Alveolar Epithelial Disintegrity in Pulmonary Fibrosis

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Abstract

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease characterized by progressive decline in lung function, resulting in significant morbidity and mortality. Current concepts of the pathogenesis of IPF primarily center on dysregulated epithelial cell repair and altered epithelial-mesenchymal communication and extracellular matrix deposition following chronic exposure to cigarette smoke or environmental toxins. In recent years, increasing attention has been directed towards the role of the intercellular junctional complex in determining the specific properties of epithelia in pulmonary diseases. Additionally, recent genome-wide association studies (GWAS) suggest that specific genetic variants predictive of epithelial cell dysfunction may confer susceptibility to the development of sporadic idiopathic pulmonary fibrosis. A number of genetic disorders linked to pulmonary fibrosis and familial interstitial pneumonias are associated with loss of epithelial integrity. However, the potential links between extra-pulmonary clinical syndromes associated with defects in epithelial cells and the development of pulmonary fibrosis are not well understood. Here, we report a case of hereditary muco-epithelial dysplasia that presented with pulmonary fibrosis and emphysema on high-resolution computed tomography. This case illustrates a more generalizable concept of epithelial disintegrity in the development of fibrotic lung diseases, which is explored in greater detail in this review article.

New and Noteworthy:

There is increasing evidence implicating defects in host-defense and cell-cell adhesion in the development of Idiopathic Pulmonary Fibrosis (IPF). This article explores the role of developmental and acquired defects in alveolar epithelial cell-cell adhesion in the pathogenesis of pulmonary fibrosis.
**Introduction**

Interstitial lung diseases represent a heterogeneous group of diffuse lung diseases characterized by chronic, progressive dyspnea that occurs primarily in older adults. Idiopathic pulmonary fibrosis (IPF) is the most common among the idiopathic interstitial pneumonias (IIPs) and is characterized by usual interstitial pneumonia on high-resolution computed tomography (HRCT) and lung biopsy (45). Two drugs, pirfenidone and nintedanib, were approved by the United States Food and Drug Administration (FDA) for IPF based on reduction in the rate of lung function decline (24, 46); however, these drugs do not appear to arrest (or reverse) fibrosis. The mechanisms of their anti-fibrotic actions remain unclear, and treatment efficacy may be influenced by effects on the epithelium and/or mesenchymal cells.

The fibrosing varieties of IIPs are thought to be associated with alveolar epithelial cell (AEC) dysfunction, characterized by progressive loss of the normal alveolar architecture. The current paradigm is that recurrent injury to AECs followed by aberrant repair/ regeneration of epithelial barrier, persistence of activated fibroblasts and alterations in extracellular matrix (ECM) result in progressive pulmonary fibrosis (52, 71). In recent years, increasing attention has been directed toward the role of the intercellular junctional complex in determining the specific properties of epithelia in pulmonary diseases (19, 23, 26, 29, 50, 54). Identification of familial cases of interstitial pneumonias with gene mutations in surfactant protein C (40) or telomerase (3), and clinical syndromes such as Hermansky-Pudlak syndrome (11, 67) further support a critical role for AEC injury in disease pathogenesis. Additionally, recent population studies in patients with IIP demonstrate multiple susceptibility loci that indicate an increasingly important role of specific genetic variants associated with defects in host-defense and cell-to-cell adhesion (8, 17, 41).
A broad spectrum of inherited and acquired conditions, including infections or autoimmune diseases, in which essential components of intercellular junctional complexes are missing or structurally altered, can lead to epithelial barrier disintegrity. In this report, we describe a case characterized by epithelial cell defects: hereditary muco-epithelial dysplasia (HMD), presenting with features of pulmonary fibrosis. Translating this finding “bedside to bench”, we explore the role of dysfunction of components of the intercellular junctional complex in the alveolar epithelium in the development of fibrotic lung diseases, in general. Further, we discuss emerging concepts in the involvement of epithelial cell defects and polymorphisms of genes associated with lung epithelium in the pathogenesis of IPF.

