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Mini Review

Engineering of Pulmonary Microvasculature for Whole Lung Engineering

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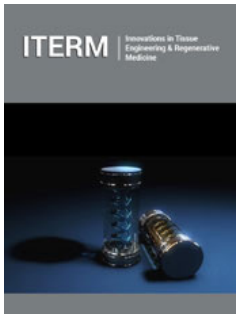
Abstract

Lung transplantation is an effective treatment for severe and end stage lung diseases. A readily available functional bioengineered lung from an autologous source is critical for lung transplantation. Previous studies have decellularized lungs and repopulated them with functional cell population, however, it failed due in large part to impaired formation of vascular compartment. Pulmonary vasculature not only exerts a physical barrier to separate blood and tissue but is a metabolically active barrier that controls exchange of gas, macromolecules, and immune cells and as such is very critical for lung tissue engineering. Many strategies including proper endothelial cell populations, seeding techniques and culture conditions have been optimized to achieve endothelial coverage of entire lung scaffold. In this article, strategies and challenges related to vascularized lung tissue engineering will be reviewed.



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Introduction

The focus of lung tissue engineering has been the development of synthetic or natural scaffold with stem or progenitor cells to regenerate lung tissue with functional pulmonary vasculature. However, current technologies failed to recapitulate the complex 3-dimensional architecture of the lung and further, clinical translation of this technique remains unknown. In 2010, two separate groups made modest progress by decellularizing rodent lungs and demonstrated the decellularized organ not only preserved the 3D architecture of alveoli but also removed cellular components that minimized immunogenicity, making 'off the shelf' bioengineered lung possible [1,2]. They have further repopulated the decellularized lung with endothelium and epithelium and managed to obtain cell growth in vitro. However, after several hours of transplantation, bioengineered lung failed due in large part to impaired formation of the vascular compartment, leading to thrombosis and pulmonary edema. Therefore, regeneration of functional pulmonary vasculature is critical for lung tissue engineering.

Pulmonary Vasculature

Pulmonary circulation consists of three major parts: arteries, capillaries, and veins. Morphometric approaches indicate that capillary walls dominate ~2% of lung anatomic volume and are responsible for gas exchange and nutrient transport to the whole parenchyma, which comprises 70 - 80% of lung anatomic volume (reviewed in [3]). Extra-alveolar structures, on the other hand, including extra- alveolar blood vessels such as pulmonary arteries and veins take a relatively small fraction of lung volume, and supply nutrients to airways that are ~7% of lung volume (reviewed in [3]). Pulmonary capillaries are small vessels from 5 to 8μm in diameter ([4-6] and reviewed in [3]), having a wall of one endothelial cell thickness. The endothelial cells, which form a monolayer lining the inner surface of blood vessels, synthesize many factors such as nitric oxide, prostacyclin, tissue plasminogen activator, thrombomodulin, heparan-sulfate, and the endothelial protein C receptor (a receptor associated with the activation of protein C) to control thromboresistance, vascular barrier, and vessel wall inflammation ([7-15] and reviewed in [16]). Dysfunction of pulmonary microvascular endothelial cells increases permeability, leading to extravascular leak of protein-rich edema, polymorphonuclear leukocyte influx, microvascular thrombosis, and further lung dysfunction (e.g. acute lung injury, sepsis) [17]. Thus, pulmonary vascular

endothelial cells, especially microvascular endothelial cells, play a regulatory role in pulmonary vascular homeostasis, alveolar function, and further lung regeneration.

Endothelial Cell Sources for Lung Tissue Engineering

Pulmonary endothelial cell

It is well known that endothelium from different organs is phenotypically distinct (reviewed in [18]). For example, microvascular ECs from lung secrete 1.5-15 times more urokinase-type plasminogen activator antigen, an enzyme involved in alveoli repair during injury [19] and surfactant protection [20], than human hepatic microvascular ECs, human umbilical vein ECs, angioma ECs, and lung fibroblasts [21]. Lung microvascular ECs uniquely expressed higher levels of factor VIII, vWF and UEA binding, and have lower Dil-Ac-LDL uptake compared to ECs from brain and liver [18]. Under treatment of endothelial barrier disruptor thrombin, lung microvascular ECs display the highest affinity and response, followed by brain ECs and liver ECs [18]. Therefore, lung ECs display a significantly different phenotype from ECs of other organs, and as such the ideal way to repopulate lung vasculature would be to use cells directly from lung vasculature.

