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Review Article

Engineering Airway Epithelium

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Airway epithelium is constantly presented with injurious signals, yet under healthy circumstances, the epithelium maintains its innate immune barrier and mucociliary elevator function. This suggests that airway epithelium has regenerative potential (I. R. Telford and C. F. Bridgman, 1990). In practice, however, airway regeneration is problematic because of slow turnover and dedifferentiation of epithelium thereby hindering regeneration and increasing time necessary for full maturation and function. Based on the anatomy and biology of the airway epithelium, a variety of tissue engineering tools available could be utilized to overcome the barriers currently seen in airway epithelial generation. This paper describes the structure, function, and repair mechanisms in native epithelium and highlights specific and manipulatable tissue engineering signals that could be of great use in the creation of artificial airway epithelium.

1. Structure and Function of Airway Epithelium in the Airway Tract

The airway tract can be divided in two zones: the conditioning zone in which the inhaled air is cleaned, moistened, and transported to the distal part of the airways and the respiratory zone where the blood is oxygenated. The conditioning zone consists of the nasal cavities, pharynx, larynx, trachea, bronchi, and large and terminal bronchioles, and the respiratory zone of respiratory bronchioles and alveolar duct and sac [1]. A layer of epithelium lines the interior of the airway tract. Through most of the conditioning zone, the airways are lined with epithelium containing various cell types: ciliated, goblet, brush, and basal cells [2]. All cells are in contact with the basement membrane; however, basal cells do not reach the airway lumen. This organization, called pseudostratified, gives the impression of a multilayered tissue.

The main cell types of the airway epithelium are the ciliated, goblet, and basal cells. Goblet cells secrete mucus to the airway lumen. This mucus lubricates the apical surface of the epithelial layer, moistens the inhaled air, helps to trap potential harmful foreign particles from the environment, and can absorb harmful gases such as ozone [1, 4]. Ciliated cells have specialized organelles called motile cilia, which can be found as clusters of 100–300 motile cilia on the apical surface. The cilia have motor proteins that allow them to beat in coordinated waves allowing the movement of mucus and foreign particles toward the throat [4, 5]. Basal cells have been categorized as epithelium-resident stem cells and, therefore, their function is to maintain the homeostasis of the normal epithelium after an injury or during tissue renewal [6, 7]. Brush cells are characterized by microvilli in the apical cell surface and although their function has not been completely defined, recent evidence suggests that these cells are chemosensory cells that sense bitter compounds in the airway lining fluid [8].

At the end of the conditional zone (i.e., the last branches of bronchi and the bronchioles), the epithelium changes from pseudostratified to a simple cuboidal epithelium.

Ciliated, goblet, and basal cells are gradually reduced, and nonciliated cells called Clara cells increase in number [1, 9]. Clara cells are dome-shaped cells that protrude into the airway lumen [9]. They are multifunctional cells that secrete proteins such as CCSP, mucins, and antimicrobial peptides into the airway lumen and act as progenitor cells repopulating ciliated cells [7, 10]. In the respiratory zone, the epithelium becomes thinner and changes from simple cuboidal epithelium in the respiratory bronchiole to simple squamous in the alveolar ducts and sacs [1]. The alveolar ducts and sacs are lined by an epithelium composed of two specialized cells: type I and type II alveolar cells, which will not be discussed further.

The airway epithelium is constantly exposed to the environment and dangerous pathogens. Therefore, a primary role of the epithelium is as a protective barrier. The epithelial surface is covered by a layer of airway surface liquid (ASL), mainly produced by the epithelial cells. The ASL is composed of a mucus layer overlying a watery periciliary liquid (PCL) layer [11]. In the mucus layer, the pathogens are trapped, killed, and removed by the beating cilia, a process known as mucociliary clearance [2]. The mucus layer is mainly composed of mucin glycoproteins that are important for the mucus structure. In humans, there are two major forms of mucins: MUC5AC and MUC5B, which are mainly produced by goblet cells and submucosal glands, respectively [12, 13]. The specific role of the mucins in the host response defense is not completely clear, but it is believed that they are involved in the response to infection, inflammation, and the presence of foreign particles [12, 13]. The PCL surrounds cilia providing hydration and facilitating mucus transport and clearance [14]. Epithelial cells maintain ASL composition and volume through secretion of Cl^- ions and the absorption of Na^+ ions [15].