Case report

A 39 year-old Caucasian male was evaluated for progressively worsening dyspnea on exertion and persistent non-productive cough for over 5 years. He has had alopecia, skin scaling and visual disturbances since childhood. He denied any other rashes, joint pains or mucosal lesions. He was a lifelong non-smoker without identifiable environmental exposures. He has a family history of HMD confirmed by pathology reports in a sibling (sister) who died at age 12 of pulmonary complications. His father also died at an early age (24 years) of an unknown lung disease. With this family history and characteristic clinical presentation during childhood, he was given the diagnosis of HMD prior to his presentation to our institution. Physical exam at presentation to our clinic was remarkable for fine crackles and scant expiratory wheezes bilaterally, digital clubbing and skin scaling of the arms. Pulmonary function tests (PFTs) revealed severe obstruction [Forced Expiratory Volume in 1 second (FEV1) = 1.57 L (37%)];
Forced Vital Capacity (FVC) = 3.36 L (59%); FEV1/FVC ratio = 47%; air trapping [Total Lung Capacity (TLC) = 6.45 L (81%); Residual Volume (RV) = 3.10 (135%)] and moderately reduced diffusion [DLCO = 13 (43%)]. During the 6-minute walk test, he was able to walk 351 meters without oxygen desaturation. High resolution computed tomography (HRCT) scans showed predominantly reticulations with honeycombing and traction bronchiectasis in a peripheral and basilar distribution with co-existent paraseptal emphysema (Figure 1). Complete blood count and routine chemistry panels were normal. Tests for specific antibodies including antinuclear factor (ANA), anti SS-A and SS-B antibodies, antineutrophilic cytoplasmic antibodies (ANCA), anti-DNA antibodies, anti-Jo-1, anti-Scl-70 and anticyclic citrullinated peptide (CCP) antibodies were all negative. Over the course of 4 years of follow-up in our clinic, his PFTs showed a decline in FVC to 2.40L (43%), TLC 4.82 L (61%) and DLCO 9.5 (35%). He has been treated with inhaled corticosteroids and bronchodilators.

Pathogenesis of Hereditary Muco-epithelial Dysplasia

HMD is a dyshesive, dyskeratotic epithelial syndrome caused by an abnormality in desmosomes and GJs with autosomal dominant inheritance (64). It can present with phenotypic variants involving mucosae, skin, hair, lungs and eyes. Patients have severe airflow obstruction with combined interstitial fibrosis and emphysema; lung involvement commonly presents with spontaneous pneumothorax from rupture of giant bullae (4, 32, 63, 64). There are fewer reports of an association with pulmonary fibrosis, perhaps related to availability of HRCT until more recently. A 1979 report indicated the presence of fibrosis with thickened septa and small cysts throughout the lung parenchyma on post-mortem examination of lung tissue section in a patient with HMD (63); in this report, histolopathology of oral and vaginal mucosa demonstrated
dyshesive epithelium with lack of maturation and atrophy, dyskeratosis and unusual cytoplasmic inclusions. Ultrastructuraal studies showed a paucity of desmosomes and the presence of perinuclear filamentous inclusions resembling internalized GJ and desmosome material in dyskeratotic cells (63). A more recent report of mucosal biopsies of eight patients with HMD indicated the presence of numerous cytoplasmic vacuoles and the filament bundles interspersed between these vacuoles expressing keratin; however, the expression of junctional and cytoskeletal proteins was normal (4). These findings suggest that this disease likely represents a defect in the development of cytoskeletal components and/or assembly of intercellular gap junctions. The desmosomal cadherin gene cluster in chromosome 18q12.1 including desmoglein and desmocollin were excluded as possible candidate genes in the haplotype analysis of one family (4). However, the specific genetic mutation(s) involved in HMD is yet to be identified.

Epithelial barrier composition and dynamics in pulmonary fibrosis

Persistent exposure to cigarette smoke or environmental toxins results in AEC injury with associated basement membrane damage. This may lead to dysregulated repair/regeneration and altered epithelial-mesenchymal communication with subsequent progressive fibrosis (13, 25). Cell-cell adhesion is critical for maintaining the integrity of alveolar epithelium, thus, maintaining its protective barrier function against toxic agents or pathogens, and allowing intercellular transfer of molecules and signals. Cell adhesion molecules (CAMs), a group of specialized proteins, are found on epithelial cell surfaces and mediate the adhesive cell-cell interactions between adjacent epithelial cells. The intercellular junction complexes at cell-cell contact sites of epithelial cells are comprised of the tight junction (TJ), adherens junction (AJ), gap junction (GJ) and desmosomes (Figure 2).
Epithelial barrier dysfunction due to repetitive tissue injury leads to host responses involving a myriad of interactions among various cells and soluble factors that are orchestrated to restore normal lung structure and function. Few studies have examined the potential role of intercellular junctional complex proteins in maintenance of the epithelial barrier integrity and development of pulmonary fibrosis following loss of this barrier function. In this section, we will briefly review the role of each component of the alveolar epithelial intercellular junctional complex.

