Biomaterials 35 (2014) 2664–2679

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Three-dimensional scaffolds of acellular human and porcine lungs for high throughput studies of lung disease and regeneration

Darcy E. Wagner [a], Nicholas R. Bonenfant [a], Dino Sokocevic [a], Michael J. DeSarno [b], Zachary D. Borg [a], Charles S. Parsons [c], Elice M. Brooks [a], Joseph J. Platz [c], Zain I. Khalpey [d], David M. Hoganson [e], Bin Deng [f], Ying W. Lam [f], Rachael A. Oldinski [g], Takamaru Ashikaga [b], Daniel J. Weiss [a,*]

[a] *University of Vermont, Department of Medicine, 226 Health Sciences, Research Facility Center, Burlington, VT 05405, USA*
[b] *University of Vermont, Department of Medical Biostatistics, 27 Hills Building, Burlington, VT 05405, USA*
[c] *University of Vermont, Department of Surgery, Fletcher House 301, Burlington, VT 05405, USA*
[d] *University of Arizona, Department of Surgery, 1501 North Campbell Avenue, Tucson, AZ 85724, USA*
[e] *Washington University in St. Louis, Department of Surgery, 1 Barnes Jewish Plaza, 3108 Queeny Tower, St. Louis, MO 63110, USA*
[f] *University of Vermont, Department of Biology, 311 Marsh Life Sciences, Burlington, VT 05405, USA*
[g] *University of Vermont, College of Engineering and Mathematics, 301 Votey Hall, 33 Colchester Ave, Burlington, VT 05405, USA*

A R T I C L E I N F O

Article history:
Received 4 November 2013
Accepted 26 November 2013
Available online 8 January 2014

Keywords:
Acellular matrix
Endothelial cell
Epithelial cell
Extracellular matrix (ECM)
Human lung fibroblast
Mesenchymal stem cell

A B S T R A C T

Acellular scaffolds from complex whole organs such as lung are being increasingly studied for *ex vivo* organ generation and for *in vitro* studies of cell–extracellular matrix interactions. We have established effective methods for efficient de and recellularization of large animal and human lungs including techniques which allow multiple small segments ($\sim$1–3 cm^3) to be excised that retain 3-dimensional lung structure. Coupled with the use of a synthetic pleural coating, cells can be selectively physiologically inoculated via preserved vascular and airway conduits. Inoculated segments can be further sliced for high throughput studies. Further, we demonstrate thermography as a powerful noninvasive technique for monitoring perfusion decellularization and for evaluating preservation of vascular and airway networks following human and porcine lung decellularization. Collectively, these techniques are a significant step forward as they allow high throughput *in vitro* studies from a single lung or lobe in a more biologically relevant, three-dimensional acellular scaffold.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Rapid developments in tissue engineering utilizing decellularized whole organs as biologic scaffolds coupled with advances in stem and endogenous lung progenitor cell biology have offered the potential of using acellular suboptimal donor or cadaveric lungs recellularized with appropriate cell types for *ex vivo* lung regeneration [1–6]. Acellular lungs are also powerful *in vitro* platforms for studying cell–extracellular matrix (ECM) interactions in lung diseases such as emphysema and fibrosis, and in aging [7–9]. The resulting acellular scaffold retains characteristic disease architecture and recellularization attempts have shown differential responses, consistent with disease phenotypes [7,8].

As acellular scaffolds derived from rodent lungs are readily available and easily handled, high throughput approaches can be utilized to study cell–matrix interaction or to assess the multiple conditions needed for effective recellularization and development of functional lung tissue [5,7,10,11]. However, there is a more limited supply of larger lungs, including those from humans and potential xenogenic sources (e.g. porcine). Further, approaches utilized in rodent models may be insufficient for adequate decellularization of lungs obtained from larger animals and humans. As such, a limited number of reports have examined the feasibility of decellularization in large organs [1,8,12,13].

Technical difficulties in handling larger tissue represent a major challenge in attempting to scale up rodent model decellularization

* Corresponding author. Tel.: +1 802 656 8925; fax: +1 802 656 8926.
E-mail addresses: Darcy.wagner@med.uvm.edu (D.E. Wagner), Nicholas.Bonenfant@med.uvm.edu (N.R. Bonenfant), Dino.Sokocevic@med.uvm.edu (D. Sokocevic), mdesarno@uvm.edu (M.J. DeSarno), zachary.borg@med.uvm.edu (Z.D. Borg), charles.parsons@vtmednet.org (C.S. Parsons), elice.brooks@med.uvm.edu (E.M. Brooks), Joseph.Platz@med.uvm.edu (J.J. Platz), zkhalpey@surgery.arizona.edu (Z.I. Khalpey), hogansondm@wudosis.wustl.edu (D.M. Hoganson), bdeng@uvm.edu (B. Deng), Ying-Wai.Lam@uvm.edu (Y.W. Lam), oldinski@uvm.edu (R.A. Oldinski), Takamaru.Ashikaga@uvm.edu (T. Ashikaga), dweiss@uvm.edu (D.J. Weiss).