In addition to promoting the airway luminal clearance, epithelial cells also initiate host defense mechanisms, forming a first line of protection against pathogens. Airway epithelial cells recognize pathogens through specific receptors such as Toll-like receptors and RIG-I-like receptors [16] and secrete defense molecules such as mucins, antimicrobial peptides (AMPs), reactive oxygen species (ROS) [16], antivirals such as interferon-β (IFN-β), and proinflammatory such as tumor necrosis factor (TNF) and interleukin-1 (IL-1) [17, 18].

2. Epithelial Repair

A variety of factors and stimuli can cause damage to the airway epithelium. As cells within the airway have a low rate of turnover, normal maintenance is provided by a subset of slowly renewing progenitor cells [19]. However, due to its role in providing a barrier to protect against environmental exposure, rapid and effective repair of the epithelium after injury is vital. This repair can be divided into three stages: dedifferentiation, proliferation, and differentiation [20]. Alterations to the normal repair process have been suggested as a cause for multiple airway diseases.

After injury, deepithelialization occurs via epithelial shedding, exposing the basal membrane, and triggering neighboring cells to dedifferentiate [21]. Repair begins immediately and occurs via migration and spreading of cells adjacent to the wound edge [21–23]. This process serves as a temporary "patch" to provide a cell barrier quickly and efficiently. The migrating cells are also responsible for secreting matrix components that stimulate further migration and act as a scaffold for the cells to build on. Once cells have migrated into the wound site, they begin proliferating to fully close the wound [23]. Full barrier function is restored only after the formation of a squamous metaplastic epithelium which eventually gives rise to a pseudostratified epithelium [24].

Dedifferentiation of the epithelial cells results in a flattened cell with a more mesenchymal phenotype capable of rapid migration [25]. Cell migration to cover the defect occurs through a combination of extracellular matrix (ECM) production and secretion of cytokines by both the remaining epithelial cells and the bronchial wall fibroblasts [26–28]. By the release of ECM components such as fibronectin and collagen IV, epithelial cells are able to self-regulate their rate of migration to eventually fill the defect. Fibronectin not only provides an adhesive platform for the cells, but it has also been shown *in vitro* and *in vivo* to be a key regulator of directional migration of bronchial epithelial cells [23]. As the cells migrate, the secretion of matrix metalloproteinases (MMPs) is necessary to allow for the release of focal adhesion sites at the rear of the cell [24]. MMP-9, in particular, is secreted by multiple cell types, including basal and epithelial cells after wounding. Blocking MMP-9 causes a decrease in the rate of cell migration [29]. In small mammals, the migration phase lasts about 8–15 hours, after which the wound is covered with a layer of flattened dedifferentiated cells [22, 23, 30].

In the first few hours after cell migration, an increase in proliferation occurs mainly at the region adjacent to wound edge, filling the voids left by the migrating cells [23]. Proliferation is mediated by factors secreted by a combination of resident cells and infiltrating leukocytes [22]. Of the many soluble factors, member of the epidermal growth factor (EGF) and transforming growth factor (TGF) families have been found to have a profound effect on the rate of repair [31]. Cell proliferation is a highly organized event that lasts for days to weeks depending on the size of injury [28]. This process peaks between 24 and 48 hours after injury in mouse models [23, 30] and takes even longer in humans [32]. Heguy et al. [32] examined injury caused by airway brushings. They found that by 7 days after injury, most of upregulated genes were late-stage cell cycle genes involved in G2 and M phase, showing that proliferation is very synchronized. By 14 days, these genes were back to normal levels [32].

The final stage in epithelial repair is the redifferentiation of the cells and restoration of full function [19]. Whilst the exact mechanisms controlling cell fate are not clear, it is a highly complex process that ensures the correct number of each cell type is formed [33]. Both multiple paracrine factors and cell-cell contact are likely required for correct cell differentiation. Transcription factors such as β-catenin, Foxa1, Foxa2, Foxj1, and Sox proteins are upregulated during repair of murine airway after naphthalene injury. These factors are

also expressed during embryonic lung development and are thought to have a role in the redifferentiation of ciliated cells during the repair process [34].

