University of Groningen

Three dimensional lung models - Three dimensional extracellular matrix models

Nizamoglu, Mehmet; Joglekar, Mugdha M.; de Hilster, Roderick H.J.; Ngassie, Maunick Lefin Koloko; Teitsma, Greta J.; Migulina, Nataliya; Blokland, Kaj E.C.; Burgess, Janette K.

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2022

[Link to publication in University of Groningen/UMCG research database](#)

Three dimensional lung models - Three dimensional extracellular matrix models

Mehmet Nizamoglu[1,2], Mugdha M. Joglekar[1,2], Roderick H.J. de Hilster[1,2], Maunick Lefin Koloko Ngassie[1,2], Greta J. Teitsma[1,2], Nataliya Migulina[1,2], Kaj E.C. Blokland[1,2,3,4,] and Janette K. Burgess[1,2,5,*]*

[1]University of Groningen, University Medical Center Groningen, Department of Pathology and Medical Biology, Groningen, The Netherlands [2]University of Groningen, University Medical Center Groningen, Groningen Research Institute for Asthma and COPD, Groningen, The Netherlands [3]School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, NSW, Australia [4]National Health and Medical Research Council Centre of Research Excellence in Pulmonary Fibrosis, Sydney, NSW, Australia [5]University of Groningen, University Medical Center Groningen, W.J. Kolff Institute for Biomedical Engineering and Materials Science-FB41, Groningen, The Netherlands

Introduction

The noncellular component in all tissues and organs is the extracellular matrix (ECM). The ECM fulfils an essential role, acting as a physical scaffolding, while initiating critical biochemical and biomechanical cues for all cells that reside within it [1]. The ECM mainly consists of fiber-forming and interfibrillar molecules. Collagen and elastin are categorized as fiber-forming molecules, whereas proteoglycans (PGs) and glycoproteins are considered interfibrillary molecules [2]. Healthy ECM is crucial for cells, as it modulates events such as migration and adhesion [3]. All tissues and organs have their own specific ECM, adapted to meet their individual needs to ensure that the tissue or organ can fulfill its defined roles. Within the lung the ECM consists of two main compartments: the basal membrane, which is a specialized layer under the epithelial and endothelial cells, and the interstitial matrix [4]. Fibroblasts are recognized as the cell type that is responsible for the maintenance and majority

*Equal contributions.

of ECM production [5]. During lung development the ECM not only provides the structure of the developing organ but also is important for the regulation of cell functions such as proliferation, migration, and differentiation [6]. Fibroblasts are crucial during development and homeostasis in their role as the gatekeepers that are responsible for production, deposition, and maintenance of the ECM, residing throughout the organ [7,8]. Dynamic changes within the ECM are associated with several chronic lung diseases, including chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF), illustrating the essential role of the ECM in the lung at all stages of life [9]. In addition to its role as a scaffold, the ECM has been recognized as a key regulator of a myriad of biological processes, including cell migration, growth, survival, differentiation, and metabolism [8,10].

The lung ECM consists of a multitude of macromolecules of which the major constituents include elastin, collagens, glycosaminoglycans (GAGs), PGs, and glycoproteins (Fig. 7.1). These macromolecules specifically fulfill roles that contribute to the maintenance of the ECM biomechanical characteristics and function of the lung. Elastin is one of the principal constituents of lung ECM and represents 20%−30%, 7%−16%, and 3%−5% dry weight of parenchyma, blood vessels, and airways, respectively [11]; providing the lung its extension and recoil properties [12]. One of the most abundant ECM proteins in the lung is collagen, which comprises 28 different types, which are categorized according to their structure and function as fibrils, fibril-associated collagens with interrupted triple helices, network-forming, beaded filaments, and anchoring fibrils collagens [10,13]. GAGs are polysaccharides with highly hydrophilic proprieties that contribute to the viscoelasticity of the lung [14]. Additionally, GAGs are known as regulators of immune responses, growth factor activity, and tissue modeling and remodeling [14,15]. Four subfamilies of GAGs can be distinguished in the lung; heparan sulfate, hyaluronic acid (HA), chondroitin sulfate/dermatan sulfate, and keratan sulfate [16]. HA is not synthesized as a PG; the other GAGs are synthesized as PGs, which consist of a protein backbone (referred to as the core protein) to which GAGs bind covalently [17]. Thus major proteoglycan families can be classified on the basis of their GAG composition, molecular weight, and function [14]. Extracellular PGs can be classified in two groups: aggregating and small leucine-rich repeat PGs [16]. Known for their role in matrix-cell interactions, glycoproteins, including fibronectin, laminins, vitronectin, thrombospondin, tenascin, and nidogens, play a crucial role in regulating cellular responses [18]. A more in-depth discussion of the composition and function of ECM is provided in Chapter 3.

To investigate the role of the ECM in the development of lung pathologies, different in vitro experimental models, such as traditional two-dimensional (2D) and, more recently, innovative three-dimensional (3D) cell culture systems, have been developed. The 2D culture system is a well-established and broadly used system that (as discussed in Chapter 2), while having facilitated the generation of valuable data as the main workhorse of the in vitro lab for many years, has now been recognized to be limited with respect to its ability to mirror the physiological microenvironment from which cells are derived. Therefore the importance of model systems that further mimic the natural physiological microenvironment of cells is necessary for a better understanding of cellular responses during homeostasis and disease.

In this chapter we discuss the 3D in vitro models for representing lung disease, repair, and regeneration. First, we outline the changes in the ECM during chronic lung diseases and summarize the major differences between 2D and 3D culture systems. Next, we describe the single ECM protein-based models used for advanced 3D lung modeling. Then we illustrate
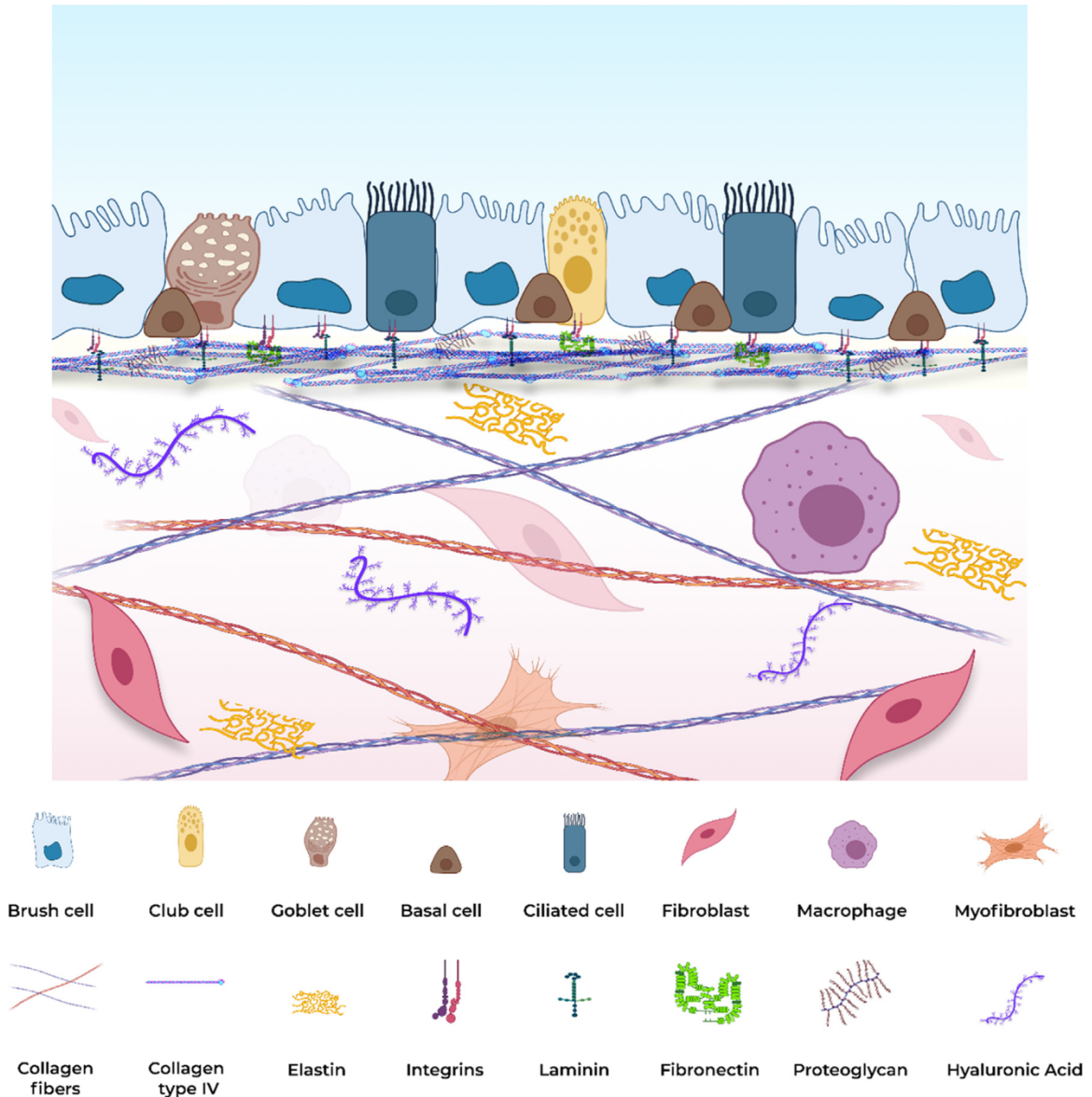
FIGURE 7.1   A schematic representation of lung extracellular matrix and tissue structure. Lung epithelium is located above a basement membrane, which consists mainly of collagen type IV fibers and laminin. Below the basement membrane the interstitial matrix is formed by various types of collagen fibers, PGs and elastin.

state-of-the-art research that has been per- formed using the complete lung ECM as a basis for the model. In conclusion we discuss the lim- itations of actual conceptualized and available 3D culture systems, the challenges that have been encountered, and the novel approaches to improve the quality and robustness of 3D cul- ture systems.

