (B) laminin, (C) fibronectin, and (D) elastin. All images were acquired with a ×10 objective. Scale bar: 100 mm. (CHAPS = 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate; SDS = sodium dodecyl sulfate.)

Fig 6. Ultrastructure of decellularized lung tissues. Representative scanning electron micrographs are shown for all experimental groups. Scale bar: 50 mm. (CHAPS = 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate; SDS = sodium dodecyl sulfate.)
differences were observed (Figs 8A, 8B). Both the tangential modulus and peak stress experienced at a 20% strain were higher for human than for porcine LECM. CHAPS decellularization resulted in the highest tangential modulus and peak stress for human and porcine LECM. The most compliant LECM was observed for the three-step and SDS methods in the human and porcine LECM, respectively. The peak stress and tangential modulus for all decellularization methods correlated with changes in elastin content, as shown in Figures 8C and 8D.

Because CHAPS-decellularized tissue showed the best retention of LECM structure, hMRC-5s, hSAECs, and human adipose-derived mesenchymal stem cells hMSCs were cultured on CHAPS-decellularized human and porcine LECM. All cell types proliferated at comparable rates over a 7-day period (Fig 9), were fully viable over 7 days of culture (Figs 10A, 10B), and had comparable metabolic rates (Figs 10C, 10D). However, hSAECs were more metabolically active in human than in porcine LECM over 7 days of culture (Figs 10C, 10D).

Comment
The present study assessed the feasibility of using porcine lung as a source of LECM for lung tissue engineering, toward engineering a whole lung or lung lobe for transplantation. Tissue slices were decellularized by three different methods: (1) SDS, (2) CHAPS, and
investigated, CHAPS resulted in the best retention of LECM integrity and composition compared with the SDS and three-step methods. Except for mechanical properties and the metabolism of hSAECs, a few differences were found between the human and porcine LECM.

In previous studies, whole rat lungs were decellularized by perfusion decellularization and then repopulated with neonatal rat lung epithelial cells and vascular endothelial cells [3, 4, 11]. Pulmonary epithelial cells attached and grew within the matrix much better than on tissue culture plates, suggesting that the acellular matrix provided important cues for directing cellular organization and growth.

Other studies have investigated the effects of different decellularization methods on the composition and re-cellularization of mouse lungs [13, 14], as the first step toward engineering ex vivo functional lung tissue. However, perfusion decellularization described for mouse and rat lungs may prove more challenging for human lungs because of significant differences in size, diffusion distances, and the anatomy of the airway and vascular networks [3, 4, 11, 13, 14]. To maintain uniform decellularization of human-sized lung, it is important to understand the effectiveness of each of the reagents used and how they affect lung matrix structure. In addition, access to an abundant source of human-sized lungs is needed for in vitro testing and the development of an optimal perfusion system that would fully decellularize human lungs. To assess these parameters, we analyzed the ultrastructure, biomolecular composition, biomechanical properties (in transverse sections of the lower left lobe, to minimize the effects of lung anisotropy), and cellular attachment and growth of three relevant cell types for decellularized human and porcine lungs.

The results of the present study are consistent with those of Peterson and colleagues [3, 15], in which CHAPS (a zwitterionic detergent) was the best reagent for decellularizing lung tissue based on the greatest retention of most of the LECM proteins studied. The effects of SDS and CHAPS on lung matrix protein retention are also consistent between the 2 studies.

Decellularization reduces the total amount of collagen and sulfated glycosaminoglycans to approximately 80% and 20% of native tissue, respectively (Figs 3A, 3B). In our study, elastin content was also significantly higher in the porcine CHAPS group than in the human CHAPS group (Fig 3C), a result that may be related to the increased compliance observed in the mechanical testing of CHAPS-decellularized porcine LECM. Although it is difficult to conclusively correlate the retention of LECM components with the mechanical behavior observed, there appears to be a relationship between the amount of elastin and the stiffness of LECM at low strain (Figs 3C, 8D). Similar trends can be found for collagen and sGAG content (Fig 3D). Alternatively, the relationship between LECM content and the mechanical properties may be due to the decellularization method itself, not strictly to the retention of LECM components. The specific contributions of structural proteins and tissue anisotropy

Fig 9. Growth curves of 3 human cell types on lung scaffolds decellularized by 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS). Growth curves for 7 days culture of three different types of cells on CHAPS-decellularized human and porcine lung extracellular matrix (ECM). (A) Human lung fibroblasts (hMRC-5s). (B) Human small airway epithelial cells (hSAECs). (C) Human adipose-derived mesenchymal stem cells (hMSCs). Data represent mean ± SE (n ≥ 9). * p < 0.05.
to the mechanical properties of LECM remain to be investigated in greater detail.