As with other epithelial tissues, repair is also mediated by a population of stem or progenitor cells. Due to the complexity of the pulmonary organ, it is thought that multiple stem cell niches exist, each one containing a population of cells capable of regenerating particular cell types [35]. Work by Giangreco et al. [36] has suggested that resident progenitor cells are required for normal tissue maintenance. As the lung is a slowly renewing tissue, there is no requirement for highly proliferative progenitor cells. In the case of acute injury, activation of these progenitor populations is enough to elicit repair [19, 36, 37]. However, when more widespread injury occurs, depletion of the resident progenitor cells can result in the activation of stem cells [36]. Recently, putative stem cell populations have been identified in human lung, suggesting that even a multipotent stem cell might be involved in airway repair after injury [38]. In addition to the stem cell in the lung, it is also clear that bone marrow-derived cells (BMCs) can traffic into injured lungs, aid in repair, and reduce inflammation [39, 40].

3. Engineering Approaches to Control Epithelial Regeneration and Repair

Tissue engineering (TE) strategies offer another option to promote and accelerate macroscopic and microscopic epithelial repair by controlling cell organization using chemical and mechanical signals. Applying TE strategies to organize airway cells into specific and controlled structures will also improve the performance of these cells as an *in vitro* model of epithelial tissue.

The gold standard for the repeatable manufacture of adult airway epithelium *in vitro* is transwell culture [41, 42]. Transwell culture is based on two-compartment culture where primary airway epithelial cells are seeded on porous, collagen-coated membranes in liquid culture. After reaching confluence, liquid from the top compartment is removed leaving the epithelial sheet exposed to air. This is known as air-liquid-interface (ALI) culture. Over a two-week maturation period, epithelial cells form motile cilia at the apical surface signifying apical-basal polarization. However, transwell culture does not create correctly aligned epithelium with coordinated beating of motile cilia [43, 44]. A myriad of TE tools exist to direct cell organization. These tools, when adapted for epithelial TE, may prove useful for generating more appropriate cell organization and ciliary alignment in *in vitro* epithelium.

It is well known that cells are instructed by and modify the materials they grow on over time. Regulating these instructive signals over time and space is a key challenge of TE. A wide variety of tools have been developed to study the effect of different chemical and mechanical signals on cell behavior. Most TE tools, however, have been developed for endothelial, muscle, and nerve cells. These cell types do not polarize in an apical-basal fashion and are grown on solid culture substrates. We speculate that little work has been reported using these tools to organize epithelium due to the necessity of special culture conditions required to produce a functional epithelium. To apply TE strategies to align structural components of epithelial cells, it is necessary to adapt existing methods for use on the porous membrane of a transwell plate that allows nutrient diffusion to the apical surface of the cells. Here, we describe some tools that are currently used in TE which have the potential to be relevant and useful for engineering epithelium if adapted appropriately. These tools can be classified based on the signal type, and method presented. As seen in Figure 1, we will focus this paper on chemical and mechanical signal types. Chemical signals can be presented in a mobile or immobilized state, while mechanical forces can be presented in a constant or inducible fashion.

Chemical signals can be immobilized on biomaterial scaffolds in a graded fashion to guide cell movement and organization (Figure 1(a)), [45]. For example, using an immobilized concentration gradient of NGF and NT-3 on a poly(2-hydroxyethyl-methacrylate) and poly(L-lysine), Moore et al. were able to guide neurite outgrowth of primary neurons [46]. The effect of utilizing two growth factors together was shown to increase the biological response in chick neural cells. This approach has been used to successfully guide the behavior of fibroblasts [47], endothelial cells [48, 49], osteocytes [50], and human mesenchymal stem cells [51].