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.11.078

techniques to porcine and human lungs or lobes. To address this issue, we assessed several methods of decellularization in porcine and human lungs using peristaltic pump-driven flow of decellularization reagents into both the airways and vasculature, with or without physical agitation, and compared these to manual instillations with static incubations, a technique commonly utilized for decellularizing rodent lungs [5,7,10,11]. Optimization of fluid volumes was assessed for each step of the decellularization protocol.

Attempts to recellularize acellular scaffolds of larger lungs present additional hurdles. Recellularization of an entire acellular lobe or lung will require extremely large cell numbers. Supply of adequate nutrients and oxygen to maintain tissue vitality will be particularly challenging. Additionally, study of entire lungs or lobes does not readily lend itself to high throughput studies. Prior approaches have utilized thin slices of acellular human or porcine lungs onto which cells were non-specifically layered or thicker acellular tissue slices into which cells were non-specifically injected [1,8,12,14]. While these studies demonstrated cell survival and even phenotypic changes following inoculation or seeding, these methods do not permit the selected study of cells with their respective ECM components (i.e. endothelial cells introduced into the vasculature or epithelial cells in airspaces). Rather, cells were heterogeneously introduced throughout the acellular scaffold and study of specific cell–ECM interactions is limited.

Methods to introduce cells in a biologically relevant fashion, as done in acellular rodent lungs [1–4,7,10,15–17] (e.g. airway or vasculature instillation), and which allow high throughput study approaches from larger lungs would be a significant step forward. We have developed a method of excising small ($\sim$1–3 cm^3) segments of acellular human and porcine lung scaffolds that maintain 3-dimensional structure and into which cells can be selectively inoculated via the preserved vascular and airway conduits. Excised segments can be obtained from select regions of the lung to study specific regional cell–matrix interactions. However, as excision of individual lung segments damages the integrity and function of the lung pleura, we have also developed a synthetic pleural coating that encapsulates the recellularized segments to provide an isolated lung unit for culture and analysis. Recellularized segments can then be sliced for high throughput studies or left as 3-dimensional segments.

2. Methods

2.1. Cell culture

Human bronchial epithelial cells (HBEs) (courtesy of Albert van der Vliet, University of Vermont, originally from Drs. J. Yankaskas and R. Wu) [18], human lung fibroblasts (HLFs) (ATCC, CCL 171), and human bone marrow-derived mesenchymal stromal cells (hMSCs) (obtained from the Texas A&M Stem Cell Core facility) [19] were cultured and expanded on tissue culture treated plastic at 37 °C and 5% CO_2. Human vascular endothelial cells CBF12 (courtesy of Mervin Yoder, Indiana University – Purdue University Indianapolis), were similarly cultured and expanded on collagen I coated tissue culture plastic. HBEs were cultured in serum free DMEM/F-12 (50/50 mix) (Corning), 10 ng/ml Cholera toxin (Sigma), 10 ng/ml epidermal growth factor (Sigma), 5 μg/ml insulin (Gemini Bio-Products), 5 μg/ml transferrin (Sigma), 0.1 μM dexamethasone (Sigma), 15 μg/ml bovine pituitary extract (Lonza), 0.5 mg/ml bovine serum albumin (Life Technologies), and 100 IU/ml penicillin/100 μg/ml streptomycin (Corning). HLFs were expanded in DMEM/F-12 (50/50 mix) (Corning), 10% fetal bovine serum (Hyclone), 100 IU/ml penicillin/100 μg/ml streptomycin (Corning), 2 mM L-glutamine (Hyclone). CBF12 cells were grown in cEGM-2 (Lonza) supplemented with 10% fetal bovine serum (Hyclone), and 100 IU/ml penicillin/100 μg/ml streptomycin (Corning). hMSCs were expanded in culture with Modification of Eagle Medium-Earle's Balanced Salt Solution (MEM-EBSS) (Hyclone), 20% fetal bovine serum (Hyclone), 100 IU/ml penicillin/100 μg/ml streptomycin (Corning), 2 mM L-glutamine (Hyclone), and used only at passage 7 or 8.

2.2. Human and porcine lungs

Cadaveric human lung lobes were obtained from 33 different patients (a total of 51 lobes) from the autopsy services at Fletcher Allen Hospital in Burlington, Vermont. In addition one failed donor lung was obtained from the University of Arizona, courtesy of Zain Khalpey; and three surgically explanted lungs obtained from patients with interstitial lung disease (ILD, either idiopathic pulmonary fibrosis or autoimmune-associated interstitial lung disease) from Washington University in St. Louis, courtesy of David Hoganson. Lungs were categorized as normal or diseased based on review of available clinical records, including known history of COPD or other lung diseases, smoking history, chest radiographs, and use of respiratory medications. A total of 29 lobes from 19 patients, classified as normal, were utilized in this current study. Clinical characteristics of the lungs and time from death until autopsy are summarized in Supplementary Table 1A. Seven lobes from the three patients with ILD were also used to compare the effect of lung fibrosis on the ability of lungs to be effectively decellularized (Supplemental Table 1B).

