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Production and Utilization of Acellular Lung Scaffolds In Tissue Engineering

Joan E. Nichols,[1*] Jean A. Niles,[1] and Joaquin Cortiella[2]

[1]*Department of Internal Medicine Infectious Diseases, University of Texas Medical Branch at Galveston, Galveston, Texas 77555*

[2]*Department of Anesthesiology, University of Texas Medical Branch at Galveston, Galveston, Texas*

ABSTRACT

Pulmonary disease is a worldwide public health problem that reduces the quality of life and increases the need for hospital admissions as well as the risk for premature death for those affected. For many patients, lung transplantation is the only chance for survival. Unfortunately, there is a significant shortage of lungs for transplantation and since the lung is the most likely organ to be damaged during procurement many lungs deemed unacceptable for transplantation are simply discarded. Rather than discarding these lungs they can be used to produce three-dimensional acellular (AC) natural lung scaffolds for the generation of engineered lung tissue. AC scaffolds are lungs whose original cells have been destroyed by exposure to detergents and physical methods of removing cells and cell debris. This creates a lung scaffold from the skeleton of the lungs themselves. The scaffolds are then used to support adult, stem or progenitor cells which can be grown into functional lung tissue. Recent studies show that engineered lung tissues are capable of surviving after in vivo transplantation and support limited gas exchange. In the future engineered lung tissue has the potential to be used in clinical applications to replace lung functions lost following injury or disease. This manuscript discusses recent advances in development and use of AC scaffolds to support engineering of lung tissues. J. Cell. Biochem. 113: 2185–2192, 2012. © 2012 Wiley Periodicals, Inc.

KEY WORDS: DECELLULARIZED LUNG; ACELLULAR NATURAL SCAFFOLD; NATURAL MATRIX; LUNG EXTRACELLULAR MATRIX; TISSUE ENGINEERED LUNG

For end-stage lung disease the only therapeutic option is often lung transplantation [Studer et al., 2004; McCurry et al., 2009]. In the United States, although the time spent on lung transplantation wait lists has declined the waiting time to transplantation for most patients is still long [McCurry et al., 2009]. At this time there continues to be a significant shortage of donated lungs and because of this many patients die before receiving a transplant. Although the standards set for determining the fitness of lungs for transplantation are appropriate they often result in the rejection of the majority of procured organs [US Organ and Transplantation Network, 2009; Medeiros et al., 2012; Zych et al., 2012]. This is because the lung is the most likely organ to be compromised during the process of organ donation and retrieval. The retrieval process often results in poor lung function which can be a cause of donor organ rejection. This has led to an innovative solution to offset the shortage of donor lungs. Development of lung perfusion techniques which recondition donor lungs not initially meeting standards for transplantation can increase the pool of acceptable organs [US Organ and Transplantation Network, 2009; Medeiros et al., 2012; Zych et al., 2012]. Work continues in this area to optimize organ retrieval procedures and to develop better methods for maintenance of donor lungs after procurement, thereby enhancing their viability. Although better procedures will increase the pool of lungs we will still experience a shortage of available organs for transplantation. For those lungs that do not meet the US organ procurement standards there is still one remaining option. Rather than discarding damaged lungs they can be used to produce acellular (AC) natural lung scaffolds for generation of engineered lungs or tissue. Although not a viable treatment option at this time it is possible that in the future tissue engineering may present an innovative solution to the organ shortage problem. The driving force behind the development of the field of tissue engineering in general has been the lack of appropriate tissues or organs to meet current transplantation needs. Tissue engineering for regenerative medicine purposes is the reconstruction of tissue equivalents to replace physiologic functions of tissues lost due to disease or injury. Some progress has been made in the engineering of organs such as urinary bladder [Atala, 2011] and trachea [Macchiarini et al., 2008; Bader

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*Correspondence to: Joan E. Nichols, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-0435. E-mail: jnichols@utmb.edu

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and Macchiarini, 2010] for clinical applications. Engineering of complex organs has not yet been realized and progress towards engineering tissue such as liver, pancreas, kidney, heart, or small intestine have been reviewed elsewhere [Bernstein, 2011]. There has been significant work in the development of AC scaffolds for use in tissue engineering of organs other than the lung [Gilbert et al., 2006; Badylak et al., 2009, 2011]. Recently, some progress has been made in both development of AC scaffolds [Cortiella et al., 2010; Ott et al., 2010; Petersen et al., 2010; Price et al., 2010] and engineering of lung tissue for evaluation in animal models [Ott et al., 2010; Petersen et al., 2012; Song et al., 2011].

