# Lung Extracellular Matrix

The lung extracellular matrix (ECM) comprises of a network of protein–protein and protein–proteoglycan interactions that modulate the biophysical environment of cells through ECM stiffness, porosity, topography and insolubility. In response, the lung ECM is remodelled by cells, whereby its components are deposited, degraded or modified in a reciprocal relationship. Changes in the ECM dynamics is associated with chronic fibrotic lung diseases such as asthma, chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis.

## 1. Introduction

The lung extracellular matrix (ECM) plays a key role in the normal architecture of the lung, from embryonic lung development, alveolarization at birth, to mechanical stability and elastic recoil of the breathing adult lung [1][2]. The lung ECM provides both biochemical and biophysical cues, which direct cellular functions and differentiation. The lung ECM can also alter the biochemical environment of surrounding cells, by storing and sequestering growth factors and cytokines to regulate spatially and temporally their bioavailability. The lung ECM can modulate the biophysical environment of cells through ECM stiffness, porosity, topography (spatial arrangement and orientation) and insolubility. Lung ECM molecules connect to cells via integrins, syndecans and other receptors to influence cell signalling, migration and proliferation. In response, the lung ECM is remodelled by cells, whereby its components are deposited, degraded or modified in a reciprocal relationship [3][4].
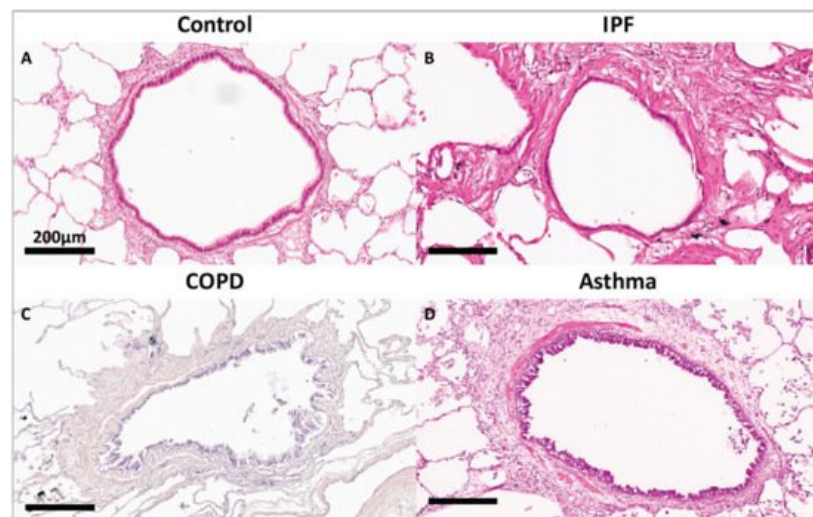
## 2. The Lung Extracellular Matrix

The "core matrisome" of the lung ECM comprises over 300 proteins [5], and each has distinct physical and biochemical properties that dictate its function. The lung matrisome is organized into two main structural subtypes: (1) basement membranes that provide the anchorage site for epithelia, endothelia, muscle, fat and peripheral nerves; and (2) the interstitial matrix that connects the structural cells within the lung. The predominant components of the basement membrane include collagen IV, collagen V, laminins (which are also the most abundant non-collagenous component), chondroitin sulphate proteoglycans (perlecan, agrin and dystroglycan), entactin, fibronectin, fibulin I and fibulin II [6][7]. The interstitial matrix comprises largely a meshwork of elastin, collagen I, collagen III, fibronectin, vitronectin, tenascin, versican and decorin [8][9]. The matrisome network of protein–protein and protein–proteoglycan interactions form the supramolecular assemblies of collagen and elastic fibers that shape the structural scaffold of the ECM within the lung. The arrangement of this meshwork allows for the non-linear stress–strain behaviour within the lung, with elastin providing viscoelasticity and collagen tensile strength.

## 3. How Cells Modify the Lung Extracellular Matrix

The lung ECM functions as a physical barrier, an anchorage site and a migration highway for cells within the organ. The ECM dynamics within each cellular niche are tightly regulated to ensure normal development, physiology and homeostasis of organ systems. Alterations in the lung ECM environment result from the synthesis, degradation or altered organization of ECM components by surrounding structural and immune cells. This is achieved by redundancy in the mechanisms used to modulate the expression and function of the ECM and ECM-modifying enzymes. When such control mechanisms are corrupted, ECM dynamics become deregulated, leading to various congenital defects or disease.