**Tight junctions:**

Tight junctions form the apical component of the junction complex and are essential for innate immunity, as well as cellular differentiation and proliferation. They comprise the membrane proteins, claudins and occludins, in addition to scaffold proteins known as zona occludens (ZO-1, ZO-2 and ZO-3) (48). Claudins, particularly claudin 18, are the major proteins contributing to the epithelial barrier function of TJs in the lungs and maintain alveolar fluid homeostasis (33) (47). Disruption of TJs can result in increased paracellular permeability, thus permitting entry of antigens, toxins and protein-rich fluid into alveolar spaces. Reduced expression of claudins, particularly, claudin-18, along with lower levels of mRNA encoding TJ proteins was reported in an experimental bleomycin-induced lung injury model (42). Differential claudin and cadherin expression in hyperplastic AECs compared to normal AECs during an aberrant repair process suggests focal changes in permeability of this barrier (19, 29). Although the lower expression of claudins could simply be due to epithelial cell death from bleomycin exposure, the structural disruption of TJs in fibrotic lesions suggests that bleomycin injury causes alveolar barrier dysfunction by other possible mechanisms. Transforming growth factor-β1 (TGF-β1), a well-established pro-fibrotic cytokine, has been shown to cause disruption of TJs in
human alveolar epithelial cells and induce epithelial-to-mesenchymal transition (EMT) (42). Additionally, TGF-β1-induced TJ disruption was augmented in a bleomycin injury model of phosphatase and tensin homolog (pten)-null mice (38). In this study, pten-null mice demonstrated disassembly of TJs of AECs and exacerbated lung fibrosis following injury. Furthermore, this study also demonstrated decreased PTEN expression in AECs of human IPF lungs. Taken together, these studies suggest that structural disruption of TJs with subsequent loss of alveolar epithelial integrity play an important role in the development of pulmonary fibrosis.

In addition to preservation of barrier function, adequate expression of claudins may be essential to restore alveolar epithelial barrier during normal injury-repair responses. Increased expression of both TJ and AJ proteins were demonstrated in regenerative alveolar epithelium (29). However, expression of claudin-1, claudin-3, and claudin-4 in fibrotic lung were shown to be similar to or even lower than those measured in the healthy controls (29). Interestingly, claudin-KO mice demonstrated impaired alveologenesis and alveolar barrier dysfunction (28). It is possible that the diminished capacity of epithelial cells to produce claudin could lead to incomplete repair and differentiation of epithelial cells, thus resulting in hyperplastic type II AECs seen in pulmonary fibrosis.

Adherens junctions:

Located more basal to TJs, AJs consist of cadherin and the nectin family CAMs; they are linked to the actin cytoskeleton through the binding proteins, catenins and afadin, respectively. AJs function to stabilize cell-cell adhesion, and regulate actin cytoskeletal organization, intracellular signaling and gene transcription. Epithelial cadherin (E-cadherin) is a calcium-dependent CAM with pivotal roles in epithelial cell behavior (20), and it is often used as a
marker of epithelial cells. Expression of E-cadherin is reduced in lung sections of patients with IPF; cytoplasmic localization of this protein during EMT is associated with disruption of epithelial barrier function and increased cell migration \(\text{(20, 61)}\). Cigarette smoke impairs the proteins, ZO-1, ZO-2 and E-cadherin in human bronchial epithelial cells, thus resulting in altered permeability of the epithelial barrier and, potentially, mesenchymal differentiation of epithelial cells \(\text{(49, 69)}\). Thoracic radiation and bleomycin-induced lung injury can also decrease E-cadherin and aquaporin-5 expression in AECs, increasing plasma/water permeability into alveolar spaces \(\text{(2, 9)}\). Immunohistochemistry of lung tissue in aquaporin-5 knockout mice demonstrated fibrosis with increased deposition of type I collagen in alveolar walls \(\text{(9)}\). Although these changes in permeability and increased alveolar fluid accumulation is typically associated with acute lung injury, persistent injury to the epithelial barrier may lead to a cascade of reactions with release of soluble factors that promote myofibroblast differentiation and ECM deposition.