Extracellular matrix changes in chronic lung diseases

The vital functioning of the lung is crucially dependent on maintenance of the homeostatic balance between the cells and the ECM in which these cells reside. While cellular mechanisms underlying the pathology of various (chronic) lung diseases have been thoroughly investigated to date; the pivotal functional impact of the changes that are observed in the ECM during these diseases has recently been recognized. Of importance, the acknowledged heterogeneity of chronic lung diseases is thought to be reflected by changes in the ECM during the disease course. Changes in the composition, organization, and structure of the ECM consequently affect the biomechanics of the lung tissue at both the macro and micro scales, influencing the biomechanics of breathing and gas exchange but also local forces and mechanotransduction that are experienced at the cellular level.

Asthma

The main characteristics of asthma include chronic inflammation and airway hyperresponsivenes, accompanied by airway remodeling which includes goblet cell hyperplasia [19−22]. Changes in the ECM structure in asthma include thickening of the basement membrane and increased deposition of ECM in and around the airway smooth muscle (ASM) bundles. These changes have been summarized elegantly elsewhere [8,22−27], and the complete details are outside the scope of this chapter. Specifically, fibronectin and (fragments of) elastic fibers have been found to be increased in the ASM bundles in patients with clinically severe asthma compared to controls [20]. Similarly, the ASM bundles contains fewer PGs in patients with severe asthma compared to patients with moderate asthma [28]. The collagen type IV in the basement membrane has also been shown to decrease in asthma patients, despite the increase in basement membrane thickness [29]. Interestingly, tumstatin, the degradation product of collagen type IV α3, was found to be absent in the lungs of asthma patients compared to healthy controls [30]. Fibrillar collagen organization was also found to be disorganized in the airways of asthma patients compared with healthy controls [31]. Higher levels of periostin were found in both the epithelial and subepithelial layers in asthma patients in comparison to controls [32]. Next to the deposited factors, soluble ECM proteins are contributors to the changes in ECM in asthma: Higher levels of fibulin-1, a secreted glycoprotein, were found in asthma patients compared to healthy controls [33] and the presence of this glycoprotein in the ECM was important for the regulation of ASM proliferation [33] and the development of airway wall fibrosis [34]. Considered together, these studies illustrate an altered ECM composition in the asthmatic airway wall.

Chronic obstructive pulmonary disease

COPD is a chronic lung disease that is characterized by airway obstruction caused by several factors, including pulmonary inflammation accompanied by bronchitis, mucus hypersecretion, remodeling of the small airway wall, and emphysema [35]. The last two phenotypes, airway wall remodeling and emphysema, are the consequences of alteration in lung ECM homeostasis [22,23,27]. Emphysema is caused by the degradation of elastic fibers and collagens by proteolytic enzymes, including matrix metalloproteinases (MMPs) and elastase [22]. Samples from different origins, including sputum, bronchoalveolar lavage, lung parenchyma, and lung cells, showed an increase in MMP expression and their enzymatic activity in patients with COPD [36]. Merrilees et al. demonstrated that elastin fibers were decreased in the alveoli of lungs of patients with COPD [37].

Comparably, immunohistochemical analysis demonstrated a reduction in fractional area of elastic fibers in COPD [38]. Additionally, degradation products of elastin were increased in urinary excretions of patients with COPD patient compared with non-COPD controls [39]. The destruction of elastin fibers results in loss of lung elasticity, an alteration of the transpulmonary dissemination of the transpulmonary pressure that results in lung hyperinflation [23]. Additionally, immunohistochemical analyses revealed that the fractional area of collagen type I was significantly reduced in the inner layer and muscle layer of the small airways in COPD when compared with nonsmokers [38]. Interestingly, no changes were reported for other collagen types, including type III and IV, or PGs, including decorin, biglycan, and lumican [38]. This was in contrast to the findings of van Straaten et al., who reported reduced decorin and biglycan in the peribronchiolar regions from COPD patients with emphysema, compared to those of controls or patients with lung fibrosis [40]. The levels of heparan sulfate were reduced in the airway walls of patients with both COPD and lung fibrosis. However, fibronectin was found elevated in the inner and outer layer and the muscle layer of the small airways in COPD patients in contrast to smokers and nonsmokers, while tenascin was increased only in the inner layer of the small airways of patients with COPD compared to controls [38]. Collagen organization also plays an important role in ECM remodeling and the regulation of cellular function. Using second harmonic generation, Tjin and colleagues demonstrated that the organization of collagen type I in the airway wall was significantly different in COPD lung tissue compared with nondiseased controls [41]. There are excellent reviews summarizing the changes that are observed in the lung ECM of COPD subjects [15,24,26,42].

Changes in the expression and organization of ECM molecules in the lung may have an important consequence for the mechanical properties of the lung ECM in the COPD lung. The stiffness of the tissue plays a critical role. Suki and colleagues extensively discussed the importance of collagen and elastin for the mechanical properties of lung parenchyma. Since the stiffness of elastin was demonstrated to be twofold smaller than that of collagen [43], a decrease of elastin may lead to an imbalance in ECM composition and an increase in lung stiffness, which in turn can negatively affect lung function.

Idiopathic pulmonary fibrosis

IPF is a chronic fibrotic lung disease of unknown etiology, characterized by abnormal deposition of ECM in the lung parenchymal regions [44]. While repeated microinjuries to the epithelial layer are thought to be the initiator of an aberrant wound healing response, miscommunication between the epithelial and stromal cells, senescence of these cells, as well as increased numbers of profibrotic cells such as myofibroblasts or profibrotic macrophages, are all thought to contribute to the perpetuation of the fibrotic response in the lung tissue [45,46]. The changes in ECM in IPF and their associated influences on the cells are reviewed elsewhere [22,23,47−51]. Among these changes, greater deposition of collagens type I, III, and VI has been well documented in IPF patients compared to healthy controls [52]. Similarly, elastic fibers are more abundant in the lung parenchyma of IPF patients compared to non-IPF controls [53,54]. In addition to the increased deposition of ECM proteins, posttranslational modifications such as fiber cross-linking are more prominent in lung tissues of patients with IPF compared to healthy controls [55,56]. Lung biomechanical properties are altered as a result of changes in ECM structure; the lung tissue of IPF patients is significantly stiffer in comparison to healthy tissue (16.52 ± 2.25 versus 1.96 ± 0.13 kPa), with an accompanying decrease in the viscoelastic

relaxation properties (72.1% ± 13.1% versus 88.7% ± 10.4%)) [57,58]. The instructiveness of ECM has been shown to provide a positive feedback loop between fibrotic ECM and fibroblasts [59−61]. Similar to other chronic lung diseases, secreted or released ECM fragments and growth factors deposited in the ECM could be playing crucial roles in the pathophysiology of IPF. Levels of TGF-β, latent TGF binding protein, and several ECM protein degradation fragments have been found to be higher in IPF patients compared to healthy controls [62−64]. Likewise, IPF patients were found to have higher levels of fibulin-1 in both serum and lung tissue compared to healthy controls [65]. The enzymes that are responsible for regulating cross-linking of collagen and elastin fibers, lysyl oxidases, and transglutaminases are also recognized to be dysregulated in IPF [55,60,66]. In summary, the amounts and composition of ECM proteins present in the lung tissues are drastically altered in IPF, which also leads to biomechanical changes in the lung microenvironment.