Immobilized chemical signals on biomaterials could provide a useful tool for epithelial TE as multiple growth factors acting together could promote more physiological tissue proliferation, motility, and differentiation in an airway epithelial model. In addition to gradients, patterns of immobilized growth factors [52, 53] could also prove to be of great use in epithelial TE. Although there has not yet been work specifically on epithelium, it appears likely that airway epithelial maturation could be controlled by generating immobilized, through covalent bonding of growth factor to a substrate, (Figure 2(a)). The various growth factors would conceivably interact with the immature epithelium to drive differentiation to specific epithelial cell types in a repeatable fashion based on the organization of the immobilized growth factors. Another use of immobilized chemical signals on a scaffold is to drive cells down a specific differentiation pathway. A single growth factor on a scaffold to multiple growth factors on solid substrates has been shown to modulate oligodendrocyte differentiation [45] and stem cell fate [54]. This approach of presenting a chemical signal using a biomaterial to guide differentiation is conceivably useful in epithelial TE as certain differentiation pathways leading to specific lineages could be developed as a model, or a graft of distinct areas of airway epithelium.

While the above techniques have been developed for surface culture, encapsulation of cells within a hydrogel presents an opportunity for cells to be delivered to necessary sites both *in vitro* and *in vivo* within a chemically defined 3D environment. Hydrogels can present different chemical groups and can be bio- or nondegradable over time. Guiding cells using immobilized chemical signals in defined 3D environments within hydrogel scaffolds has been seen to have great value in treating retinal degenerative diseases