\(\alpha_3\beta_1\) integrin is a laminin receptor that promotes cell-cell communications through its interactions with the E-cadherin/\(\beta\)-catenin complex \(\text{(34, 59)}\). It was recently reported that \(\alpha_3\beta_1\) integrin interacts with E-cadherin and the TGF-\(\beta\) receptor to form a tri-molecular complex, which triggers phosphorylation of \(\beta\)-catenin at Y654 in AECs \(\text{(23)}\). Phosphorylated \(\beta\)-catenin subsequently interacts with phosphorylated Smad2, a TGF-\(\beta\) receptor-regulated effector protein, to induce fibrotic gene expression. This study suggests a role for interactions between \(\alpha_3\beta_1\), E-cadherin and \(\beta\)-catenin signaling in the development of lung fibrosis. Additionally, increased expression of cadherin-11 (CDH11) in IPF patients and animal models of lung fibrosis have been reported \(\text{(50)}\); treatment with CDH11-blocking antibody or genetic deletion of \(Cdhh11\) protects mice against bleomycin injury-induced lung fibrosis. These data support a pivotal role of CDH11
in pulmonary fibrosis. Given the fact that multiple cell populations including AECs, fibroblasts and macrophages express CDH11 in pulmonary fibrosis, it is likely that CDH11 regulates multiple steps in the fibrotic process.

**Gap junctions:**

Gap junctions are essential for intercellular communication and secretion of surfactant necessary for barrier function. They consist of an array of transmembrane channels composed of connexins (Cx) that connect to similar structures in the adjacent cells. Differential expression of various connexins in the lung, especially Cx43 and Cx46, has an important role in the regulation of normal lung homeostasis and remodeling in response to epithelial cell injury (1, 31). Fibroblasts from patients with IPF demonstrate significant reduction in Cx43 mRNA with alteration to gap junction intercellular communication compared to fibroblasts from normal subjects (53). Additionally, mice deficient in vascular endothelial cell-specific Cx43 and Cx40 develop spontaneous fibrosis with fibroblast accumulation and aberrant alveolar remodeling (26). Further studies are required to determine if abnormalities in alveolar epithelial cell-specific connexins and epithelial cell gap junctions predispose to lung fibrosis.

**Hemidesmosomes:**

Hemidesmosomes are specialized multiprotein transmembrane complexes that facilitate the binding of keratin IF in epithelial cells to the underlying basement membrane and ECM. This binding is essential in maintenance of integrity and mechanical stability of the lung. The hemidesmosomes are formed by integrin α6β4, laminin 5 and tetraspanin CD151 (37, 56). Tetraspanins belong to a family of proteins that form multimolecular complexes with a variety of other proteins including integrins.
Tetraspanin CD151 is predominantly expressed in the basolateral surface of epithelial cells and is crucial for the maintenance of epithelial integrity via adhesion of the basal surface of AECs to basement membrane. Deletion of CD151 in AECs results in alterations to the cell structure and degradation of the epithelial integrity due to impaired adhesion to basement membrane (54). In this study, CD151 knockout mice were shown to spontaneously develop age-related pulmonary fibrosis; AECs from these mice exhibit fibroblast-like changes through upregulation of TGF-β1 signaling and augmented phosphorylated Smad2. Furthermore, decreased CD151 expression was observed in AECs from patients with IPF (54), supporting a role for loss of CD151 and epithelial integrity in at least a subset of IPF patients. Interestingly, tetraspanin CD151-integrin α3β1 association has been shown to be functionally important for α3β1-integrin mediated cell migration and matrix remodeling (21). Further studies to understand the role of tetraspanin CD151-integrin interactions and their effects on TGF-β1 in the development of pulmonary fibrosis are warranted.

Genetic variants affecting epithelial cell integrity

Over the past decade, there has been remarkable progress in the understanding of IPF pathogenesis. In addition to acquired defects in epithelial barrier integrity, there have been recent advances in identifying polymorphisms of genes associated with lung epithelium and their association with higher risk of IPF. Population studies initially implicated the role of specific genetic variants including MUC5B, TERT, TERC, SFTPC and SFTPA2 in the development of IPF and other fibrosing IIPs (8, 17, 41). Additional genetic variants including desmoplakin (DSP) and dipeptidyl peptidase 9 (DPP9) genes associated with cell-to-cell adhesion were
recently identified in patients with fibrotic IIP (8).