The end goal of decellularizing human lung tissue is to obtain a native-like scaffold for repopulation with lung cells or their precursors. Inasmuch as the ultrastructure of the matrix and the specific extracellular proteins and molecules it contains may provide lung-specific cues for stem cells, it is important to identify the decellularization protocol that best preserves this environment. With the exception of mechanical properties under low strains (0% to 20%), there appear to be parallels between human and porcine lungs based on the degradation or loss of LECM proteins during decellularization (Figs 4, 5).

Strains of up to 20% were considered based on the radial volumetric expansion of lungs at inspiratory capacity. Differences between the stress and tangential modulus at 20% strain in human and porcine lungs (Figs 8A, 8B) could be a result of storage at −80°C before processing and testing. Other possibilities include differences in the health and age of the human and porcine lungs tested. Although the porcine lungs came from healthy pigs, the human lungs were those rejected for transplantation that underwent changes in their LECM related to the reason for rejection. Additionally, older tissues may have more cross-linking and different overall distributions of fibers. The exact reason for this mechanical difference between human and porcine LECM remains unknown.

Three cell types were cultured on slices of human and porcine LECM to determine whether the decellularization method affected cellular attachment and growth. CHAPS-decellularized human and porcine LECM showed similar growth rates for hMRC-5s, hSAECs, and hMSCs, suggesting that all three cell types attach and proliferate at similar rates (Fig 9). These findings obtained for slices of human and porcine lungs decellularized on a shaker are consistent with those in previous studies of perfusion decellularization of mouse lungs [14]. In contrast to previous studies, our study showed substantial growth of hMSCs on LECM [13]. Interestingly, hSAECs displayed higher mitochondrial activity on human LECM when compared with those on porcine LECM (Figs 10C, 10D). Whereas epithelial cells may be more sensitive to species-specific matrix, the exact reasons remain unknown.

In summary, our study suggests that porcine lung is a promising substitute for human lung for in vitro studies of decellularization and human cell–ECM interactions for lung tissue engineering. Porcine lungs can serve as the platform to optimize decellularization protocols for eventual translation to human lungs. Inasmuch as all donor human lungs that meet transplantation criteria are used for transplantation, an alternative source of lungs is needed for research. Our data show that porcine LECM serves as a suitable and readily available substitute for human lung tissue for pulmonary tissue engineering research and applications.

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DISCUSSION

DR SETH FORCE (Atlanta, GA): That was an excellent discussion. I know that you're early on in this research before taking it to the in vivo step, but have you thought about in the future trying to use your scaffold matrix to put on a membrane, such as an oxygenator, to use an extracorporeal membrane oxygenation (ECMO) circuit? Because one of the main problems we have is that the main area of thrombosis and problems with clotting, and it seems like that might be kind of a natural barrier that could prevent that. I mean, it would be a nice way potentially to look at your scaffold in vivo in an animal model.

DR SINGH: Dr Bacchetta, the director of the ECMO program, is currently doing that. We're providing him with the extracellular matrix which he's putting on the oxygenators, and we're testing flow and hypercoagulability.

DR FORCE: I mean, do you have any early results, just like anecdotal results from that?

DR SINGH: We'll be able to submit those for June and present those hopefully next year, but I think right now it's too soon to say exactly what's going to happen.

DR DAVID MASON (Cleveland, OH): I think that was a fascinating talk. One question is, just logistically, how does it work? You would envision implanting a lung and sewing on an artery and a vein in the standard fashion? So these would be scaffolds that would be implanted like a regular lung?

DR SINGH: A regular lung implant.

DR MASON: So an artery would eventually get out to the matrix which you're creating and then exchange gases and then return it.

DR SINGH: We're repopulating both the airway and the vasculature.

DR SINGH: I mean in a rat you'd need a couple of million cells. But in a human lung you'd need a couple of hundred million, to put it mildly.

DR MASON: I see. And when you decellularize it, I couldn't tell, is there a vessel that remains at the end, like a large vessel, for example? I mean, I assume that the matrix isn't recreating a pulmonary artery. Does that remain? How does that work?

DR SINGH: Well, we're currently decellularizing sheets. We haven't gotten to the stage of actually doing a pig lung or even a human lung. We hope to scale up the work that Ott and colleagues have done to possibly decellularize, repopulate a lobe of a pig lung and a human lung, and evaluate them for function on an ex vivo perfusion apparatus in the near future.