The pulmonary vasculature composes of three anatomic compartments connected in series: The arterial tree, an extensive capillary bed, and venular tree (reviewed in [22]). Capillary endothelial cells, the most abundant EC, make up 30% of the cells in human lungs [23]. These cells are adjacent to alveoli and directly responsible to form a metabolically active surface for exchange of gas, macromolecules, and immune cells, and display a significantly different phenotype from ECs in large vessels. Endothelium in vessels with internal diameters less than 38μm uniformly binds Griffonia simplicifolia, a lectin that specifically interacts with α-galactose, and have no Weibel-Palade bodies ([24-26] and reviewed in [27]). Emerging evidence indicates pulmonary microvascular endothelial cells form significantly tighter barriers than do pulmonary artery endothelial cells. Trans-electrical resistance and hydraulic conductivity data revealed that microvascular ECs in basal conditions form tighter cell-cell barriers compared to ECs from vein and artery [28,29]. This may be due to the differential expression of activated leukocyte cell adhesion molecule (ALCAM), a cell adhesion molecule of the immunoglobulin super-family, which is significantly more abundant in pulmonary microvascular ECs than in pulmonary artery ECs. This glycoprotein has been shown to co-localize with adherens junctions such as vascular endothelial cadherin (VE-Cadherin) [30]. Knockout of this gene results in reduced expression of barrier junction proteins such as occludin, α-catenin, claudin-5, and ZO-1 and increased blood permeability [30]. Microvascular ECs also proliferate faster than do ECs from large vessels. In basal culture conditions and under the same medium, the population doubling times of microvascular ECs and artery ECs are 39h and 58h, respectively. This finding was correlated with the higher proportion of endothelial colony forming cells in microvascular ECs compared to those in macrovascular ECs [28]. We have recently repopulated rat lung microvascular ECs into a decellularized lung scaffold, and demonstrated ECs not only maintained their expression of CD31 but also displayed tight junctions observed by TEM after 8 days culture [1]. Recent work has co-cultured pulmonary microvascular ECs with arterial and venous ECs in decellularized lung scaffolds and demonstrated that positive VE-cadherin staining was only observed once three cell types are present, suggesting a necessity of cell-cell cross-talk for barrier formation [31]. However, it is well documented that lung allograft endothelium can initiate immune rejection through presentation of alloantigen's to circulating T cells, natural killer cells and macrophages (reviewed in [32]), which limits the use of primary pulmonary ECs for bioengineered lung applications.

Blood outgrowth endothelial cell

A subpopulation of circulating, bone-marrow-derived cells, termed endothelial progenitor cells, has been shown to contribute to neovascularization, and home to sites of vascular trauma potentially participating in re-reendothelialization of damaged or denuded surfaces [33-35]. These cells can be easily derived from a standard apheresis procedure from autologous sources, and if maintained in culture they undergo a transition to cells with a phenotype similar to mature endothelial cells [36,37). These blood outgrowth endothelial cells (BOECs) can achieve at least 30 population doublings in serial passaging, re-plate into at least secondary and tertiary colonies, and retain high levels of telomerase activity [38,39]. They also exhibit surface marker profiles (i.e. CD31, 133, 144, 15, 146, and KDR) and cytoplasmic vWF production similar to mature ECs [40]. Various groups have demonstrated that protein and gene expression between BOECs and mature endothelial cells are highly similar [37,40-42]. For example, Medina and colleagues used 2D-PAGE to investigate the proteomes of BOECs and human dermal microvascular endothelial cells (DMECs) and observed BOECs demonstrated over 90% of the tested protein profile in common with DMECs [40]. When combined with synthetic or natural materials, BOECs can form de novo functional blood vessel in vivo. Yoder et al. [28] have isolated and cultured BOECs in collagen/fibronectin gels and transplanted them into immunodeficient mice. At 14 to 30 days, BOECs formed chimeric vessels, which were perfused with mouse red blood cells [43], suggesting that BOECs resemble mature ECs in phenotype and can be directly involved in vessel formation.

Multiple in vitro studies have seeded BOECs on surfaces of synthetic vascular grafts or vascular scaffolds and demonstrated that human BOEC adherence, growth, and phenotype are supported on various synthetic coatings (e.g. poly (1, 8-(octanediol citrate), polyurethane, polyglycolic-acid) under both static and flow culture conditions [44-48]. When seeded and cultured on three-dimensional biodegradable vascular scaffolds (polyurethane foam or polyglycolic acid/poly-4-hydroxybutyrate mesh) under dynamic culture in vitro, human umbilical cord blood OECs display suitable cell-to- polymer attachment and growth on both polymers while maintaining endothelial phenotype (CD31, vWF, and eNOS) after 12 days of culture [47]. Further preclinical studies have demonstrated autologous BOECs-seeding improved thromboprotective properties of vascular implants [49-51].