Desmosomes are intercellular junctions located in the basolateral membranes of epithelial cells. There is calcium-dependent transmembrane interaction between the extracellular domains of the desmosomal cadherins between adjacent cells. The cadherin cytoplasmic tails then associate with the linker proteins, plakoglobin and plakophilins. Linkage of this desmosomal assembly to the cytoskeleton is mediated through a series of interactions between binding proteins, desmoplakin and linker proteins (7). Thus, desmosomes primarily provide mechanical support for maintenance of tissue architecture by tethering the keratin intermediate filament (IF) network to the plasma membrane (10). This is particularly important in maintaining the integrity of tissues that experience mechanical stress such as peripheral portions of the lungs, myocardium, skin, bladder and gastrointestinal mucosa.

Mutations in expression of DSP gene that encodes for desmoplakin have been associated with several skin diseases including keratoderma, alopecia and severe acantholytic epidermolysis bullosa (18, 60). More importantly, DSP mutations that affect exon 24 encoding the C-terminal domain have been associated with cardiac interstitial fibrosis and arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) (35, 66). This was shown to be due to disruption of mechanical linkage between cells and modifications to cell-cell adhesion proteins. The minor allele of variant rs2076295 in intron 5 has been associated with decreased whole lung DSP expression and higher risk of IPF (8, 36). The differential expression of DSP in association with this variant suggests that disruption of desmosomal integrity with resultant impairment of cell-cell adhesion and aberrant epithelial barrier injury-repair response may participate in the development of IPF.

DSP has been shown to inhibit Wnt/β-catenin signaling pathway through regulation of
plakoglobin, β-catenin and matrix metalloproteinase 14 in a lung cancer model (65). Aberrant activation of Wnt/β-catenin signaling pathway has been implicated in the development of pulmonary fibrosis, (6) and is, thus, one potential mechanism for a pro-fibrotic role of DSP in IPF. Interestingly, increased DSP gene expression was demonstrated in lung tissue of IPF patients without the DSP gene variant rs2076295 (36). This increase could be the consequence of epithelial cell injury-repair response to maintain epithelial barrier integrity in response to persistent epithelial injury. Desmosomes have intra- and extra-cellular components and changes to any part of this structure could lead to alterations in, yet undetermined, cell signaling pathways.

DPP9 is an enzyme ubiquitously expressed by epithelial cells and fibroblasts and is necessary for intracellular signaling, cell adhesion and migration (68). DPP9 gene silencing or enzyme inhibition has been shown to suppress the adhesion-signaling pathway through decreased phosphorylation of focal adhesion kinase and paxillin (68). Thus, alterations in this gene may result in impaired cell movement during repair and lead to aberrant healing. Taken together, these findings suggest that genetic variations in the expression of DSP and DPP9 contribute to biochemical and biomechanical modifications that alter cell-cell adhesion in the lung. The presence of these genetic variations may increase epithelial cell susceptibility to barrier disintegrity in response to persistent injury, thus resulting in pulmonary fibrosis.

Epithelial-mesenchymal crosstalk

Epithelial and mesenchymal cell interactions are critical in the process of lung development, homeostasis during adulthood and injury-repair responses (5). Loss of epithelial barrier integrity and the altered alveolar microenvironment disrupts the tightly orchestrated
temporal and spatial regulation of epithelial-mesenchymal crosstalk (52). Injured alveolar epithelial cells and macrophages release/activate pro-fibrotic mediators, in particular TGF-β1 (22, 30). TGF-β1, activated by AEC-integrin mediated process (39) or by biomechanical signals (62), induces myofibroblast differentiation and activation. Recent studies indicate that myofibroblasts in IPF acquire a senescent and apoptosis-resistant phenotype (15). These mesenchymal cells secrete additional paracrine factors, such as hydrogen peroxide (16, 55), angiotensin-II (43, 57), Fas-ligand (12, 58) and TGF-β1 (14, 51), that may potentiate or perpetuate injury/apoptosis of AECs. In turn, the accumulation of a highly crosslinked and stiff matrix may perpetuate mesenchymal activation and progressive fibrosis (27, 70) (Figure 3).