DEVELOPMENT OF SCAFFOLD FOR LUNG ENGINEERING

Of critical importance in the selection of any scaffold for development of lung tissue are the strength and elasticity of the scaffold as well as the adsorption kinetics or capacity for cellular remodeling [Nichols and Cortiella, 2008]. The scaffold should allow the normal physiologic functions of the lung in terms of gas exchange to continue unimpeded. Lung scaffold design should also consider the geometry of the lung and the capability of the scaffold material to support both cell movement throughout the scaffold and movement of nutrients into the tissues while allowing waste removal from tissues. Until the recent development of AC lung we have not had a scaffold that met these requirements. The main requirement for any scaffold used in regenerative medicine practices is biocompatibility of the material and this is especially true with regards to the lung. Use of materials that do not possess degradation profiles similar to that of normal lung ECM or that produce immunogenic intermediates can induce inflammation and result in development of fibrosis.

Both natural and synthetic polymers have been used in the past to support engineering of small pieces of lung tissue. Use of materials other than AC lung scaffold, to engineer lung tissue has been reviewed previously by the authors and will not be discussed at length [Nichols and Cortiella, 2008; Nichols et al., 2011]. In brief, degradable synthetic matrices that have been used to engineer lung tissue include polyglycolic acid (PGA) [Cortiella et al., 2006] and PGA combined with pluronic F-poly-lactic-co-glycolic acid (PLGA) or poly-l-lactic-acid (PLLA) [Mondrinos et al., 2006]. Natural materials include development of scaffolding formed from collagen [Chen et al., 2005], and Gelfoam [Andrade et al., 2007]. While each of these materials was shown to be adequate for the development of small amounts of lung tissue none met the specific needs of the lung in terms of capacity for biodegradability, elasticity, strength, shape and pore size, and subsequent tissue development was not notable. For development of lung tissue, the scaffolding must remain long enough to provide the framework necessary to support cell attachment, cell growth and tissue development without impeding the elasticity or altering the elastic recoil of the engineered tissue or adjacent normal lung tissue. A biomaterial not as elastic as normal lung tissue may cause a restrictive condition similar to the disease process caused by scar tissue formation seen in idiopathic pulmonary fibrosis (IPF) or sarcoidosis patients [Nichols et al., 2011]. Implantation of a mixture of autologous ovine somatic lung progenitor cells (SLPCs) on a PGA/pluoronic F-127 (PGA/PF-127) scaffold into a large animal model (sheep; Fig. 1A) produced a fleshy tissue piece (Fig. 1B). The tissue fragment was well vascularized but showed little lung epithelial cell development and no obvious development of lung morphology due, the authors felt, to the inadequacy of the matrix used (Fig. 1C) [Cortiella et al., 2006]. Although the sheet of PGA/PF-127 did not support good lung tissue development, use of a small piece of PGA/PF-127 seeded with SLPCs and placed into a wedge resection site supported limited development of lung tissue at the site [Cortiella et al., 2006]. Similar results were shown with fetal lung cell (FLC)/Gelfoam constructs implanted into the lung parenchyma where there was some development of alveolar-like structures but little evidence of development of alveolar caplillary junctions or even areas of close connection between the epithelial cells and the endothelial cells comprising the blood vessels formed in the scaffold [Andrade et al., 2007]. The inadequacy of most commercial, natural, and synthetic scaffold materials to meet the specific and exacting needs of lung caused a number of investigators to consider using AC natural lung as a scaffold for the development of engineered lung.