To maintain homeostasis, the lung ECM undergoes remodelling, a process whereby old or damaged ECM proteins undergo a series of proteolytic events and are replaced by newly synthesized proteins. Lung ECM remodeling is modulated by the synthesis rate of new ECM molecules and the surfeit of proteases released by specialized

mesenchymal cells, predominantly fibroblasts and, to a lesser extent, airway smooth muscle, immune cells and epithelial cells of the lungs [4][10]. The remodelling process is directed by the interaction between the different cell types, cytokines, growth factors and enzymes within the lung ECM. The levels and ratios of ECM proteins during the repair response must be maintained to avoid disrupting the lung ECM characteristics, including tensile strength and elastic recoil. Upon lung injury, activation of the coagulation process initiates damage control and a provisional matrix (fibronectin), which is then followed by the development of an acute inflammatory response that recruits polymorphonuclear leukocytes (PMNs) and macrophages to protect against pathogens and remove debris from dead and dying cells. These important early steps prepare the site for activation of local fibroblasts, migrating myofibroblasts and recruited fibrocytes that remodel collagenous and noncollagenous tissues into scar tissue. Following a single injury, inflammatory cells usually leave the site before the resident fibroblasts and migrating myofibroblasts, and fibrocytes are activated to drive the tissue repair process. Over time, the provisional ECM is degraded via cell-mediated or proteolytic pathways and replaced with a restorative ECM consisting mainly of collagen I and collagen III, before these cells then undergo apoptosis [11]. In sharp contrast, repetitive injury is associated with the persistence of inflammatory immune cell infiltration during the remodelling of the ECM, which increases the potential for the development of an abnormal repair process creating further injury with abnormal scar formation, remodelling or permanent destruction of damaged tissue. Repeated lung injury and repair can trigger a vicious cycle of aberrant ECM protein deposition, accompanied by elevated ECM stiffness, further resulting in changes in cell phenotype and function, and this has a lasting effect on tissue function and ultimately disease progression [12]. Although the processes governing the resolution of injury repair are regulated by several pathways, in chronic fibrotic lung diseases the processes are compromised, thus resulting in impaired fibroblast proliferation, apoptosis and aberrant ECM remodelling [3][11]. Disruption of this balance changes the dynamics of the lung ECM with characteristics such as stiffness and elastance, as seen in asthma, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF) [13][14][15]. Compared to the normal lung ECM (0.5–5 kPa), studies have shown that a stiff ECM (15–100 kPA) [16][17][18][19] can exacerbate TGF-β activation leading to enhanced pro-fibrotic signalling. Disruption of homeostasis within the lung ECM can also lead to the dysregulated synthesis of ECM proteins, such as fibrillar collagen I, by activated fibroblasts, leading to tissue fibrosis regardless of the inflammatory response [20]. The interplay between several diverse factors (cell types, genes, cytokines, growth factors, enzymes and epigenetics) is essential for the normal repair and remodeling processes within the lung [21]. Studies have shown that deviations in the posttranslational modification of ECM proteins, including enzymatic cross-linking, glycation and oxidation, impact the tensile strength, biomechanics and cell–ECM interactions and are contributors to lung diseases [22][23]. As shown in **Figure 1**, compared to a terminal bronchiole (last generation of conducting airways within the lung) from a donor control, there is extensive fibrosis surrounding the terminal bronchiole airway walls in the lungs from patients with IPF, COPD and asthma.



**Figure 1.** Representative image of a terminal bronchiole stained with hematoxylin and eosin from (**A**) donor control lung, (**B**) a lung from an end-stage idiopathic pulmonary fibrosis patient, (**C**) a lung from a patient with mild chronic obstructive pulmonary disease. (**D**) Terminal bronchiole stained with Masson's trichome from a lung from a patient with asthma.