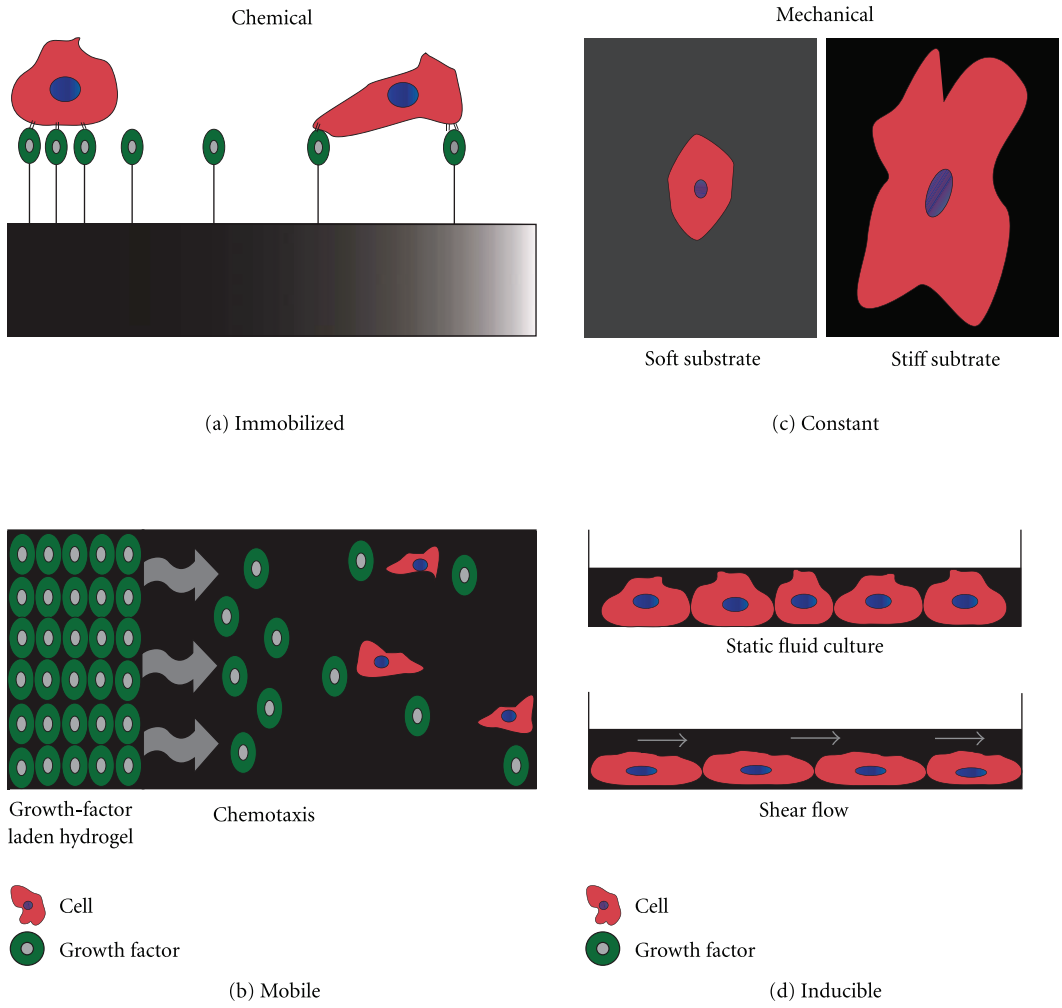

Figure 1: Examples of the tools of tissue engineering. Tools that manipulate the timing and appearance of chemical and mechanical signals offer opportunities to organize and direct the differentiation of developing tissue. Chemical signals can be immobilized, (a) in the form of covalently bonded growth factors that direct cell migration, or mobile in a hydrogel, (b) to create a chemotactic signal, through diffusion, for cells to respond to. Mechanical signals can be presented as a constant force, such as substrate stiffness, (c) to modulate cell spreading or as an inducible force, (d) such as shear flow, to organize cells in the direction parallel to flow.

[55] and spinal cord injuries [56]. While the majority of hydrogels with immobilized signals has been developed for nonepithelial cells, some materials for epithelial applications are already available. To create an oral mucosa equivalent, Kinikoglu and others developed a coculture system on a scaffold that presented specific chemical properties [57]. Fibroblasts and oral epithelial cells were seeded on this scaffold to create stratified and differentiated epithelium-like oral mucosa. In a refinement of their research, Kinikoglu and colleagues used recombinant DNA technology to develop an epithelial TE tool that presented the RGD peptide sequence within a biocompatible polymer that was then electrospun onto elastin and collagen foam, thereby creating a 3D coculture system of fibroblasts and oral epithelium on scaffolding that presented a static chemical signal to promote specific types of integrin binding [58].While these tools were developed for oral epithelium, their adaptation to air-liquid-interface culture would involve a transfer to 2D patterning technologies to be useful to airway epithelial maturation.

Javaherian and colleagues created [59] and adapted [60] a fast and facile 2D technique for patterning multiple epithelial cell populations into a specific organization. This allowed use in a permeable support culture system while still allowing the development of normal polarized epithelium. This technique could conceivably be adapted to expose airway epithelium to various patterned growth factors to study the effect on differentiation with the goal of finding the correct growth factor pattern necessary for *in vivo-like* epithelial morphogenesis.

Others in the field of lung tissue engineering have looked at the effect of polymer chemistry on epithelial maturation. Lin and colleagues studied the efficacy of polyglycolic acid (PLGA) as a hydrogel matrix for lung tissue engineering [61], while Cortiella and colleagues did a comparative study of PLGA and Pluronic F-127 (PF-127) hydrogel constructs impregnated with lung cell progenitors [62]. Both found evidence that suggested that PLGA would be an excellent lung matrix substitute *in vitro*. The construct was capable