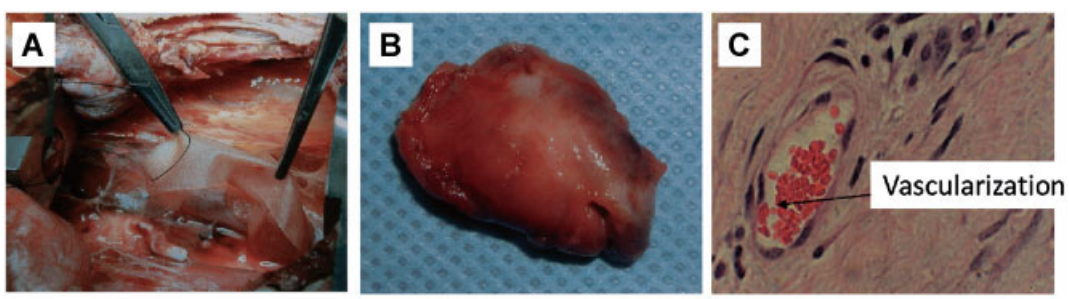

Fig. 1. Results of PGA/cell construct implantation. A: Surgery showing placement of the PGA/PF-127 SLPC seeded construct into the thoracic cavity at the pneumonectomy site. The tissue construct was attached to the right main stem bronchus. B: Flesh tissue growth harvested after 3 months. C: Sections of the tissue in (B) stained with hematoxylin and eosin showing vascularization of the tissue and presence of fibroblasts. 400× Magnification [adapted from Cortiella et al., 2006]. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

BENEFITS OF NATURAL ORGAN-SPECIFIC ACELLULAR SCAFFOLDS

AC natural scaffolds are composed of the extracellular matrix (ECM) secreted by the resident cells of the tissue or organ from which they are produced. As a result, these organ-specific scaffolds already possess the correct anatomical, chemical, and morphological structure of the natural tissue and have been shown to facilitate the constructive remodeling of many different organs in both preclinical animal studies and human clinical applications [Badylak et al., 2009]. The composition and structure of AC scaffolds and organ ECM depends on the origin of the tissue and the physiologic functions provided by the tissue [Dunsmore, 2008]. Separate from its biological requirements, the biophysical cues originating from ECM microstructure and mechanical properties have been shown to be a major influence on the growth and differentiation of cells and regulation of cell behavior [Pizzo et al., 2005]. It has been suggested that organ-specific ECM structure and mechanics may actually guide tissue patterning [Ingber, 2003; Engler et al., 2007]. ECM can also provide influences that are polar opposites of these positive biophysical cues in support of tissue development. It is important to remember that ECM remodeling, particularly of collagen and elastic fibers, may in fact interfere with respiratory mechanics [Suki et al., 2005; Anciães et al., 2011; Phillips et al., 2011].

PRODUCTION OF AC LUNG SCAFFOLD

We know that the fundamental physical properties of the lung and its functions are influenced by the composition of the ECM and that ECM proteins play an important role in influencing lung strength, flexibility, and elasticity. The structure of the lung is largely determined by the connective tissue network of the ECM and the organization of the nonlinear mechanical properties which lead to the complex mechanical behavior of the lung [Suki et al., 2005]. The interstitium of the lung parenchyma is composed primarily of collagen I, III and elastin and the primary function of these components is to form the mechanical scaffold that maintains the structure of the lung during the process of ventilation. Processes that remove cells from the lung may alter the ECM composition and affect the physical characteristics of the scaffold material. Development of ideal procedures for production of decellularized lung must allow for retention of key ECM components which support lung functions while facilitating removal of cell debris and nucleic acids. Effective decellularization procedures are dictated by factors such as tissue density, tissue organization, or organ structure [Crapo et al., 2011]. This is of particular importance for the lung since tissue density and structure vary considerably among main stem bronchi, bronchioles, and distal lung. A variety of methods exist which have been used to produce AC scaffolds from many tissues or organs [Gilbert et al., 2006; Badylak et al., 2011; Crapo et al., 2011], but none of these processes are lung specific and do not take into account the complexity of lung structure. Techniques used for tissue and whole organ decellularization have been reviewed, including descriptions of solvents, detergents, physical agents, and enzymes [Crapo et al., 2011]. Currently, there are only a few

TABLE I. Comparison of Current Protocols for Lung Decellularization, ECM Components Evaluated, Cell Source Used for Recellularization and Cell Responses