### 3.1. Asthma

Asthma affects an estimated 262 million people and caused over 461,000 deaths in 2019[24]. Asthma is characterized by chronic airway inflammation, bronchial hyperresponsiveness and airway remodeling. Airway remodeling was first described in cases of fatal asthma by Huber and Koessler[25]. Since then, features of airway remodeling have been documented for all stages of asthma severity and have been linked to reduced lung function, airways hyperresponsiveness, and greater use of asthma medications[26][27][28]. The deposition of ECM is a prominent feature of

lung remodelling in patients with asthma irrespective of age, disease severity or steroid use [28][29][30]. ECM remodelling in asthma involves the airway epithelial basement membrane, laminar propria, bronchial and pulmonary vasculature [28][31] [32]. The compositions of the airway smooth muscle (ASM) ECM, epithelial basement membrane and interstitial ECM have also been shown to be altered in asthma, with a predominant deposition of fibronectin, collagen I and III [33]. Most recently, the topography of fibrillar collagen fibers has also been assessed and shown to be more disorganized and fragmented in the airway laminar propria and pulmonary vasculature [28][34]. However, recent emerging studies using various samples including serum, ASM, bronchial fibroblasts and airway epithelial cells have shown miRNAs that are differentially expressed in asthma pathology and contribute to ECM remodelling.

### 3.2. Chronic Obstructive Pulmonary Disease

COPD is one of the most common lung diseases worldwide, and the third leading cause of death[35] due to exposure of tobacco smoke and/or environmental pollutants[36]. COPD is characterized by chronic lung inflammation and irreversible airflow limitation. COPD is caused by the chronic inhalation of cigarette smoke or other harmful particles, which cause pulmonary injury, leading to chronic airway inflammation and tissue remodelling. The major histopathological changes observed within the lung include chronic bronchitis, small airway disease and emphysema. Recent studies have shown that small airway disease precedes emphysematous tissue destruction, suggesting a temporal pattern of ECM deposition and destruction in the disease progression [37]. Using a large cross-sectional study, Hogg and colleagues showed that the progression of COPD from GOLD (Global Initiative for Obstructive Lung Disease) stage 0 to GOLD stage 4 is strongly associated with thickening of the airway wall and each of its compartments by remodelling of the ECM, which affects airway wall elasticity, thickness and resistance [38][39]. In addition, loss of elastic recoil from the destruction of the ECM within the parenchyma (emphysematous tissue destruction) is a well-described feature of COPD [40], with several studies showing decreased protein expression and volume fraction of elastin in both the conducting airways and the parenchyma of patients with mild, moderate or severe COPD [41][42].

### 3.3. Idiopathic Pulmonary Fibrosis

IPF is the most common and progressive type of idiopathic interstitial pneumonia that is unresponsive to treatment, leading to a median survival of 3–5 years [43][44][45][46]. The pathology of IPF is characterized by heterogeneous interstitial fibrosis, honeycomb cysts and fibrotic foci associated with excessive deposition of ECM proteins resulting in aberrant matrix metalloproteinases, connective tissues, morphogens and impaired signalling of growth factors [47][48][49][50][51]. More recently the early pathology of IPF has been shown to involve the loss of small airways[52].The current hypothesis for tissue destruction in IPF involves continuous damage and senescence of the alveolar epithelium, leading to the destruction of the basement membrane and activation of myofibroblasts [53]. As the disease progresses, the composition of the ECM has been shown to vary, with an accumulation of versican in the onset of IPF, whereas collagen I and collagen III accumulation is observed in both the early and late stages of IPF [54]. The severity of fibrosis in IPF has also been shown to correlate with the number of elastic fibers, with a higher elastic fiber score being related to worse disease outcomes [55]. The diseased ECM is a critical linchpin in IPF, and it serves as a causal link for alteration in cell gene expression patterns at the translational level [3].

## 4. Conclusions

The initiation and progression of chronic lung diseases are modulated by complex environmental and epigenetic factors. In the initiation and progression of these diseases, there is an intricate interaction between cells and the ECM through various molecules and signalling pathways. Alterations in the lung ECM are driven by synthesis, degradation, and changes in topography by multiple cell types in the lung, but more importantly, dysregulation of the ECM seems to provide a positive feedback loop to drive fibrosis progression. The development of complex models and screening of human tissues is essential to understand how ECM regulation is abnormal in lung disease and how it could be targeted therapeutically.

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