DR STEPHEN CASSIVI (Rochester, MN): I congratulate you on your work. Clearly, the big question is: Can we scale this up from what Laura Nikason and Harald Ott have done in the small animal? We're trying to do that ourselves at our institution. I can give you one of the pitfalls that we've had so far in scaling it up to the pig: it is the issue of pressurization. As you decellularize, it is important to make sure that you are not blowing out the cell-cell junctions, especially as you decellularize from the airway portal, because there is no egress for the SDS or the CHAPS. There is no normal egress from the airway like there is from the vascular side. And so I would encourage you, when you do ramp up to the large model, to pay attention to the pressures as you decellularize.

My question to you is, what's your strategy (I know it's early days), but what's your strategy to achieve the populations necessary of stem cells to populate the big huge playing field that is the pig or human lung? It's a little bit more conceivable at the rat and the mouse level. But how are you going to achieve that at your institution?

DR SINGH: Well, we're hoping that Dr Vunjak-Novakovic, who is a leader in stem cell research in New York and at Columbia, would help us come up with such a strategy. Because you're right—I mean in a rat you'd need a couple of million cells. But in a human lung you'd need a couple of hundred million, to put it mildly.
We’re going to have to come up with a multiflask system to grow cells on a large scale to be able to deliver those cells to repopulate a lung, which is why we go for repopulating a lobe first—you know: go that way, evaluate function, and then step up further.

DR DANIEL BOFFA (New Haven, CT): Have you ever tried this strategy using the scaffold of end-stage lungs to see if just putting stem cells into damaged matrix or architecture has any restorative capabilities?

DR SINGH: We have not gotten lungs and tried that. But we’re in the process of using a similar method for acute lung injury, and we hope that we’d be able to repair those lungs and evaluate those in our next few apparatus, but we have not tried it yet.

INVITED COMMENTARY

Lung transplantation remains the only definitive treatment for end-stage lung disease. However, its clinical effect is limited by donor organ shortage, the need for immunosuppression, and chronic rejection leading to graft failure. As of April 2013, 1,690 Americans were waiting for a donor lung, and nearly half of them will be waiting for more than 2 years [1]. Patient survival and graft function after lung transplantation are continuously improving, but still reach only 50% to 60% at 5 years after transplantation [2]. A bioartificial lung derived from the patient’s cells that can be implanted similar to a donor organ could become a theoretic alternative to allotransplantation.

Tissue engineering relies on the concept of using an extracellular matrix scaffolds to place cells into their physiologic 3-dimensional context, thereby enabling the formation of functional grafts for implantation [3]. One approach toward the engineering or “regeneration” of functional lung grafts for transplantation is based on native extracellular matrix scaffolds. These can be generated by perfusion decellularization of cadaveric organs, a process that ideally removes all of the cells and leaves only extracellular matrix components behind. In small-animal experiments, such whole organ scaffolds have been successfully repopulated with vascular and epithelial cells and matured to functional lung grafts [4, 5]. In orthotopic transplant experiments, these grafts were maintained by the recipient’s blood supply and functioned for several days in vivo [6].

As a next step toward moving this technology closer to a potential clinical validation, human-scale lung scaffolds have to be generated. In the present report, O’Neill and colleagues [7] compare different decellularization protocols for human and porcine lung sections. The authors did not perfuse the cadaveric lung samples but submerged lung slices in different chemicals to examine composition, mechanical properties, and biocompatibility of the resulting tissue. Acellular scaffold slices allowed for cell attachment and survival in a 2-dimensional culture system, suggesting nontoxicity of the native extracellular matrix. Importantly, human cells thrived equally on porcine and human matrix sections, a promising finding considering the nearly unlimited supply of porcine lungs not only as a test bed for organ regeneration but also as potential “off-the-shelf” organ scaffolds. In this data set, all tested decellularization protocols led to a decrease in elastin content and changes in mechanical properties, which is consistent with other publications, and a detail that warrants further investigations given the physiologic need for elasticity during ventilation [8, 9].

As the authors suggest, the end goal of decellularizing human or porcine lungs is to obtain native-like scaffolds for organ engineering. The use of perfusion as a delivery method for the tested decellularization agents may provide the unique possibility to maintain the entire organ’s architecture, including a hierarchic vasculature and airways, while creating a biocompatible scaffold material for cell seeding. Scaling the data presented in their study to whole lungs of human size will provide further insight into the choice of ideal decellularization protocol and help to assess the translational potential of lung engineering based on native extracellular matrix.

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