Protocol	Tissue origins	Detergent used	Condition for DC	Processing time	ECM remaining in AC lung	Cell source recellualarization	Cell responses or products
Lwebuga-Mukasa et al. [1986]	Human lung	Distilled water then 0.1% Triton X-100 followed by 2% Sodium deoxycholate and 1M NaCl	Immersion	48+ h	Collagen I, III, IV, V; laminin and fibronectin	Rate Type II AEC	Loss of lamellar bodies and change in morphology of AECs to a approximate a Type I AEC type
Cortiella et al. [2010]	Rat lung	1% SDS	Infusion followed by Bioreactor Immersion	3–5 weeks	Collagen I and elastin. Loss of laminin and collagen IV	mESC (C57BL6)	Pro surfactant protein C, surfactant protein A, CD31, cytokeratin- 18, CCSP and TTF-1
Ott et al. [2010]	Rat lung	0.1% SDS	Infusion stem and immersion	120 min	Collagen, proteoglycans, and elastin fibers	Rat FLC	Surfactant protein A, SPC and TTF-1 T1 alpha by FLCs
Petersen et al. [2010]	Rat lung	CHAPS	Infusion and immersion	180 min	Collagen I, elastin, and laminin	Neonatal rat epithelium and microvascular lung endothelial cells	Pro surfactant protein C and pro-SPB Clara Cell Secretory Protein Aquaporin-5
Price et al. [2010]	Mouse lung	0.1% Triton X-100 followed by 2% sodium doxycholate	Infusion and immersion	48+ h	Collagen I, elastin and laminin, but low levels of GAGs-	Murine FLC (E17)	Pro-surfactant protein C Cytokeratin-18
Daly et al. [2012b]	Mouse lung	0.1% Triton X-100 followed by 2% sodium doxycholate	Infusion and immersion	3 days	Collagen I, collagen IV, laminin, fibronectin but low levels of elastin and GAGs	Mouse bone marrow MSCs	Transient expression of TTF-1 but no other lung lineage markers

GAGs, glycosamineoglycans; mESC, murine embryonic stem cells; AEC, alveolar epithelial cells; FLC, fetal lung cells; MSC, mesenchymal stem cells; proSPC, pro-surfactant protein C; pSPB; prosurfactant protein B; CCSP, Clara cell secretory protein; TTF-1, thyroid transcription factor-1; AEC, alveolar epithelial cells.

protocols specific for decellularization of the lung and these are presented in Table I. These protocols differ in the detergent used, the physical conditions for decellularization, and the length of time of the process and subsequently in the ECM composition of the AC scaffold produced. Detergents used for the decellularization of lung include Triton X-100, Sodium dodecyl sulfate and 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS; Table I). Triton X-100 ($C_{14}H_{22}O(C_2H_4O)_n$) is a nonionic surfactant which has a hydrophilic polyethylene oxide group and a hydrocarbon lipophilic or hydrophobic group. Sodium dodecyl sulfate (SDS), is an organic compound with the formula $CH_3(CH_2)_{11}OSO_3Na$). It is an anionic surfactant used in many cleaning and hygiene products. Sodium deoxycholate (deoxycholic acid) is a water soluble, bile acid, ionic detergent generally used in methods for protein isolation or as a component of many cell lysis buffers (e.g., RIPA buffer). CHAPS is a zwitterionic detergent used in the laboratory to solubilize biological macromolecules such as proteins or as a non-denaturing solvent in some procedures for protein purification. Comparisons of matrix composition after decelluarization of rat or mouse lungs has indicated that variations in the ECM composition may occur depending on the detergent used (Table I). All of the detergents listed in Table I removed the cellular components of the lungs completely. The basement membrane is composed of collagen type IV, Laminin, and proteoglycans. It is important to remember that collagen IV, laminin, and proteoglycans are part of the basement membrane which epithelial cell integrins attach to. The interstitial matrix is composed of fibrilar collagens types I, II, and VII as well as elastin, proteoglycans, and hyaluron. Use of Chaps or Triton X-100 combined with sodium deoxycholate in the decellularization process allowed for retention of varying amounts of basement membrane components such as collagen IV, laminin, and proteoglycans, as well as components of the interstitial matrix collagen 1 and elastin. SDS use resulted in removal of most basement membrane components but left interstitial matrix components collagen I and elastin. Infusion of tissues with detergent, using perfusion systems (Table I) or bioreactor methods (Fig. 2A) produced AC whole trachea with attached lung scaffolds (Fig. 2B; Table I). Processing times varied greatly for these methods based on the choice of detergent used.

Regardless of the method used in Table I to produce AC scaffolds, the cell source, or the composition of the remaining ECM, selected cells were capable of attaching to the lung scaffolds and depending on the cell source used, differentiated and exhibited some expression of lung-specific protein markers. Although ECM is influenced by the decellularization process growth of cells on the AC scaffold immediately results in modifications to the composition of the ECM. Although the SDS decellularization method used by Cortiella et al. [2010] did not allow for retention of components of the basement membrane, murine embryonic stem cells (mESCs) had no problem attaching to the AC scaffold, differentiating or developing into rudimentary tissues [Cortiella et al., 2010]. From the moment of cell attachment to the scaffold, the mESCs immediately initiated remodeling of the ECM resulting in production of basement membrane components lost during the process of decellularization including collagen IV and laminin.