23 porcine heart–lung blocs were obtained at the University of Vermont in Burlington, Vermont from 6 to 11 week old male Sus Scrofa pigs, courtesy of Helene Langevin, MD under all appropriate IACUC and AAALAC standards. The average time from death until necropsy was 100 min.

2.3. Decellularization of human lungs

Human lung lobes were decellularized by sequential treatment with detergent and enzymatic washes. The sequence of detergent instillations and incubation times is based on previous work reported by us and others [2,5,7,8,10,11]. In order to assess optimal means of decellularizing human lungs, individual human lobes were assigned to one of 5 methodological groups: manual instillation of detergents (M), perfusion with varied flow rates from 0.5 L/min to 2 L/min (VP), or perfusion with a peristaltic pump at constant flow rates (CP) of either 1 L/min, 2 L/min, or 3 L/min (CP1, CP2, CP3, respectively). CP methods also included physical agitation on a shaker table during incubations. All lungs in the VP or CP groups were perfused with a precision peristaltic perfusion pump (Stockert-Shiley, Multiflow Roller Pump 10-00-00). Variable perfusion was initially utilized to test the feasibility of instilling decellularization fluids into porcine lungs and human lobes with a perfusion pump and to establish a range of flow rates which allowed inflation of the lungs and lobes without concomitantly causing gross damage to the decellularized scaffold. In instances where more than one lobe was available from an individual patient, upper lobes were exposed to 2 L/min, lower lobes were exposed to 3 L/min, and middle lobes at 1 L/min. Complete details, including reagent volumes and incubation conditions are listed in Table 1. All ILD samples were decellularized using 2 L/min (CP2). The order of decellularization reagents—distilled water, 0.1% Triton X-100, 2% sodium deoxycholate (SDC), 1 M sodium chloride (NaCl), DNAse, followed by terminal sterilization with peracetic acid (PAA)—is based on our previous work in rodent and non-human primate models [5,7,10,11,16,17,20]. All human and porcine lungs decellularized using the CP methods also received an increased final rinse volume at the completion of the decellularization protocol as compared to M and VP methods (Table 1).

Prior to decellularization, individual human lobes were separated and the main stem bronchus and major vasculature inlets and outlets (see Supplementary Fig. 1) were rinsed with deionized (DI) water containing 5× penicillin/streptomycin (Lonza) using the assigned method of instillation. Half of the volumes used in each step were flushed through the airways via the trachea and the other half through the vasculature as detailed in Supplementary Fig. 1. All subsequent instillations into individual human lobes followed this manner of main bronchus airway instillation and all major vascular ports with half of the volumes being instilled through the main bronchus and half through the vasculature. The flow rates in VP were continuously adjusted from 0.5 L/min to 2 L/min until the lobe was maximally inflated. Lobes were not exposed to flow during incubation steps, but were exposed to agitation on a shaker table in the CP methods.

Using the different manual or perfusion approaches, 0.1% Triton-X100 (Sigma) in DI water containing 5× pen/strep was infused through airway and all major vascular ports. Lungs were then submerged in a Triton-X100 solution and incubated for 24 h at 4 °C (with or without physical agitation, as outlined in Table 1). On day 2, the lungs were removed from the Triton-X100 solution and manually manipulated to assist in removal of decellularization fluids. DI water and 5× pen/strep were then instilled into the lobe as previously described. Removal of prior decellularization fluids was achieved in this manner after each step. Next, 2% sodium deoxycholate (SDC) (Sigma) in DI water containing 1× pen/strep was instilled into individual lobes, the lobe was then submerged in SDC solution and placed on a shaker table for 24 h at 4 °C. The next day, lungs were removed from the SDC solution and rinsed with DI water and 1× pen/strep. 1 M NaCl (Sigma) and 5× pen/strep in DI water were then instilled into the lung and allowed to incubate for 1 h at room temperature ($\sim$25 °C). Lungs were removed from the NaCl solution, rinsed with DI water and 1× pen/strep, and then instilled with 30 μg/ml porcine pancreatic DNase (Sigma), 2 mM $CaCl_2$ (Sigma), 1.3 mM $MgSO_4$ (Sigma), and 5× pen/strep in DI water. Lobes were then submerged in the DNase solution and incubated for 1 h at room temperature. The lungs were removed from the DNase solution and rinsed with DI water and 1× pen/strep. Human lungs were terminally sterilized using a 0.1% peracetic acid (Sigma) in 4% ethanol rinse for 60 min at room temperature. Finally, lungs were removed from the peracetic acid solution and rinsed with the storage solution (1× PBS, 5× pen/strep, 50 mg/L gentamicin (Cellgro), 2.5 μg/ml Amphotericin B (Cellgro)). Lungs were subsequently stored submerged in storage solution at 4 °C until needed for up to three weeks. Owing to the shelf-life of the anti-microbial agents, lungs were removed and re-instilled with 4 L of fresh storage solution every three weeks.

Download English Version:

https://daneshyari.com/en/article/10227824

Download Persian Version:

https://daneshyari.com/article/10227824

Daneshyari.com