Jantzen et al. [50] seeded autologous porcine BOECs at the point-of-care in the operating room onto Titanium (Ti) tubes for 30 minutes followed by implanting into the inferior vena cava of swine (n=8). After 3 days, a blinded analysis revealed that all 4 cell-seeded implants are free of clot, whereas 4 controls without BOECs were either entirely occluded or partially thrombosed [50]. Quint and coworkers have cultured aortic smooth muscle cells on a degradable polyglycolic acid mesh scaffold in bioreactors for 10 weeks followed by decellularization. Porcine BOECs were seeded on the lumen of vessels for 3 days, and these tissue-engineered blood vessels were implanted as porcine carotid interposition grafts. After 30 days of implantation, they found that all BOEC-seeded TEBV remained patent, whereas only 3/8 control vein grafts were patent. They have also determined that less neointimal hyperplasia were observed compared to control vein grafts [52]. Thus, BOECs potentially preserve endothelial properties on biomaterial scaffolds and may be a proper candidate for vascularization of lung scaffolds. However, the phenotype and proliferative potential of BOECs can be impaired in cardiopulmonary disease patients (reviewed in [53]) who are the major cohort requiring lung transplantation. Furthermore, reseeding with BOECs necessarily requires large volumes of apheresis product, and can lead to risk of several complications related to leukapheresis procedures such as hypocalcemia due to citrate anticoagulation (reviewed in [54]).

iPS-EC

As a source for regenerative therapies, human induced pluripotent stem cells (iPSCs) offer immense potential and was recognized by the 2012 Nobel Committee in Medicine. These cells, derived from differentiated adult cells through genetic reprogramming, have provided an exciting alternative for bypassing ethical concerns related to embryonic stem cells derivation and potential issues of allogeneic immune rejection [55-57]. Differentiation toward endothelial cells from iPSCs has been extensively studied and reviewed elsewhere [58,59]. The iPSCs are usually cultured into three- dimensional embryoid bodies in suspension culture followed by differentiation into 3 germ layers: endoderm, ectoderm, and mesoderm, where endothelial cells belong. Culture strategies including using growth factors, co-culture with parenchymal cells, and 2D culture on coatings enriched with ECM proteins have been used to differentiate ECs from mesodermal cells (reviewed in [58,59]). For example, inhibition of TGF-β in pluripotent stem cell differentiation increased VE-Cadherin+ cells by 10-fold. Subsequent inhibition of TGF-β in culture improved the proliferation of purified endothelial cells up to 36-fold [60]. Co-culture of mouse bone marrow stromal cell line OP9 with iPSCs improved the emergence of CD31+ endothelial cells to as early as 8 days [61]. Transduction of Oct4 and Klf4 into human neonatal fibroblasts in the presence of soluble factors promotes the induction of endothelial reprogramming. Clusters of induced endothelial cells resemble primary human ECs phenotype and protein profiles such as CD31, VE-Cadherin, and vWF were obtained at 28 days [62].

Many studies have tried to improve the yield of iPSC-differentiated ECs for scale-up applications. Sahara et al. [63] screened > 60 small molecules that would promote endothelial differentiation and found that administration of BMP4 and a GSK-3β inhibitor in an early phase and treatment with VEGF-A and inhibition of the Notch signaling pathway in a later phase led to the emergence of endothelial cells within six days. These ECs were able to form functional capillaries in vivo with anastomosis to the host vessels when transplanted into immunocompromised mice. The iPSC:EC ratio of 1:20 was reported to be a significant improvement over prior publication [63]. A recent work by Prasain et al. [64] have significantly improved the yield of iPSC: EC ratio to 1:1 × 108. They have identified a Neuropilin- 1 (NRP-1) +/CD31+/CD144+ subpopulation from iPSC-differentiated endothelial cells displays functional properties similar to umbilical cord blood endothelial colony forming cells, with high clonal proliferative potential and robust in vivo vessel-forming ability. The ESC-NRP-1+CD31+ECFC and iPSC-NRP-1+CD31+ECFC maintained a stable endothelial phenotype and function and did not undergo replicative senescence for 18 passages in vitro. These iPSC-NRP-1+CD31+ECFC have shown capacity to form human vessels in mice and to repair the ischemic mouse retina and limb for > 6 months with lack of teratoma formation potential [58,64]. Thus, these iPSC-NRP-1+CD31+ECFC may provide clinically relevant number of functional cells and may be a proper EC candidate for vascularization of lung tissue engineering.

Conclusion

Pulmonary vasculature not only exerts a physical barrier to separate blood and tissue but is a metabolically active barrier that controls exchange of gas, macromolecules, and immune cells and as such is very critical for lung tissue engineering. However, many challenges such as proper EC population, seeding strategy, and culture strategy, remain to be overcome to achieve a vascularized lung scaffold. Endothelial cells display significant phenotypic heterogeneity and as such the ideal cell population for lung tissue engineering should be from lung itself. However, the allogeneity of endothelium necessarily initiates immune rejection through presentation of alloantigens to circulating T cells, natural killer cells, and macrophages. Endothelial cells isolated from autologous leukapheresis products are readily available, functional, and proliferative, however their functions are largely impaired if harvested from cardiovascular patients, which are the major cohort of lung transplantation recipients. iPSC-derived ECs are highly proliferative, purified and functional and as such may be a proper cell candidate for vascularization of lung tissue engineering. However, iPSC differentiation toward lung-specific endothelium has been sparsely investigated, and therefore phenotypic comparison between iPSC-ECs and pulmonary ECs may help to understand their differences.

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