IN VITRO USE OF AC LUNG SCAFFOLDS

One of the first descriptions using a preparation of human AC lung to support cell adherence describes using a strip of AC alveolar matrix seeded with rat type II alveolar epithelial cells (AEC) to examine the influence of ECM on cell attachment and morphology. The AC scaffold described contained Collagen I, II, IV, and V as well as laminin and fibronectin [Lwebuga-Mukasa et al., 1986]. Rat type II AEC seeded on the human AC lung scaffold took on some of the morphological characteristics of type I AECs such as loss of lamellar bodies and cytoplasmic flattening. Similar results were described when rat type II AECs were cultured on strips of AC human amniotic membrane [Lwebuga-Mukasa et al., 1984]. From 1986 until 2010

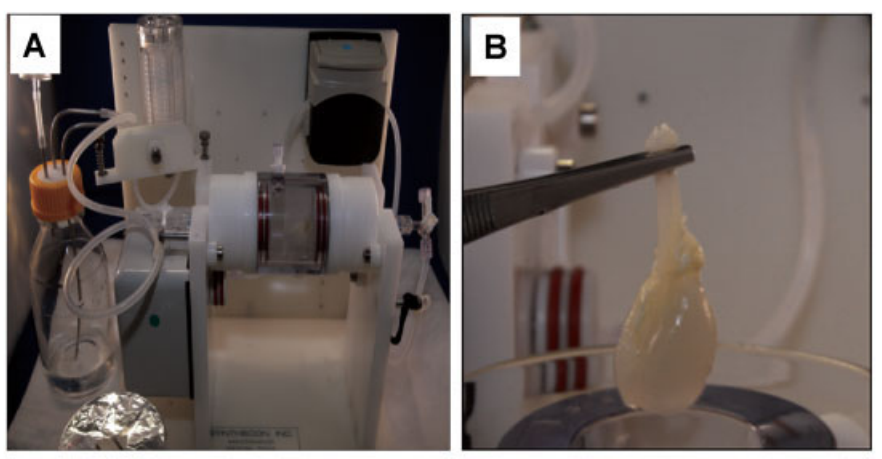

Fig. 2. Example of bioreactor system for decellularization. A: Picture of the bioreactor system used to produce acellular lung scaffold. Fresh 1% SDS was continually pumped through the chamber. B: Acellular whole rat trachea and lung scaffold after full decellularizarion [Photographs by Kenneth D. Frohne]. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

there was no progress in the development of AC lung scaffolds for the engineering of lung tissue. This changed in 2010 when four research groups published their findings related to the development of engineered lung using whole trachea–lung mouse or rat AC scaffolds [Cortiella et al., 2010; Ott et al., 2010; Petersen et al., 2010; Price et al., 2010]. In these studies, whole AC trachea–lung scaffolds were implanted with mouse [Price et al., 2010] or rat FLCs [Ott et al., 2010; Petersen et al., 2010] or with mESCs [Cortiella et al., 2010] (Table I). All groups reported good cell attachment, survival of cells and limited differentiation of cells into lung-specific cell phenotypes. All groups also reported that the recelluarization was not complete and that there were areas of the the lung scaffolds that did not contain cells. Recently, whole rat AC lung scaffold has also been shown to support attachment of murine bone marrow-derived mesenchymal stromal cells (MSCs) or C10 mouse lung epithelial cells following intratracheal inoculation [Daly et al., 2012b]. Although the MSCs were cultured in small airways growth media (SAGM) the MSCs predominately expressed genes consistent with a mesenchymal or osteoblast phenotype and no airway genes or vascular genes were expressed.