Two-dimensional versus three-dimensional cell culture systems

Two-dimensional cell cultures have been used since the beginning of the 20th century. Basic 2D cell culture models include adherence of cells to Petri dishes, tissue culture flasks, or well plates made from glass or tissue culture polystyrene. The 2D culture systems described in this section do not consider suspension cell cultures. Classic 2D systems are simple to handle, are easy to reproduce, and facilitate the growth of large volumes of cells. Also, methods such as single-cell imaging and profiling of cells are easy to apply using such 2D model culture systems. Moreover, 2D cell culture procedures are generally standardized and reproducible [67,68], and these models are widely applied for the study of the mechanisms underlying lung

diseases [69]. However, several limitations in using 2D models have been identified in trying to replicate the 3D in vivo environment, including differences in cell-cell and cell-ECM interactions, as summarized in Table 7.1. Other limitations of 2D models include adhesion of cells only in a 2D plane and induction of apical-basal polarity of the cells, which can influence apoptosis-signaling pathways [67,70]. The stiffness of basic 2D culture surfaces, such as tissue culture plastic (TCP), is significantly higher than the in vivo microenvironment, resembling cartilage or bone tissue rather than soft lung tissue. Even in fibrotic diseases such as IPF, the increased Young's modulus is not as high as that of TCP, which is 1 GPA compared to 16 kPa, respectively [49,71]. Increased substrate stiffness leads to greater proliferation of cells and differentiation of fibroblasts to myofibroblasts [72,73]. The highlighted differences between 2D and 3D culture models affect cell behavior, which contributes to cellular alterations that affect phenotype, differentiation, proliferation and gene and protein expression, cell signaling, and behaviors [67,68,73]. Classical 2D models provide limited opportunities for studying cell migration and tissue remodeling, with perhaps the exception of information that can be garnered from wound-healing experiments. However, in recent years, novel 2D models that feature properties that further reflect those of lung tissue have been developed. For example, to study the effects of mechanical properties on cell behaviors, mechanically tunable polydimethylsiloxane substrates and polyacrylamide (PA) hydrogels can be used. Moreover, ECM proteins can be covalently cross-linked to the PA hydrogels [74]. The growth of cells on a basement membrane extract, such as Matrigel, provides signaling engagement, cell integrity, and structural support, which are unattainable in the basic 2D models [75]. Another semi-2D model that has been adopted is an air-liquid interface culture system, in which epithelial cells are grown on an upper surface of a porous membrane. The apical side of the

TABLE 7.1 Comparison of two- and three-dimensional culture systems.

Property	Two-dimensional	Three-dimensional
Adhesion	One plane	Three-dimensional
Polarity	Basal-apical	No forced polarity
Stiffness	High stiffness (megapascal to gigapascal range)	Variable stiffness (kilopascal range)
Soluble gradients	Absent	Present
Access to nutrients, GF, oxygen	Very accessible	More complicated with increasing thickness, similar to in vivo
ECM	One layer of matrix (coating)	Surrounded by matrix
Motion	Unconstrained spreading and migration	Spreading and migration is hindered due to surrounding matrix
Culturing Cost	Cheap	Expensive
Throughput	High	Low
Visualization	Easy	Difficult
Interactions	2D cell interaction, basolateral ECM interactions, no niches	2D and 3D cell interactions, cell-ECM/scaffold, niches
Culture protocols	Simple, methods are known, various kits are available, easy to reproduce	Complex methods that are not standardized, not easy to reproduce, more difficult to maintain the culture
Reproduction of key aspects of the tissue	Stiffness ~ 100 MPa (stiffer than fibrotic tissue), cells change behavior (gene, RNA, protein expression, adhere more strongly, proliferation is higher)	Stiffness comparable to tissue and highly adaptable, cells behavior is closer to the behavior in vivo

2D, 2-dimensional; *3D*, 3-dimensional; *ECM*, extracellular matrix; *GF*, growth factors.

membrane can be used to expose cells to air. The basal side is maintained in contact with the culture media to provide a continuing supply of nutrients. This model is commonly used for the culture of bronchial epithelial cells, which differentiate into pseudostratified mucociliated epithelial cells after exposure to air, to test the influence of external stimuli on these cells [76].

Over the past decades, 2D cell culture models have provided a wealth of knowledge and insight into some of the physiological and pathophysiological mechanisms of human biology and disease [77–79]. Nevertheless, the lung, as well as all other organs, is a 3D tissue structure in vivo. The 3D structure of every tissue is highly reliant on its native ECM, which is specialized for that tissue and the mechanical stresses that it must endure in situ. The cells residing in the lung naturally experience physical interactions with their relative ECM in either 2D or 3D. The endothelial cells and the alveolar and airway epithelium experience a 2D interaction with the basement membrane that lies beneath their basal surfaces, and the mesenchymal cells that reside in the interstitium experience 3D interactions with their surrounding ECM. When put together, the whole model is a complex 3D structure with intricate interactions between the resident cells and the ECM. The inflow of transient immune cells is an element that is not within the scope of the models that are discussed in this chapter.

In culturing cells in a 3D environment using models such as hydrogels or matrices, the cells have the opportunity to adhere in all dimensions to the matrix fibers that surround them and consequently do not experience forced polarity [77,80,81]. These engagements occur via the binding of cell surface integrins to defined motifs [e.g., arginine-glycine-aspartate (RGD) motifs on fibronectin] that are present on ECM proteins. Through this binding, cells sense the stiffness of the surrounding ECM, a process that is referred to as mechanosensing [82–86]. The mechanical stimuli from the ECM are then converted into biochemical activity activating intracellular signaling pathways resulting in gene expression responsible for regulating cell survival, proliferation, differentiation, apoptosis, ECM protein synthesis, and secretion [85]. In addition, matrix fibers sequester soluble factors (e.g., growth factors, cytokines, extracellular vesicles, chemokines) and nutrients through entrapment and binding to PGs and GAGs, potentially exposing cells to a gradient of nutrients, growth factors, and soluble factors [77,79,87,88]. The addition of a third dimension is a logical step forward to make the models that are used for studying cell interactions in lung diseases more translational, and with the incorporation of the ECM these innovative models will allow the creation of more intricate coculture systems to answer more complex questions about lung pathophysiology [89].

Three-dimensional models—1: single-protein models

Collagen

Among the most commonly used 3D models, which consist of one ECM protein, are models based on collagen type I. Collagen type I is commercially available, but it can also easily be derived from rodent, porcine, bovine, or human sources and can be relatively cheap to produce in large quantities [90]. Second, collagen can be used in different forms of biomaterials such as hydrogels or sponges [91]. Since collagen is the most abundant ECM molecule in native tissues, which modulates and supports the survival of different cell types, it makes collagen scaffolds a good model for mimicking in vivo tissue. Cells can be cultured in 3D collagen models for days and weeks. Cells that are grown in such an environment are likely to maintain behavior, migration, attachment through GFOGER, GVOGEA, GLOGEN, and other sites, and signaling pathways similar to those enacted in the lungs [92,93] (Fig. 7.2). Recently, 3D collagen scaffolds have been used for exploring alveolar recovery and angiogenesis after lung injury. After the implantation procedure a collagen scaffold lost 30% of its size by the 14th day and had almost completely degraded by the 90th day in vivo [94]. In other research, a 3D collagen model has been used to deliver epithelial cells and fibroblasts to rabbit trachea in vivo [95]. Third, the ability to modify gel stiffness or pore size (the distance between fibers) by changing the number of cross-links and/or collagen concentration makes this model interesting for studying conditions that alter the biomechanical properties of surrounding tissue [96]. The stiffness of collagen substrates can be regulated from 100 Pa up to several kilopascals, which mimics the biomechanical conditions of fibrotic, normal, or emphysematous lungs. Finally, collagen models are compatible with using standard commercially available kits for endpoint measurements such as RNA, DNA, protein isolation, and immunokits. All these advantages make collagen gels a good model for studying lung diseases, metastatic growth, wound repair, and fibrosis [55,66,90,97].

Collagen models have several limitations that should be recognized. Fibroblasts and other mesenchymal cells will contract or rearrange the hydrogel [98]. The structure of a collagen hydrogel, particularly the fiber arrangement
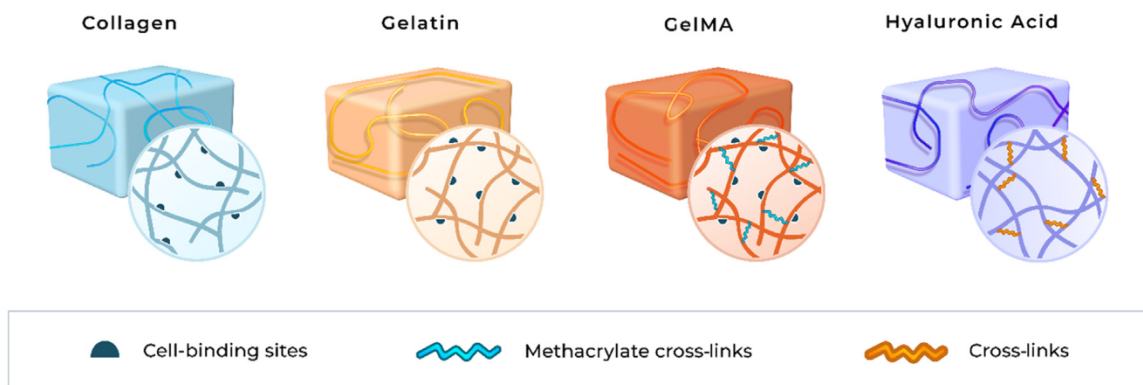
FIGURE 7.2 Schematic representation of different types of hydrogels made with single ECM proteins. Hydrogels of collagen, gelatin, and their derivatives present cell-binding sites that facilitate cell attachment. Hyaluronic acid hydrogels in their native form do not contain any cell-binding sites. GelMA and hyaluronic acid hydrogels usually have cross-links between the protein chains, reinforcing the overall structure and providing mechanical support.

within the hydrogel, can be altered by pH, ionic concentration, and temperature through effects on collagen polymerization [96]. In the end, collagen models are limited by their simplicity: Cells interact with only one type of matrix protein, there is a lack of broad tunability, and the cells that are embedded within these hydrogels can have undesirable effects on the structural assembly of the scaffold.