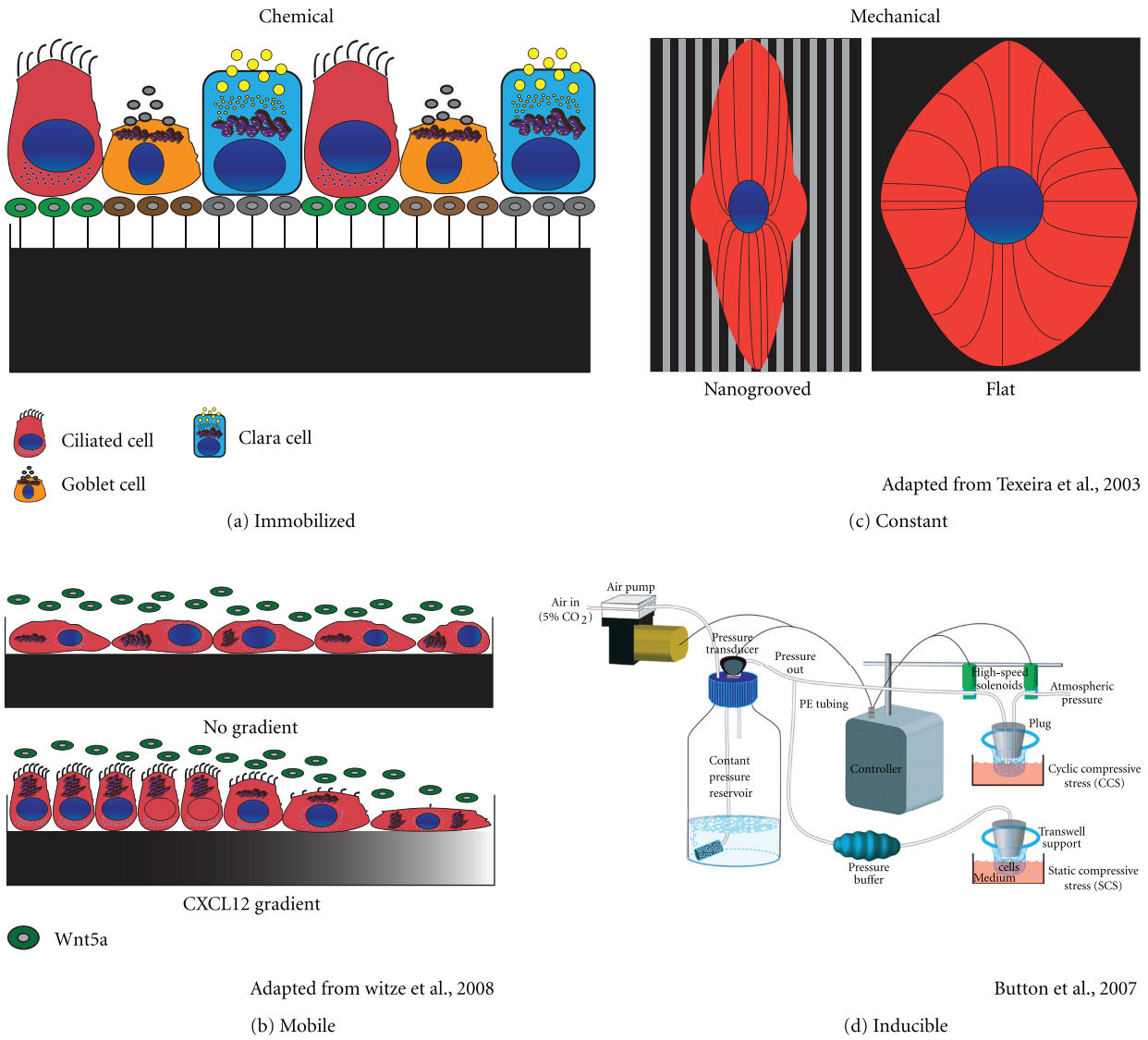

Figure 2: Specialized exemplar tools of epithelial tissue engineering. Chemical signals can be presented as immobilized growth factors (a) that promote differentiation of airway basal cells to specific cell types in a pattern that is reminiscent of *in vivo* airway epithelium, or (b) a mobile chemokine gradient of CXCL12 that promotes airway epithelium polarity in the presence of Wnt5a, based on the work of Witze et al., 2008 [68]. Mechanical signals can be presented as a constant force that organizes epithelial cells cultured on nanogrooved and flat substrates (c) based on the work of Texeira et al., 2003 [90], or as a reversible force that mimics the transluminal pressure gradient applied to airway epithelium during normal tidal breathing to modulate ciliary beat frequency [3].

of producing specific airway epithelial proteins: Clara cell protein 10 and cytokeratins; however, *in vivo*, these constructs induced potent inflammatory reactions that inhibited appropriate epithelial morphogenesis. These results lead to the conclusion that selection of the polymer based on chemistry is very important to creating functional tissue. This shows that while PLGA and PF-127 are not ideal for epithelial morphogenesis, a polymer with the correct chemical patterning would facilitate more physiologic airway epithelial differentiation and maturation.