IN VIVO USE OF AC LUNG SCAFFOLDS

Use of whole AC trachea–lungs to engineer tissue makes the process of transplantation into animal models easier since the lungs: (i) have the same dimensions as natural lung so they fit properly into the thoracic cavity, (ii) support the functions of the lung in a way similar to normal lung, and (iii) possess the appropriate anatomical structures (trachea or bronchus) that allow it to be sutured in place in order to facilitate orthotopic transplantation. Engineered lung produced on AC scaffolds has been transplanted into animal models although graft recipient survival was limited in these early studies [Petersen et al., 2010; Ott et al., 2010; Song et al., 2011]. For the first of these studies, orthotopic implantation of an engineered left lung, derived from FLCs cultured on whole AC rat lung matrix for up to 8 days was done orthotopically following a left thoracotomy and a left-sided pneumonectomy [Petersen et al., 2010] (Fig. 3A–C). When implanted into rats for short time intervals, 45–120 min, the engineered lungs were shown to participate in gas exchange although there was some bleeding into the airways (Fig. 3C). A second group also engineered lung using rat FLCs cultured on AC rat scaffold for 5 days. The engineered lung was also transplanted orthotopically following a left-sided pneumonectomy [Ott et al., 2010]. After transplantation into the rat the constructs were perfused by the recipient's circulation and the engineered lung provided gas exchange in vivo for up to 6 h following extubation. In these initial experiments, graft function in vivo was limited to a few hours due to development of pulmonary edema in the engineered lung. Later studies by the same group used engineered lungs derived from AC rat scaffolds seeded with human umbilical vein endothelial cells (HUVECS) and rat FLCs cultured for 7–10 days [Song et al., 2011]. For these experiments, engineered lungs were transplanted into recipient athymic rats following a pneumonectomy. Athymic rats receiving a pneumonectomy with no transplant, or rats receiving transplantation of cadaveric lungs from Sprague–Dawley rats were used as controls. Cadaveric lungs were shown to have slightly higher compliance levels than engineered lungs on postoperative day 7. Oxygenation levels for both engineered and cadaveric recipients was seen to be higher than for pneumonectomized animals up to day 7 but gradually declined in rats receiving an engineered lung between 7 and 14 days. Compliance and gas exchange of the engineered lung in this study gradually declined after 7 days due to progressive graft consolidation and inflammation. Engineered lung grafts but not cadaveric grafts also induced the formation of a thick fibrous scar surrounding the graft, causing restriction of graft expansion. This scarring was potentially due to a residual natural killer (NK) cell response in the athymic recipient [Song et al., 2011].

ROLE OF AC MATRIX IN DIFFERENTIATION

We are just beginning to understand the influence of lung ECM on the differentiation of stem or progenitor cells and of subsequent tissue formation. Studies to examine this role have been limited although the field of tensegrity-based mechano-sensing has long suggested that (i) regional variation of ECM remodeling that occurs

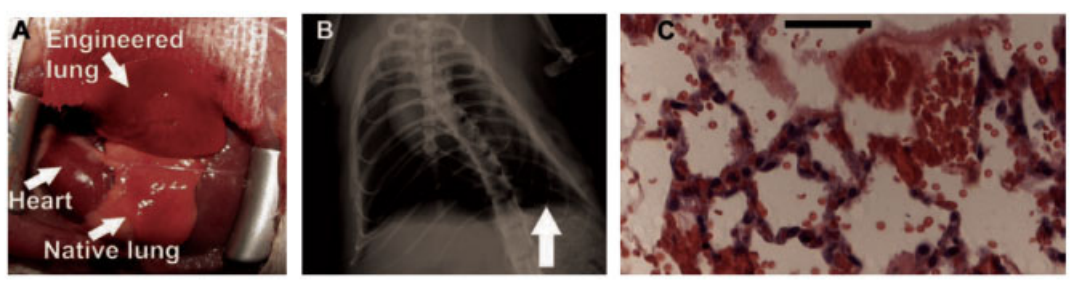

Fig. 3. Implantation of engineered lungs into rats. A: Tissue engineered rat left lung was implanted into Fisher 344 rat recipient and photographed ~30 min later. B: X-ray image of rat showing the implanted engineered left lung (white arrow) and the right native lung. C: H&E stain of explanted lung. Red blood cells perfusing the septa are evident, and some red cells are present in the airspaces. Scale bar 50 μm [Reproduced with permission of the author and the publisher, Petersen et al., 2010]. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