Gelatin and methacrylated gelatin

Gelatin is a naturally occurring and hydrophilic protein that is obtained as a result of permanent hydrolysis of collagen [99]. Depending on the method that is used for its production, there are two types of gelatin: type A and type B. Acidic hydrolysis, which yields type A gelatin, negligibly affects amide groups. Conversely, alkaline hydrolysis converts glutamine and asparagine to glutamate and aspartate residues respectively, resulting in type B gelatin [99]. Gelatin is less immunogenic compared to collagen due to fewer aromatic groups. Moreover, it retains the RGD sequence and MMP degradation sites of the parent collagen molecule that plays

an indispensable role in orchestrating cell-matrix adhesion and enabling migration and cellular remodeling respectively [99,100]. Gelatin is both biodegradable and a biocompatible material and is economical to produce and abundantly extractable from porcine skin, fish, bovine hides, and porcine, and bovine bones [101]. Given these enticing properties, gelatin is one of the most extensively used polymers in the food, pharmaceutical, cosmetic, and biomedical industries and is generally regarded as a safe material.

Gelatin is thermoreversible and forms a hydrogel as the temperature decreases below $30°C–35°C$ [102]. This occurs because gelatin sustains a conformation change from a random coil to triple helix and rearrangement of the triple helices gives rise to a huge polymer network. However, these noncovalent (hydrogen and van der Waals) interactions are broken as the temperatures rise above $30°C–35°C$. In fact, gelatin dissolves in water at $37°C$, and as a result, native gelatin hydrogels have low stability and elasticity and poor mechanical properties [102]. These limitations are often assuaged by covalently cross-linking gelatin either in its native form or following functionalization of its side chains [103]. Native gelatin can be

cross-linked chemically or enzymatically, while modified gelatin is commonly cross-linked thermally or enzymatically or by using photoinitiators [103–106] (see Fig. 7.2).

Gelatin has been used for a myriad of biomedical applications such as to produce microparticles or nanoparticles, polymeric fibers, and hydrogels for tissue-engineered scaffolds and bioadhesives [107]. Three-dimensionalized bioprinting has especially proved to be an extremely valuable technique in terms of recreating organs with complex architectures, such as lungs, as it enables layer-by-layer deposition of biomaterials and/or cells [108]. Several studies have used gelatin as a bioink to print lung scaffolds. For instance, a sodium alginate–gelatin hydrogel, encapsulating non–small cell lung carcinoma patient-derived xenograft cells and cancer associated lung fibroblasts, was 3D printed to model the tumor microenvironment in vitro [109]. The printed scaffold supported the development of 3D coculture spheroids up to 25 days. Additionally, tumor-stromal crosstalk was demonstrated by increased expression of vimentin and α-SMA (smooth muscle actin) and loss of E-cadherin in coculture spheroids [109]. This composite sodium alginate–gelatin hydrogel has also been used to support culture of lung cancer cells (A549 and 95D) for at least 2 weeks. Furthermore, 3D culture of these cancer cells enhanced their migratory properties and invasiveness compared to their 2D cultured counterparts [110].

Interestingly, gelatin has been used to produce microbubble scaffolds, using specialized microfluidic devices, to mimic alveoli structure [111,112]. A two-channel fluid jacket microfluidic device yielded 3D gelatin microbubble scaffolds that were seeded with mouse pulmonary stem/progenitor cells (mPSCs) and supported the differentiation of mPSCs into alveolar pneumocytes [111]. Additionally, a four-channel microfluidic device has been used to generate disk-shaped gelatin microbubble scaffolds with a uniform pore size of 100 μm resembling the alveoli structure [112]. A549 cells seeded in these scaffolds had higher drug resistance compared to their 2D controls; hence the 3D hydrogels are better models for anticancer drugs screening [112]. Microfluidic devices for in vitro modeling of the lung microenvironment have also gained traction. Recently, gelatin methacrylate (GelMA) was used to mimic the lung microenvironment in an airway-on-chip model [113]. In this model, the biological properties of GelMA were further enhanced by resuspending Matrigel particulates and encapsulating lung fibroblasts within the GelMA solution [113]. Furthermore, the alveolar-capillary barrier microenvironment was modeled to study the influence of the ECM structure and mechanics on epithelial cell injury during cyclic airway reopening during mechanical ventilation [114].

Another novel use of gelatin has been in the development of prosthetics for tracheal reconstruction [115,116]. Recently, it has been demonstrated that gelatin-based scaffolds are compatible with techniques such as electrospinning, micromolding, and photolithography to produce micropatterned and nanopatterned topographical features to mimic native ECM [117,118]. The mucosal folding of the respiratory track was mimicked in cell-laden GelMA hydrogels that were bonded to prestretched tough hydrogel substrates composed of interpenetrating polymer networks of PA and alginate. Relaxation of the substrate induced controlled patterns in the GelMA layer [119]. In another study, the microarchitecture of ECM fibers of healthy and diseased lung tissue was mimicked using PCL-gelatin electrospun fibers [114].

In conclusion, the ability of gelatin to support cellular activity, modifiable mechanical properties, and several functional groups for chemical modifications make it a highly desirable biomaterial for the generation of 3D models for cell culture with versatile potential for tissue engineering and regenerative medicine particularly in pulmonary diseases.

Other extracellular matrix components

Other ECM components and their derivatives have also been used for generating 3D in vitro lung models, although model types and the applications of these systems are limited. One of the most applied ECM components is HA, also called hyaluronan, which is a linear polysaccharide composed of repeating units of disaccharides (glucuronic acid and N-acetyl glucosamine) [120]. While most studies focus on drug delivery approaches using HA [121], the use of this flexible biomaterial as an in vitro model in hydrogel form has also been explored. In its native form, HA does not form viable hydrogels; however, chemical functionalization (additions of methacrylate [122], thiol [123], furan [124]) of HA as well as combining HA hydrogels with other biopolymers (gelatin [125,126], fibronectin [127], methylcellulose [124]) results in hydrogel formation (Fig. 7.2). Varying the concentration of HA and the degree of modification of HA molecules results in great variability in the mechanical properties of the resultant hydrogel (0.35 ± 0.05 kPa-1613.0 ± 248.5 kPa) [122,128]. Considering the high range, it would be possible to use such models in fibrotic lung disease research, in which hydrogels with higher stiffness can be used to mimic the fibrotic microenvironment.

Other ECM components have been used to construct 3D ECM-based in vitro models. Fibronectin, an important ECM glycoprotein, was modified with the addition of polyethylene glycol (PEG) molecules to form mechanically tunable hydrogels that could support sprouting of human umbilical vein endothelial cells in an in vitro model [129]. Similarly, fibrinogen was used in combination with collagen to formulate in vitro models with varying stiffness [130]. Fibrin hydrogels were used to create a 3D in vitro model for fibroblast—epithelial cell coculture to mimic the airway [131,132] or even a triculture model for epithelial cells, fibroblasts, and endothelial cells [133]. As in collagen, gelatin, or HA hydrogels, it is possible to modulate the mechanical properties of fibrin hydrogels within an extensive range (1.1 ± 0.3 kPa-31 ± 2.8 kPa), which increases the applicability of these hydrogels to a variety of lung diseases as well as representing specific locations within lung tissue [134].

Three-dimensional models—2: extracellular matrix models with complex extracellular matrix mixtures