Chemical signals in TE can also be presented to the cell in the form of diffusible, mobile, and chemical signals released from a material or scaffold (Figure 1(b)). In a classic example, Richardson and colleagues developed a polymeric system for dual growth factor delivery that leads to differential release kinetics of growth factors and altered the timing of the chemical signals [63]. The diffusible chemical signals directed endothelial cell migration to generate vascularized tissues. Single growth factor delivery systems have shown great utility in promoting differentiation and maturation of embryoid bodies [64], adipose-derived stem cells [65], and angiogenesis [65, 66]. More complex systems of sequential and combinatorial delivery of growth factors on cell-laden scaffolds have been developed for fibroblast culture [67].

These growth factor delivery systems could be relevant in epithelial TE epithelium as altering the presentation of a single or a combination of growth factors could be used to discern more elegant and physiologically relevant spatiotemporal effects on epithelial developmental processes as well as increasing cell viability and engraftment in *in vivo* models. In particular, organization of airway epithelium could be controlled by generating gradients of growth factors. Wiltze and colleagues described using a chemical gradient (CXCL12) to create polarized structures in response to Wnt5a in a melanoma cell line [68]. This technique to create polarized structures could conceivably be adapted to create organized-ciliated airway epithelium if cells were exposed to a similar gradient of CXCL12 (Figure 2(b)).

While the effect of growth factor gradients and patterns on epithelial morphogenesis has not been studied, the manipulation of mucociliary clearance by altering chemical signals present in the maturing epithelium is well documented [69–76]. For instance, it is well known that bitter compounds, such as the metabolites of resident bacteria found in cystic fibrosis patients, promote increased mucociliary beating [72]. Increased calcium and zinc ions increase the rate of mucociliary beating [75] as does serotonin in the trachea in an acetylcholine-independent pathway. The chemosensory nature of the epithelium could be exploited through a chemotactic signal embedded in a hydrogel that in a controlled fashion releases the signal that increases mucociliary clearance and promotes a healthier, more clinically relevant epithelium.

The use of chemical signals to organize and control airway epithelial maturation and differentiation is dependent on integrating these signals into a transwell format while maintaining the diffusion capabilities of the porous membrane. Immobilized signals can be added to the membrane through covalent modification, to pattern the growth and drive the differentiation of airway epithelial cells to create a more *in vivo* organization. Mobile gradients can be created in a permeable support through a growth-factor-laden hydrogel that creates a chemotactic signal throughout the permeable support and promote epithelial migration towards the signal source. This growth-factor-laden hydrogel contained within the permeable support system of a transwell would create a device, which would be of great use in wound repair studies.

In addition to chemical signals, mechanical signals can be controlled in the cell environment to guide cell behavior. Substrate stiffness is a well-studied example of a mechanical signal that is presented in a constant manner, (Figure 1(c)). Substrate stiffness can be utilized to manipulate cell morphology and proliferation. The classic example is the seminal work done by Pelham and Wang in 1997 [77] where polyacrylamide gels of different stiffness were created to study the effect stiffness has on various cell types. Their work found that fibroblasts cultured on more compliant substrates spread less and became more motile. This model was expanded upon by Discher et al. [78–81] and further refined to create a high-throughput technique to ascertain the appropriate stiffness for specific cell types [82]. Examples where substrate stiffness can be exploited to promote specific tissue characteristics are in the heart [83] and mammary epithelium [84]. Substrate stiffness modulation could be used on airway epithelium to ascertain and exploit the effect of different stiffness on organization, proliferation, and maturation to create a faster growing epithelial sheet that differentiates to a specific mature cell type.

Another aspect of the environment that influences the mechanical environment sensed by the cell is the local surface topography. For example, grooves in substrates can induce organization of cells in the direction of the grooves. Topographic organization of cells has been used to modulate the phenotype of osteoblasts [85], cardiomyocytes [86], and fibroblasts [87]. Nanogrooves specifically have been used to organize epithelial cells in the direction of the nanogrooves: MDCK [88, 89], human corneal epithelial cells [90] (Figure 2(c)), and in human mesenchymal stem cells [66]. Nanogroove topography could be used in a TE system to organize airway epithelium along nanogrooves.