during embryogenesis leads to local differentials in ECM structure and mechanics, (ii) changes in matrix compliance (e.g., increased stiffness when the thinned basement membrane is stretched) alters mechanical force balance across membrane receptors that mediate cell–ECM adhesion, and (iii) altering the level of forces that are transmitted to the internal cytoskeleton will produce cell distortion and change intracellular biochemistry, thereby switching cells between growth, differentiation, and apoptosis [Ingber, 2008]. In the lung, the regional variability that occurs in ECM in both composition and stiffness as one progresses from the trachea to the bronchi and bronchioles and then to distal lung is extensive. Changes in ECM structure and composition should influence cell adhesion and provide critical cues that orchestrate tissue formation and cell function. One of the first reports regarding the influence of ECM on ESC differentiation into lung epithelial and endothelial lineages examined the efficiency of differentiation after allowing mESCs to attach to individual components of ECM. In these studies ECM proteins collagen I, laminin and fibronectin, were shown to induce production of type II AEC from mESC cultured in 2D or 3D [Lin et al., 2010]. Efficiency of differentiation of mESCs into lung lineage phenotypes was measured by expression of SPC-eGFP in cells using microscopy and PCR. Production of surfactant proteins C and A and aquaporin-5 were enhanced by the presence of laminin. Similar results were found when culturing mESCs on whole AC rat lung scaffold [Cortiella et al., 2010]. Efficiency of differentiation was measured here by evaluation of CD31, cytokeratin-18, and pro-surfactant protein C expression was shown to be increased by mESCs cultured on AC lung compared to commercially available matrices Gelfoam, collagen I, or Matrigel. Production of organized lung tissue as well as significant production of surfactant proteins A and C were only seen for mESCs cultured on AC lung and not on any of the other matrices used [Cortiella et al., 2010]. Other reports also support the concept that organ-specific stroma or ECM may even be required for proper site-specific differentiation and organization of lung tissues [Shamis et al., 2011]. A comparison between liver- and lung-derived AC scaffolds indicated that liver-derived scaffolds maintained the differentiation state of primary hepatocytes while lung-derived scaffolds allowed for both induction of lung lineage and maintenance of site-specific development of AE type II cells [Shamis et al., 2011].

PROBLEMS ASSOCIATED WITH USE OF AC LUNG SCAFFOLDS

The clinical application and success of decellularized trachea suggests that AC scaffolds may retain adequate strength to support both physiologic and anatomic functions of the trachea [Macchiarini et al., 2008; Bader and Macchiarini, 2010]. This may or may not be true for the lung. There are some limitations that must be considered before use of decellularized natural AC scaffold for development of lung tissues for clinical applications. We must consider the effects of the decellularization process on the mechanical integrity of the lung ECM prior to and after engineering of lung tissue. As we have mentioned previously AC ECM may be weakened, damaged, or degraded during the decellularization process. Quantitative evaluation of the composition of lung scaffolds suggests that there are great variations in AC ECM related to specific detergents used [Petersen et al., 2011]. Comparisons of AC lung scaffold produced using CHAPS or SDS suggests that there is a decrease in levels of collagen I, elastin content, and mechanical properties in SDS generated scaffolds [Petersen et al., 2012]. We do not know if the alteration in ECM composition will be detrimental to the production of tissues for the purpose of transplantation. What we know is that increased ECM production leading to deposition of collagen types I and III in fibrotic lungs results in lower lung compliance during assessment of pulmonary function tests (PFTs) [Cavalcante et al., 2005; Suki et al., 2005; Dunsmore, 2008; Suki and Bates, 2011). Compliance levels are an important indicator of lung elasticity and in disease states correlate well with collagen composition of the lung ECM. Some groups reported lower compliance values for AC compared to normal lung in vitro [Ott et al., 2010; Petersen et al., 2011; Price et al., 2010] and after transplantation [Song and Ott, 2011].

Our understanding of the link between lung tissue structure and function is incomplete due to the complexity of the problem and may not be easily understood or evaluated properly without examination of the ensemble behavior of all constituents of the lung which includes cells and ECM [Suki and Bates, 2011]. Culturing of selected cells on the AC lung scaffold will result in immediate changes to the ECM. Modifications to and remodeling of the ECM begins the moment cells attach. We may need to carefully examine the modifications made by cells to the AC ECM in order to determine what components of the ECM are truly critical to development of functional engineered tissues. For example, although SDS treatment removed basement membrane components during the process of decellularization mESCs began to produce laminin and collagen IV relatively quickly following attachment and replaced the missing ECM components [Cortiella et al., 2010]. A recent comparative analysis of whole decellularized mouse lungs produced using Triton X-100/sodium deoxycholate, SDS, or CHAPs also showed that although there were differences in gelatinase activation and protein composition of the AC scaffold, binding, and initial growth following intratracheal inoculation with MSCs or C10 cells was similar [Wallis et al., 2011].