Decellularized lung scaffolds

Decellularization is the process of removing cells from tissue or whole organs while minimizing the damage to and preserving the biological integrity, composition, and mechanical properties of the ECM [135]. This technique has enabled generation of acellular, native, and 3D ECM in vitro and ex vivo models that are useful for studying tissue-specific cell—ECM component interactions in healthy and diseased states, and the dynamic reciprocity between cells and their microenvironment [136—138]. Moreover, decellularization of allogeneic and xenogeneic ECM grafts followed by recellularization can ideally provide an unlimited supply for clinical applications such as tissue reconstruction and transplantation [137—139]. Although this has been realized for 2D tissues with simpler microstructures (skin, small intestinal submucosa, and pericardium) [140], organs with higher complexity, such as lungs, have proven to be more challenging. There are several methods that can be used in combination to decellularize tissues and organs, including physical, chemical, or enzymatic, as depicted in Fig. 7.3A [141]. Decellularization of lung tissue is mainly achieved by perfusion of decellularization solutions through the airways or vasculature of the lungs or by immersion of tissue segments in these solutions with or without agitation [142].
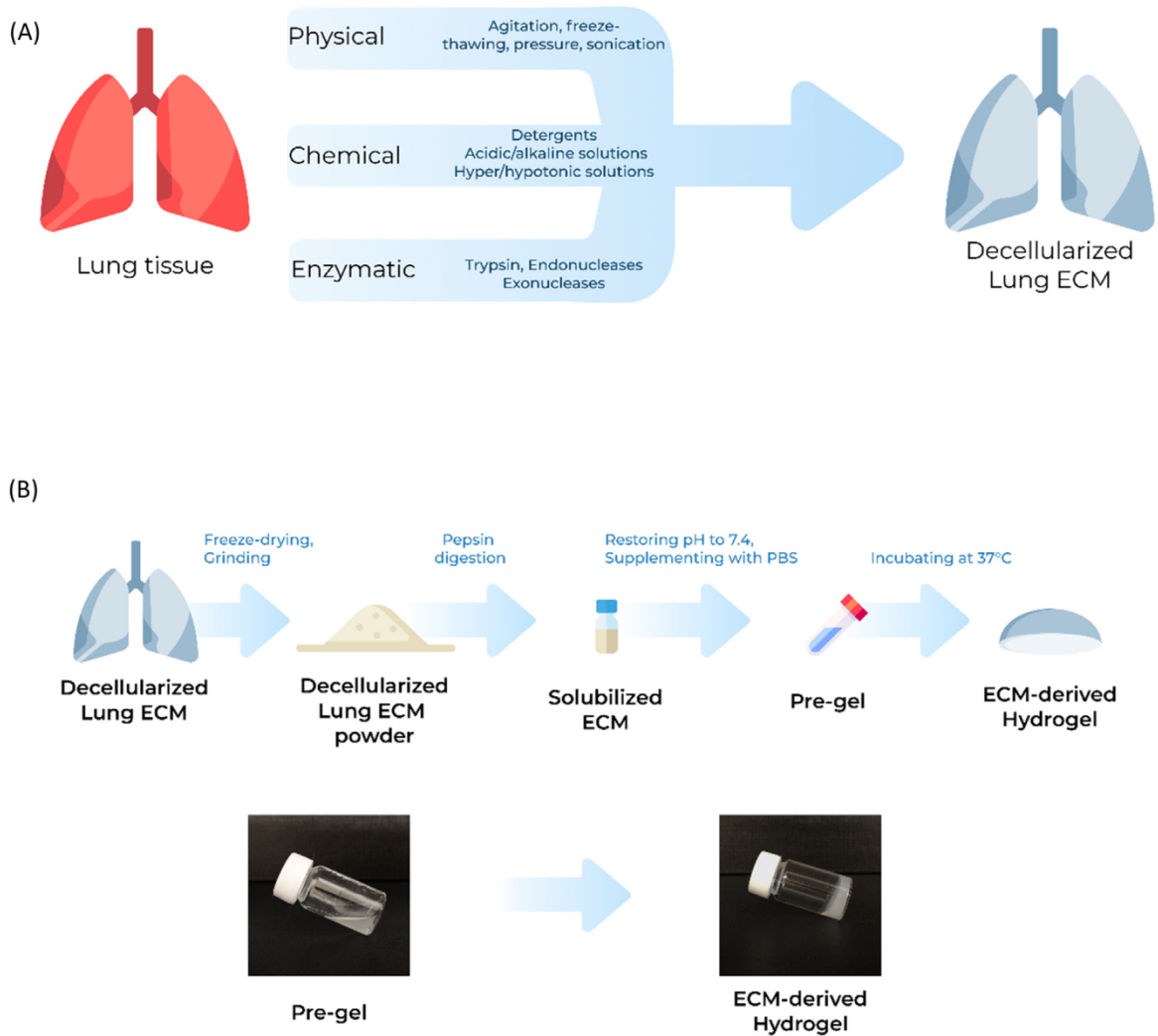
FIGURE 7.3   Generation and usage of decellularized lung ECM as a hydrogel. (A) Different methods applied to decellularize lung tissue include physical methods such as freeze-thawing or sonication, chemical methods that employ (combination of) detergents and acidic/alkaline solutions and enzymatic methods with endonucleases or exonucleases. Combinations of physical, chemical, and enzymatic decellularization methods has also been employed. (B) Schematic representation of generation of ECM-derived hydrogels from the decellularized lung ECM. Lung ECM powder, which is obtained after freeze-drying and grinding the decellularized lung scaffold, is digested by using pepsin in acidic media. The resulting solution is brought back to pH 7.4 and supplemented with phosphate buffered saline to equilibrate the salt concentration. This solution, also called pre-gel, can form hydrogels irreversibly once it is incubated at 37°C.

To mimic the lung microenvironment and stimulate functional organ regeneration, decellularized tissues have been frequently repopulated with a variety of progenitor and stromal cells [142]. Precision-cut lung slices (PCLS) have become popular ex vivo experimental models, and these can also be used in the decellularized form. A positive feedback loop

between IPF ECM and fibroblasts was demonstrated when the diseased ECM stimulated pathological gene expression enriched for ECM proteins in fibroblasts seeded on decellularized IPF PCLS [59]. Recently, ECM deposition by nondiseased lung fibroblasts seeded in acellular nondiseased lung PCLS resembled native lung tissue sections more closely compared to a monolayer of fibroblasts grown on plastic [143]. The influence of the microenvironment on cellular behavior was further demonstrated when nondiseased lung fibroblasts differentially expressed basement membrane proteins when seeded in IPF PCLS compared to nondiseased PCLS [144].

Decellularized lung models have played an indispensable role in unraveling underlying disease mechanisms that promote and enhance pathogenesis. For instance, placental microvascular pericytes sustained phenotypic transition (increased expression of α-SMA) when cultured on decellularized IPF lungs compared to decellularized nondiseased lungs, which facilitated a better understanding of the influence of pericytes in progression of IPF [145]. The crucial role of lysyl oxidase enzymes in increased tissue stiffness was uncovered when its inhibitor, β-aminopropionitrile, decreased TGF-β induced thickening of collagen fibers in nondiseased decellularized lung scaffolds seeded with nondiseased lung fibroblasts [55].

Apart from IPF, acellular scaffolds have also been derived from COPD lung tissue. COPD-derived bronchial epithelial cells had enhanced proliferative capacity and maintained basal cell phenotype when seeded on COPD-derived decellularized bronchial constructs compared to nondiseased scaffolds [146]. In contrast, no variation was observed in the differentiation or proliferative potential of emphysematous lung-derived mesenchymal stromal cells that have been grown on decellularized nondiseased and emphysematous decellularized lung tissue, although reduced growth factor production was observed in the latter [147]. These results clearly

showed that the state of the ECM largely influences the cellular response, and this response varies between different cell types. Another prominent chronic lung disease is asthma. The mechanisms that are involved in ECM remodeling in asthma have not been as thoroughly investigated using decellularized ECM models as has been done for IPF and COPD, reflecting the lack of available tissues from asthmatic donors for decellularization. Preliminary investigations using asthmatic equine models have indicated decreased levels of collagen type I and fibronectin levels in bronchi-derived acellular scaffolds [148]. However, future investigations of this model are warranted, albeit challenging.

Decellularized lung scaffolds have also been used to model the tumor microenvironment. Decellularized rat lungs that had been repopulated with human cancer cell lines and cultured in customized bioreactors produced tumor nodules and expressed MMP-9, neither of which was evident in equivalent 2D models [149]. Interestingly, murine decellularized lung matrices supported the invasion and colonization of metastatic breast cancer cells, while the majority of nonmetastatic cells were unable to survive under the same conditions [150]. These models serve as powerful tools to understand cancer metastasis and in turn will provide platforms for assessing anticancer therapies.

Several research groups have attempted whole lung decellularization for transplantation. However, these have mainly been restricted to animal models (rodents, porcine, and canine lungs) that have been recolonized for short periods with animal or human lung cells. In some studies they have been implanted in respective animal models to test the compliance and functionality of the engineered lungs [151−160]. Whole human lung or lobe decellularization and recellularization is less frequent for obvious reasons of availability and ethics [152,161]. In addition, there are several recognized limitations that must be overcome to advance this application, including the standardization of the

patient-derived lung samples and using these scaffolds as in vitro models. Moreover, more advanced methods for testing the capacity of the gas exchange in a recellularized in vitro model are required to evaluate the efficiency of the recellularization process and subsequent functionality of the engineered lung.

Decellularization presents a potent methodology for the development of in vitro models, as age- and injury-induced changes in ECM composition and characteristic anatomical alterations were retained after decellularization of lungs reflective of the original disease state [153,159,162]. Extensive information about other factors influencing cellular behavior in response to decellularized scaffolds can be found elsewhere [140]. Therefore the use of decellularized tissue for 3D modeling is an advantageous technique in terms of mimicking native tissue structure and composition. However, these models are still a long way from modeling the complexity of lung tissue. The development of long-term functional units for gas exchange is of utmost importance. However, this requires both epithelialized airways and endothelialized vessels. Vascularization and innervation of decellularized tissue, seeding, and expanding multiple cell types and lineages together and developing appropriate methods to measure experimental outcomes are some of the major challenges that have yet to be overcome.