The mechanical environment sensed by cells can also be modulated by the application of an inducible external force. One of the most common examples of an inducible mechanical force is shear flow to induce cell alignment (Figure 1(d)). Shear flow has been shown to align cells in the direction of flow and to alter responses to biological signals most clearly in endothelial cells [91–97]. The large body of work using shear flow to modulate endothelial cells has looked at how flow induced organization of endothelial cells in the direction of flow [91] and modified the inflammatory response [92], for example. Examples of shear flow used to modulate epithelium are scant within the literature; however, organized ependymal ciliary beating of the rat brain ventricle epithelium in shear flow conditions has been studied [98]. Applying dynamic shear forces to developing airway epithelium might be very useful to recapitulate physiologic development. *In utero* fetal breathing movements in amniotic fluid and adult inspiration and expiration of air are both examples of shear flow that could induce the maturation of airway epithelium.

In vivo, there are two main dynamic mechanical forces exerted on the airway epithelium: airflow-induced shear stress and transepithelial pressure [71]. Tarran and colleagues have developed two tools to deliver regulatable mechanical forces to the airway epithelium: an oscillatory rotational shear stress-inducing device which mimics inhalation and expiration stresses [69] and a compressive stress device that applies transepithelial pressure gradients [3] (Figure 2(d)). Mature human airway epithelium is most sensitive to mechanical stress within physiologically relevant boundaries [3, 69]. A tissue engineering device can be envisioned that combines airflow and transepithelial forces. In response to slight increases in shear stress and transepithelial pressure, mucociliary clearance increases. This property could be used to ensure that newly created airway epithelium is kept free of foreign bodies. Huh and colleagues utilized the mechanical stretching that occurs as transepithelial pressure fluctuates to create a lung-on-a-chip device that reconstitutes the interface and physiological activity between vascular endothelium and airway epithelium [99].

4. Signal Combinations and Dynamic Presentation

TE often involves combinations of mechanical and chemical signals in a coordinated fashion controlled over space and time. An example of controlling mechanical and chemical factors over space and time comes from the work of Sato and his colleagues [100, 101]. Building on their use of a synthetic scaffold with a collagen extracellular matrix lumen, they improved their airway prosthesis design by coating luminal collagen with a biodegradable polymer to delay collagen exposure for 10–20 days to allow for graft maturation and more complete epithelialization. Thus, by altering the timing of the exposure of their collagen lumen, they were successful in creating a more functional bronchial graft. However, incomplete epithelialization of the construct occurred which could lead to complications after transplantation such as graft-host anastomotic leaking, dehiscence, and stenosis. These results suggest that while TE strategies are utilized to control the timing and patterning of specific signals, better technologies are still required to achieve more clinically reliable constructs.

Controlling chemical and mechanical factors in a spatiotemporal manner often requires the use of a bioreactor to allow careful maturation of the engineered tissue. Within the bioreactor, chemical and mechanical signals are integrated together to provide a truly manipulatable growth environment that can be altered over time as the tissue matures.

One of the hottest areas in engineering airway epithelium is in the area of decellularizing whole organs and then recellularizing them. The benefits of such a TE system are that the chemical and physical cues naturally present in the decellularized ECM are available to influence the newly seeded cells instructing them to more closely recapitulate the native epithelial structure. Some examples are seen in decellularized lungs [102–104] and tracheas [105]. The trachea as a simpler organ architecturally has progressed into clinical use in a human subject [105]. While static cues are present in decellularized scaffolds, maturing the tissue may still require the presence of dynamic chemical and mechanical cues to generate the desired tissue organization. Such cues could be provided by maturing the seeded decellularized scaffolds in a bioreactor. Based on control of various static and dynamic chemical and mechanical signals, decellularized scaffolds will benefit from bioreactor technologies.

5. Summary

Based on the anatomy and regenerative potential of the pulmonary system, a variety of TE tools available could be utilized to overcome the barriers currently seen in airway tissue generation. Tools such as growth factor immobilization and graded morphogen release have shown great promise in epithelial and other model systems and could be rapidly adapted to an airway epithelial context. Other tools that manipulate substrate stiffness or topography could be used to promote organized epitheliogenesis by controlling proliferation and differentiation. Finally, bioreactors have shown great potential in creating whole organ grafts that could be used to study more physiologically relevant organ-level responses *in vitro* or in transplantation scenarios.

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