Another important consideration related to the host response to natural AC lung scaffolds is the immunogenicity of the material. Although cells and cell debris have been removed from the scaffold along with the human leukocyte antigens expressed on the surface of cells which are responsible for graft rejection this does not mean that the scaffolds are no longer immunogenic. The host immune response to transplanted tissues or organs is an important determinant of graft function and survival. Natural scaffolds may induce immunogenic responses and invoke immunological reactions leading to inflammation and thereby causing graft rejection. A hallmark of tissue injury is increased turnover of ECM proteins. Failure to remove ECM degradation products from the site of tissue injury or tissue remodeling can result in the induction of inflammation in the host. Hyaluronan is an important component of the lung interstitium and functions to help maintain the structural integrity of the lung. This protein also plays a major role in cell signaling following lung injury. Fragments of hyaluronan have

been shown to trigger toll-like receptor (TLR) 2- and 4-dependent inflammation activation pathways resulting in initiation of inflammatory responses [Jiang et al., 2005]. The immune response to AC scaffolds has not been studied extensively and we need to develop a better understanding of the host response to the AC scaffold alone [Keane et al., 2012]. Investigation of the immunomodulatory effects of natural ECM scaffolds has recently indicated that tissue source, decellularization method, and chemical cross-linking modifications of scaffolds can affect the presence of damage-associated molecular patterns (DAMPS) within the biologic scaffold. Presence of these DAMPS has correlated with differences in cell proliferation, cell death, secretion of proinflammatory chemokines, and upregulation of TLR-4 proinflammatory molecules and may influence remodeling of the ECM and associated [Daly et al., 2012a].

DEVELOPMENT OF AC LUNG SCAFFOLDS FOR CLINICAL USE

Before discarded human lungs can be used for clinical applications there are some issues that must be addressed. Policies need to be developed related to tissue procurement for development of AC scaffolds from discarded lungs. Production standards, processing and sterilization methods, evaluation of the product and handling requirements prior to and after production of the AC scaffold must be established. We will need to evaluate the influence of the manufacturing process on ECM composition and immunogenicity and determine what components of the AC scaffold ECM are critical for tissue engineering. Poorly manufactured lung scaffolds may also lead to an induction of immune responses in recipients, alter the process of tissue remodeling and affect the functional outcome of the engineered lung. Natural materials may also harbor bacteria or viruses if adequate steps have not been taken to ensure the cleanliness of the materials produced. Good manufacturing practices (GMP) such as training and certification of personnel and design of clean room technologies in order to guarantee the safety and quality of the AC scaffold as a product will also need to be developed. Sizing and long-term storage concerns need to be addressed as does the impact of potential complications related to the variability of the product due to donor diversity. While xenografts are regulated as medical devices, most allograft tissue is classified as a Human Cell & Tissue/Product (HCT/P) by the FDA and not as a medical device. The FDA does not require a specific sterilization technique or Sterility Assurance Level (SAL) for allografts [Jacobsen and Easter, 2008]. Similar practices will have to be developed for the cell source to be used to engineer lung tissue for transplantation. Twenty-one CFR Part 1271, became effective on April 4, 2001 for human tissues intended for transplantation that are regulated under section 361 of the PHS Act and 21 CFR Part 1270 [Food and Drugs, 2001 at www.fda.gov/CBER/tissue/tisreg.htm]. This is a comprehensive plan for regulating human cells, tissues and cellular and tissue-based products that would include establishment of registration and product listing, donor suitability requirements, good tissue practice regulations, and other requirements.

FOR THE FUTURE

Now that we have identified an appropriate scaffold that meets the needs of the lung, one of the remaining hurdles to development of engineered lung for clinical applications is the selection of an appropriate cell source. Another key hurdle that has limited the advances in the engineering of a transplantable lung is our inability to produce a fully functional and appropriately vascularized lung. We have managed to grow small pieces of distal lung or trachea but have not been able to effectively reproduce the critical functions of gas exchange in the engineered tissues due to the lack of appropriate tissue vacularization. In regards to production of engineered tissues we also need to improve alveolar barrier function and increase production of surfactant proteins. Maturation of cells needs to be enhanced to promote development of type I AEC as well as cilliary function in the engineered tissues. In the future, we hope that discarded lungs not meeting the standards for transplantation will be used to generate AC scaffolds for the production of engineered lung tissue for clinical applications.

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