Decellularized lung extracellular matrix—derived hydrogels

Although single-protein hydrogels can mimic the mechanical properties and elevate the cell culture model to 3D, they do not represent the full complexity of the matrisome. Next to using decellularized matrices, another approach for incorporating the complexity of the ECM into 3D models has emerged in recent years, namely, lung-derived ECM hydrogels [58,163]. These hydrogels are generated from solubilized decellularized lung ECM (Fig. 7.3B). The decellularized ECM is lyophilized and milled into a fine powder to increase the surface area of the ECM to aid in the solubilization process. The solubilization of the ECM has most often been performed via pepsin digestion in an acidic buffer [58,163,164]. During pepsin digestion the ECM proteins are enzymatically solubilized into a monomeric suspension, generally under constant agitation for an extended time (24−72 hours, although this varies for different tissues) at room temperature [164]. After digestion the pH of the solution is neutralized and buffered with phosphate buffered saline to prepare a thermosensitive ECM pre-gel solution that spontaneously self-assembles into a hydrogel when incubated at 37°C [165]. Recently, it has been shown that ultrasonic cavitation could also be used to solubilize the milled ECM, although the source of ECM was not lung tissue [166]. The whole process disrupts the original complex ultrastructure of the starting tissue ECM and reduces it to a suspension of its multitude of components. The pepsin solubilization process needs to be tailored to the specific organ, and the success is dependent upon the pepsin digestion time, which affects the mechanical properties such as stiffness and dictates the subsequent effect of the ECM hydrogel on cells [167]. Another important mechanical property for hydrogels is viscoelasticity. Viscoelasticity describes how a material that has both viscous (water-soluble factors) and elastic (ECM proteins) components distributes forces when a stress is applied [168]. Both stiffness and viscoelasticity have been found to influence cell behavior, such as spreading, proliferation, and differentiation [169,170]. For the lung, hydrogel models that incorporate the entirety of the ECM have been made from porcine lungs [163,171,172] and human lungs [58]. For now, most cell experiments using porcine lung ECM hydrogels have seeded cells on top of the hydrogel. Human lung fibroblasts, mesenchymal stromal cells, and pulmonary vascular endothelial cells were able to survive and grow on the

porcine lung ECM hydrogel [163]. The mechanical properties of human lung ECM hydrogels, both control and diseased (IPF and COPD), were compared to those of intact whole lung tissue pieces [58]. The differences in stiffness seen between control, COPD, and IPF tissues were still present, albeit to a lesser degree, in the corresponding ECM hydrogels. The stiffness of ECM hydrogels resembled that of whole tissue, while their viscoelasticity differed. Lung ECM hydrogels are still a very novel tool, and there remains a lot to optimize and discover. However, incorporating these into 3D coculture models will allow researchers to ask and answer more complex questions about the physiology and pathophysiology of the lung and the role of the ECM in disease pathogenesis.

Application of these lung ECM−derived hydrogels in disease-specific models can be further specialized by several different means. While using the pepsin-solubilized ECM as the bulk hydrogel [58,173] already improves the biomimicry of the 3D ECM-based in vitro lung models, using such in combination with other biopolymers could also provide extensive versatility. Although used in a 2D culture model, the combination of solubilized decellularized ECM from control or IPF lungs with PA hydrogels with defined stiffness values was shown to form hydrogels with disease-specific compositions [145]. Similarly, reinforcing the solubilized decellularized ECM with alginate resulted in the possibility of fine-tuning the mechanical properties of the resulting hydrogels [171]. The same study also elegantly demonstrated the application of the reinforced ECM hydrogel as a bioink for bioprinting of 3D lung models.

Chemically modifying the solubilized ECM to modulate the biochemical and biomechanical properties provides another alternative to improve the applicability of these hydrogels in 3D models. By functionalizing the solubilized ECM with thiol groups (thiolation) and combining this with methacrylated PEG (PEGMA)

molecules, Petrou et al. generated ECM-derived hydrogels with tunable mechanical properties in two separate steps [172]. While the initial stiffness values were adjusted by changing the concentration of the modified ECM in the solution, a second step of stiffening these hydrogels was achieved by using photo-crosslinking of the PEGMA molecules, resulting in great variability in the stiffness values (soft: 3.63 ± 0.24 kPa, stiff: 13.35 ± 0.83 kPa). The exciting opportunity of utilizing (disease-specific) lung ECM−derived hydrogels brings various levels of innovation to the development of novel 3D in vitro models for lung diseases. While alternatives to pepsin digestion have yet to be discovered to prepare solubilized ECM, ECM-derived hydrogels enhance the biomimicry of in vitro models. In addition, the possibility of fine-tuning mechanical properties either by reinforcing the solution with additional biopolymers or functionalization with chemical groups leads the way to decouple the contribution of biochemical and biomechanical changes in the progression of chronic lung diseases.

Another interesting method to prepare 3D in vitro models using solubilized decellularized lung ECM is electrospinning, a versatile scaffold preparation method that allows fine-tuning of the size and the alignment of the produced fibers [174]. Utilizing the possibility of modulating the stiffness, fiber alignment, and other mechanical properties as well as combinations of them in 3D in vitro cultures would enhance the capacity for mimicry of the in vivo lung ECM environment of these models.

Challenges

Excitingly, ECM-based 3D culture systems for the in vitro modeling of lung diseases have advanced significantly in the last decade. While these 3D culture systems indicate the limitations of 2D culturing, there are many
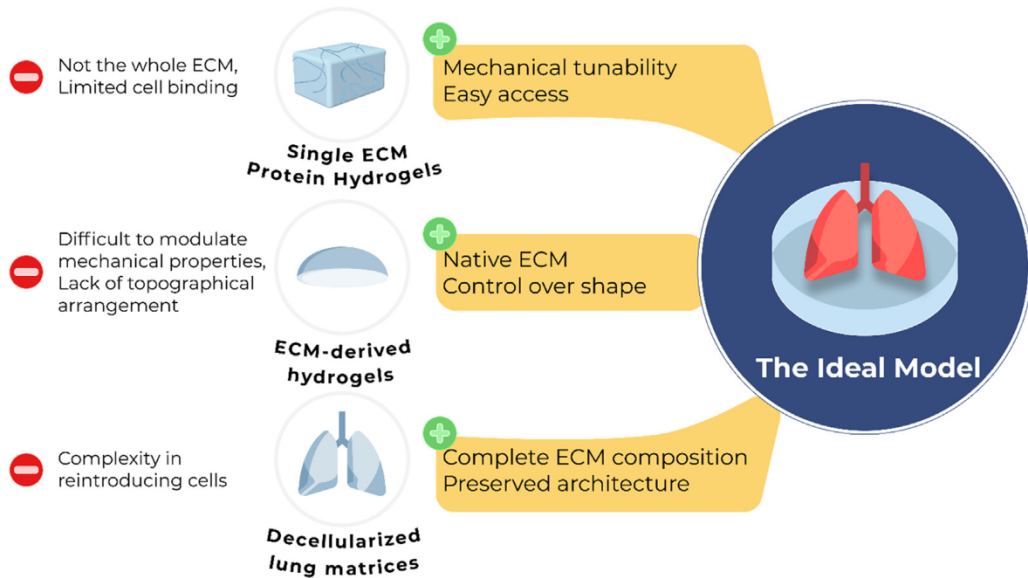
FIGURE 7.4 Summary of challenges associated with different types of ECM-based 3D lung models for advanced cell culture and the properties of the ideal model for mimicking lung disease, repair, and regeneration.

challenges that must be faced before these culture models can become mainstream tools (Fig. 7.4). Single ECM protein models such as collagen or gelatin bring a reductionist approach for research on lung diseases. The possibility of fine-tuning the mechanical properties of such hydrogels allows mimicking many different stages of lung development and disease, yet they lack the complex composition of the native lung ECM. Especially in chronic lung diseases, such as asthma, COPD, or IPF, the composition of ECM is radically altered, and such changes are not reflected in these models. The altered number and availability of cell-binding domains are another limitation of single-protein-based culture systems, mimicking the altered ECM in this aspect is not always possible with these models.

The 3D culture systems that are derived from the whole lung ECM provide advantages over their single-protein counterparts, especially in providing a more physiological composition and structural arrangement of the ECM in health and disease. Using decellularized matrices in various forms, such as tissue pieces or PCLS for disease modeling, will advance our understanding of many different mechanisms that underlie diseases; however, the current procedures for decellularization of lung tissue limit the retention of the total composition of the ECM. In particular, GAGs have been recognized to be lost during these harsh processes, and the potential impact of their loss in these culture systems has yet to be fully explored. Using the whole tissue ECM scaffold in its native form brings the opportunity of utilizing the complex architecture of lung tissue in in vitro studies; however, introducing cells back into these samples is not without challenges. Different strategies of decellularization generate differences between ECM components, structure, and mechanical properties of the obtained scaffold. The impact of these differences on recellularization remains unclear. Ensuring an appropriate 3D distribution of the cells with their correct physiological placement remains a major limitation in using decellularized

lung pieces. Additionally, factors such as cell source and seeding density, optimal medium composition, flow rate, and accessibility of the injection site also play key roles in determining the success of recellularization [175]. Long-term storage (more than 1 year) of decellularized tissue has also proven challenging because of the loss of ECM structure and reduced mechanical and angiogenic properties [176].