Future directions

In summary, there is increasing evidence indicating a role for developmental and acquired defects in epithelial cell-cell adhesion in the pathogenesis of fibrotic lung diseases. In this review, we highlight a unique case of a young man with a known genetic defect that results in loss of cell-cell adhesion that resulted in early and severe fibrosis of the lungs. We posit that developmental or acquired dysfunction of epithelial intercellular junctional complexes may have pivotal role in the pathogenesis of pulmonary fibrosis. Whether changes in the expression of specific intercellular junctional proteins are causally involved in the development of pulmonary fibrosis are yet to be elucidated. Persistent alveolar epithelial cell injury from chronic exposure to cigarette smoke or environmental toxins could alter the expression of proteins critical in maintenance of cell-cell adhesion and alveolar epithelial integrity. Although data exploring the molecular mechanisms associated with these alterations and development of IPF are limited,
emerging evidence suggest the failure of normal alveolar injury-repair responses culminate in perpetuating cycles of myofibroblast activation and ECM deposition. Recent progress in identifying genetic variants, specifically, the genes associated with alterations in cell-cell adhesion could transform our current understanding of the pathogenesis of IPF.

An integrated approach including genotyping, environmental risk factor assessment and proteomic analyses of epithelial cell junctional proteins are needed to develop personalized approaches to diagnosis and treatment of patients with IPF. It is not known whether defects in cell-cell adhesion in distal airway/bronchiolar epithelia contribute to disease pathogenesis; for example, the previously reported association between a MUC5B promoter polymorphism and development of IPF with improved survival (44) suggests loss of epithelial homeostasis in the distal airway. Further investigation of specific mechanisms involved in epithelial disintegration will be invaluable in providing genetic and molecular targets for the development of interventions that prevent/ameliorate disease progression in IPF.
References:


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Legends:

Figure 1:
High-resolution computed tomography sections of a patient with hereditary muco-epithelial dysplasia demonstrating subpleural reticulation with honeycombing and traction bronchiectasis in a peripheral and basilar distribution with co-existent paraseptal emphysema.

Figure 2:
Cell-cell junctions: Tight junctions, adherens junctions and desmosomes are the three main junctional complexes connecting adjacent epithelial cells. Tight junctions are the most apical protein complexes and regulate epithelial barrier paracellular permeability. Adherens junctions and desmosomes stabilize cell-to-cell adhesion and maintain lung homeostasis. They also regulate the actin-cytoskeletal organization and confer mechanical strength to the alveolar epithelial barrier. Gap junctions are essential for intercellular communication and secretion of surfactant. Hemi-desmosomes bind basal epithelial cells to the basement membrane. Hereditary muco-epithelial dysplasia (HMD) affects desmosomal structures. ZO: zona occludens; IFs: intermediate filaments.

Figure 3:
Epithelial-mesenchymal crosstalk in pulmonary fibrosis: Cellular homeostasis of the alveolar structure is dependent on bidirectional signaling between alveolar epithelial cells (AECs) and mesenchymal cells. Loss of homeostasis and ineffective re-epithelialization may occur with loss of cell-cell adhesion, impaired AEC migration, senescence and/or apoptosis. The resultant
epithelial disintegrity leads to integrin-mediated activation of transforming growth factor-β1 (TGF-β1), altered biomechanics, and release of proteases, cytokines and growth factors from the epithelium that activate the underlying mesenchyme. In turn, activated fibroblasts and myofibroblasts that acquire an apoptosis-resistant phenotype secrete a number of soluble factors that can induce apoptosis/senescence of AECs; these soluble factors include TGF-β1, hydrogen peroxide (H₂O₂), angiotensin-II (AT-II), and Fas ligand, thus perpetuating the injury-repair cycle leading to extracellular matrix (ECM) modification and accumulation.
Figure 2

Tight Junction

Adherens Junction

Gap Junction

Desmosome

Hemidesmosomes

BASEMENT MEMBRANE

Occludin

ZO-1, ZO-2, ZO-3

Claudin

E-cadherin

P120/Catenin

IFs

Desmoplakin

Desmoglein

Desmocollin

HMD
**Figure 3**

Ineffective re-epithelialization

Loss of cell-cell adhesion
Impaired AEC migration
Basement membrane damage

AEC Apoptosis/Senescence

Integrin-mediated TGF-β1 activation; Biomechanical signaling

H₂O₂

Fas ligand

TGF-β1

AT-II

Secretion of proteases, cytokines and soluble growth factors

Myofibroblast differentiation
Fibroblast senescence
Apoptosis resistance

ECM accumulation
ECM crosslinking
Protease resistance