Lung ECM−derived hydrogels, on the other hand, can address this problem by providing the control over shape and construction but lack the topographical organization of the ECM present in vivo. Since cells can be spatially introduced to the different parts of the hydrogel(s), placing the cells in a physiologically representative manner is possible in using these hydrogels. However, the current methodology to prepare such hydrogels is rather limited; pepsin digestion is currently the most applied method [164]. While the mechanical properties of these hydrogels can resemble the lung tissue in healthy and diseased states, the implications of the digestion procedure have recently been discussed [58,167]. Another limitation in the ECM-derived hydrogels for creating 3D models for in vitro research is the mechanical tunability. Mechanical properties can be changed by varying the concentration of the initial ECM input, but this variation also changes other properties, such as pore size and cell-binding site availability. Recent studies have focused on chemically modifying the ECM-hydrogel solutions to introduce a chemical cross-linking for the possibility of tuning mechanical properties [172], while more research is required to understand the potential implications of these modifications other than changing mechanical properties.

Conclusions

In vitro models for mimicking the lung microenvironment have advanced greatly, especially in the last two decades. While conventional 2D cell and tissue culture models are being routinely used, there is an increasing trend toward research performed using advanced models. One of the most important characteristics of these advanced models is the improved dimensionality. A 3D culture environment provides improved mimicry as a result of its resemblance to the physiological conditions, as in vivo the cells reside in a 3D network of native ECM proteins. These 3D culture setups can be realized via various means, with one of the well-characterized methods using individual components isolated from ECM. Collagen, gelatin, or HA and their derivatives have been extensively studied, and thanks to the advances in the field of biomaterials, they provide great versatility in their applications for modeling lung disease and regeneration. Tunable mechanical properties, pore size, and fiber diameter in these models allow researchers to investigate the connections between the mechanical microenvironment and the cells in lung disease. Decellularized lung ECM, on the other hand, provides the physiologic architecture of the lung tissue, as well as preserving the native composition of the ECM. Using native decellularized lung ECM to understand how disease progresses and the underlying mechanisms has provided valuable information, to understand not only how cells interact with the ECM, but also how the 3D architecture of the lung tissue microenvironment plays a role in such interactions. Recently, processing these matrices to create a hydrogel derived from lung ECM itself has been demonstrated. In addition to keeping the native ECM composition, the biomechanical properties of these hydrogels resembled the biomechanical properties of tissue as well. Using diseased or control lung ECM-derived hydrogels alone or in combination with other materials to create advanced in vitro models will further improve our knowledge of lung diseases, repair, and regeneration mechanisms.

As the fields of chemistry, molecular biology, and biomaterials improve independently, more and more advanced in vitro models for 3D modeling of lung diseases, which is one of their intersection points, will be developed. Although there are challenges for each type of material used for such 3D models, as outlined in the previous section and Fig. 7.4, combining the strengths of different models for building an ideal 3D in vitro lung model based on ECM will be possible in the near future. Such an ideal model would be easily available for both low- and high-throughput research, in addition to providing the opportunity to alter the mechanical properties without compromising the composition of the model. In concert, using native ECM for such models would enhance the physiological relevance. An ideal 3D model for the lung microenvironment would benefit from the possibility of controlling both the shape and the spatial arrangement of the cells that are introduced. As lung tissue has very well-defined ECM architecture, resembling this structure in an in vitro model would help researchers to understand the influence of ECM architecture in disease, repair, and regeneration processes.

In summary, ECM-based 3D in vitro models for modeling the lung microenvironment is a rapidly advancing field, and using such models will greatly improve our knowledge of lung disease and regeneration mechanisms.

References

[1] Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. J Cell Sci 2010;123(Pt 24):4195−200.

[2] Järveläinen H, et al. Extracellular matrix molecules: potential targets in pharmacotherapy. Pharmacol Rev 2009;61(2):198−223.

[3] Daley WP, Peters SB, Larsen M. Extracellular matrix dynamics in development and regenerative medicine. J Cell Sci 2008;121(3):255−64.

[4] Matthes SA, et al. Chapter 20—comparative biology of the normal lung extracellular matrix. In: Parent RA, editor. Comparative biology of the normal lung. 2nd ed San Diego, CA: Academic Press; 2015. p. 387−402.

[5] White ES. Lung extracellular matrix and fibroblast function. Ann Am Thorac Soc 2015;12(Suppl 1):S30−3.

[6] Upagupta C, et al. Matrix abnormalities in pulmonary fibrosis. Eur Respir Rev 2018;27(148):180033.

[7] McGowan SE. Extracellular matrix and the regulation of lung development and repair. FASEB J 1992;6(11):2895−904.

[8] Zhou Y, et al. Extracellular matrix in lung development, homeostasis and disease. Matrix Biol 2018;73:77−104.

[9] Brandsma CA, et al. Recent advances in chronic obstructive pulmonary disease pathogenesis: from disease mechanisms to precision medicine. J Pathol 2020;250(5):624−35.

[10] Theocharis AD, et al. Extracellular matrix structure. Adv Drug Deliv Rev 2016;97:4−27.

[11] Mecham RP. Elastin in lung development and disease pathogenesis. Matrix Biol 2018;73:6−20.

[12] Starcher BC. Lung elastin and matrix. Chest 2000;117(5 Suppl. 1):229S−234SS.

[13] Joyce K, et al. Bioactive potential of natural biomaterials: identification, retention and assessment of biological properties. Signal Transduct Target Ther 2021;6(1):122.

[14] Souza-Fernandes AB, Pelosi P, Rocco PR. Bench-to-bedside review: the role of glycosaminoglycans in respiratory disease. Crit Care 2006;10(6):237.

[15] Karakioulaki M, Papakonstantinou E, Stolz D. Extracellular matrix remodelling in COPD. Eur Respir Rev 2020;29(158):190124.

[16] Garg HG. Proteoglycans in lung disease. CRC Press; 2002.

[17] Couchman JR, Pataki CA. An introduction to proteoglycans and their localization. J Histochem Cytochem 2012;60(12):885−97.

[18] Xu J, Mosher D. Fibronectin and other adhesive glycoproteins. In: Mecham RP, editor. The extracellular matrix: an overview. Berlin Heidelberg: Springer; 2011. p. 41−75.

[19] Saikumar Jayalatha AK, et al. The central role of IL-33/IL-1RL1 pathway in asthma: from pathogenesis to intervention. Pharmacol Ther 2021;225:107847.

[20] Araujo BB, et al. Extracellular matrix components and regulators in the airway smooth muscle in asthma. Eur Respir J 2008;32(1):61−9.

[21] James AL, et al. The relationship of reticular basement membrane thickness to airway wall remodeling in asthma. Am J Respir Crit Care Med 2002;166(12 Pt 1):1590−5.

[22] Burgess JK, et al. The extracellular matrix—the underrecognized element in lung disease? J Pathol 2016;240(4):397−409.

[23] Burgstaller G, et al. The instructive extracellular matrix of the lung: basic composition and alterations in chronic lung disease. Eur Respir J 2017;50(1).

[24] Gu BH, et al. Matrix remodeling in chronic lung diseases. Matrix Biol 2018;73:52–63.

[25] Wight TN, et al. Interplay of extracellular matrix and leukocytes in lung inflammation. Cell Immunol 2017;312:1–14.

[26] Ito JT, et al. Extracellular matrix component remodeling in respiratory diseases: what has been found in clinical and experimental studies? Cells 2019;8(4):342.

[27] Liu G, et al. Therapeutic targets in lung tissue remodelling and fibrosis. Pharmacol Ther 2021;225:107839.

[28] Pini L, et al. Differences in proteoglycan deposition in the airways of moderate and severe asthmatics. Eur Respir J 2007;29(1):71–7.

[29] Bousquet J, et al. Asthma: a disease remodeling the airways. Allergy 1992;47(1):3–11.

[30] Burgess JK, et al. Reduction of tumstatin in asthmatic airways contributes to angiogenesis, inflammation, and hyperresponsiveness. Am J Respir Crit Care Med 2010;181(2):106–15.

[31] Mostaco-Guidolin LB, et al. Defective fibrillar collagen organization by fibroblasts contributes to airway remodeling in asthma. Am J Respir Crit Care Med 2019;200(4):431–43.

[32] Burgess JK, et al. Periostin: contributor to abnormal airway epithelial function in asthma? Eur Respir J 2021;57(2):2001286.

[33] Lau JY, et al. Fibulin-1 is increased in asthma—a novel mediator of airway remodeling? PLoS One 2010;5(10):e13360.

[34] Liu G, et al. Airway remodelling and inflammation in asthma are dependent on the extracellular matrix protein fibulin-1c. J Pathol 2017;243(4):510–23.

[35] Bidan CM, et al. Airway and extracellular matrix mechanics in COPD. Front Physiol 2015;6:346.

[36] Navratilova Z, Kolek V, Petrek M. Matrix metalloproteinases and their inhibitors in chronic obstructive pulmonary disease. Arch Immunol Ther Exp (Warsz) 2016;64(3):177–93.

[37] Merrilees MJ, et al. Changes in elastin, elastin binding protein and versican in alveoli in chronic obstructive pulmonary disease. Respir Res 2008;9(1):41.

[38] Annoni R, et al. Extracellular matrix composition in COPD. Eur Respir J 2012;40(6):1362–73.

[39] Stone PJ, et al. Elastin and collagen degradation products in urine of smokers with and without chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1995;151(4):952–9.

[40] van Straaten J, et al. Proteoglycan changes in the extracellular matrix of lung tissue from patients with pulmonary emphysema. Mod Pathol 1999;12(7):697–705.

[41] Tjin G, et al. Quantification of collagen I in airway tissues using second harmonic generation. J Biomed Opt 2014;19(3):36005.

[42] Salazar LM, Herrera AM. Fibrotic response of tissue remodeling in COPD. Lung 2011;189(2):101–9.

[43] Suki B, et al. Biomechanics of the lung parenchyma: critical roles of collagen and mechanical forces. J Appl Physiol (1985) 2005;98(5):1892–9.

[44] Martinez FJ, et al. Idiopathic pulmonary fibrosis. Nat Rev Dis Prim 2017;3(1):17074.

[45] Jenkins G. Demystifying pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2020;319(3):L554–9.

[46] Selman M, Pardo A. When things go wrong: exploring possible mechanisms driving the progressive fibrosis phenotype in interstitial lung diseases. Eur Respir J 2021;2004507.

[47] Blokland KEC, et al. Regulation of cellular senescence by extracellular matrix during chronic fibrotic diseases. Clin Sci 2020;134(20):2681–706.

[48] Vasse GF, et al. Macrophage-stroma interactions in fibrosis: biochemical, biophysical, and cellular perspectives. J Pathol 2021;254(4):344–57.

[49] Haak AJ, Tan Q, Tschumperlin DJ. Matrix biomechanics and dynamics in pulmonary fibrosis. Matrix Biol 2018;73:64–76.

[50] Herrera J, Henke CA, Bitterman PB. Extracellular matrix as a driver of progressive fibrosis. J Clin Invest 2018;128(1):45–53.

[51] Levi N, et al. The ECM path of senescence in aging: components and modifiers. FEBS J 2020;287(13):2636–46.

[52] Klingberg F, Hinz B, White ES. The myofibroblast matrix: implications for tissue repair and fibrosis. J Pathol 2013;229(2):298–309.

[53] Blaauboer ME, et al. Extracellular matrix proteins: a positive feedback loop in lung fibrosis? Matrix Biol 2014;34:170–8.

[54] Enomoto N, et al. Amount of elastic fibers predicts prognosis of idiopathic pulmonary fibrosis. Respir Med 2013;107(10):1608–16.

[55] Tjin G, et al. Lysyl oxidases regulate fibrillar collagen remodelling in idiopathic pulmonary fibrosis. Dis Model Mech 2017;10(11):1301–12.

[56] Jones MG, et al. Nanoscale dysregulation of collagen structure-function disrupts mechano-homeostasis and mediates pulmonary fibrosis. Elife 2018;7.

[57] Booth AJ, et al. Acellular normal and fibrotic human lung matrices as a culture system for in vitro investigation. Am J Respir Crit Care Med 2012;186(9):866–76.

[58] de Hilster RHJ, et al. Human lung extracellular matrix hydrogels resemble the stiffness and viscoelasticity of native lung tissue. Am J Physiol Lung Cell Mol Physiol 2020;318(4):L698–704.

[59] Parker MW, et al. Fibrotic extracellular matrix activates a profibrotic positive feedback loop. J Clin Invest 2014;124(4):1622–35.

[60] Philp CJ, et al. Extracellular matrix cross-linking enhances fibroblast growth and protects against matrix proteolysis in lung fibrosis. Am J Respir Cell Mol Biol 2018;58(5):594–603.

[61] Blokland KEC, et al. Regulation of cellular senescence is independent from profibrotic fibroblast-deposited ECM. Cells 2021;10(7):1628.

[62] Jenkins RG, et al. Longitudinal change in collagen degradation biomarkers in idiopathic pulmonary fibrosis: an analysis from the prospective, multicentre PROFILE study. Lancet Respir Med 2015;3 (6):462–72.

[63] Lepparanta O, et al. Regulation of TGF-beta storage and activation in the human idiopathic pulmonary fibrosis lung. Cell Tissue Res 2012;348(3):491–503.

[64] Nizamoglu M, Burgess JK. The multi-faceted extracellular matrix: unlocking its secrets for understanding the perpetuation of lung fibrosis. Curr Tissue Microenviron Rep 2022.

[65] Jaffar J, et al. Fibulin-1 predicts disease progression in patients with idiopathic pulmonary fibrosis. Chest 2014;146(4):1055–63.

[66] Olsen KC, et al. Transglutaminase 2 and its role in pulmonary fibrosis. Am J Respir Crit Care Med 2011;184(6):699–707.

[67] Baker BM, Chen CS. Deconstructing the third dimension: how 3D culture microenvironments alter cellular cues. J Cell Sci 2012;125(Pt 13):3015–24.

[68] Sacchi M, Bansal R, Rouwkema J. Bioengineered 3D models to recapitulate tissue fibrosis. Trends Biotechnol 2020;38(6):623–36.

[69] Krimmer DI, Oliver BG. What can in vitro models of COPD tell us? Pulm Pharmacol Ther 2011;24 (5):471–7.

[70] Weaver VM, et al. Beta4 integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium. Cancer Cell 2002;2(3):205–16.

[71] Syed S, Karadaghy A, Zustiak S. Simple polyacrylamide-based multiwell stiffness assay for the study of stiffness-dependent cell responses. J Vis Exp 2015;(97).

[72] Huang X, et al. Matrix stiffness-induced myofibroblast differentiation is mediated by intrinsic mechanotransduction. Am J Respir Cell Mol Biol 2012;47(3):340–8.

[73] Janmey PA, Fletcher DA, Reinhart-King CA. Stiffness sensing by cells. Physiol Rev 2020;100(2):695–724.

[74] Tang X, Ali MY, Saif MT. A novel technique for micro-patterning proteins and cells on polyacrylamide gels. Soft Matter 2012;8(27):7197–206.

[75] Kleinman HK, Martin GR. Matrigel: basement membrane matrix with biological activity. Semin Cancer Biol 2005;15(5):378–86.

[76] Muller L, et al. Culturing of human nasal epithelial cells at the air liquid interface. J Vis Exp 2013;(80).

[77] Duval K, et al. Modeling physiological events in 2D vs. 3D cell culture. Physiol (Bethesda) 2017;32 (4):266–77.

[78] Rozario T, DeSimone DW. The extracellular matrix in development and morphogenesis: a dynamic view. Dev Biol 2010;341(1):126–40.

[79] Schmeichel KL, Bissell MJ. Modeling tissue-specific signaling and organ function in three dimensions. J Cell Sci 2003;116(Pt 12):2377–88.

[80] Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. Science 2005;310(5751):1139–43.

[81] Liu F, et al. Feedback amplification of fibrosis through matrix stiffening and COX-2 suppression. J Cell Biol 2010;190(4):693–706.

[82] Chiquet M, et al. From mechanotransduction to extracellular matrix gene expression in fibroblasts. Biochim Biophys Acta 2009;1793(5):911–20.

[83] Humphrey JD, Dufresne ER, Schwartz MA. Mechanotransduction and extracellular matrix homeostasis. Nat Rev Mol Cell Biol 2014;15(12):802–12.

[84] Huveneers S, Danen EH. Adhesion signaling—crosstalk between integrins, Src and Rho. J Cell Sci 2009;122 (Pt 8):1059–69.

[85] Ingber DE. Tensegrity-based mechanosensing from macro to micro. Prog Biophys Mol Biol 2008;97 (2–3):163–79.

[86] Tschumperlin DJ. Matrix, mesenchyme, and mechanotransduction. Ann Am Thorac Soc 2015;12(Suppl. 1): S24–9.

[87] Hynes RO. The extracellular matrix: not just pretty fibrils. Science 2009;326(5957):1216–19.

[88] Rilla K, et al. Extracellular vesicles are integral and functional components of the extracellular matrix. Matrix Biol 2019;75–76:201–19.

[89] Pampaloni F, Reynaud EG, Stelzer EH. The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Biol 2007;8 (10):839–45.

[90] Ghodbane SA, Dunn MG. Physical and mechanical properties of cross-linked type I collagen scaffolds derived from bovine, porcine, and ovine tendons. J Biomed Mater Res A 2016;104(11):2685–92.

[91] Patil PP, Reagan MR, Bohara RA. Silk fibroin and silk-based biomaterial derivatives for ideal wound dressings. Int J Biol Macromol 2020;164:4613–27.

[92] Kanta J. Collagen matrix as a tool in studying fibroblastic cell behavior. Cell Adh Migr 2015;9(4):308–16.

[93] Castillo-Briceno P, et al. A role for specific collagen motifs during wound healing and inflammatory response of fibroblasts in the teleost fish gilthead seabream. Mol Immunol 2011;48(6—7):826—34.

[94] Wang L, et al. Biomimetic collagen biomaterial induces in situ lung regeneration by forming functional alveolar. Biomaterials 2020;236:119825.

[95] Hamilton NJI, et al. Using a three-dimensional collagen matrix to deliver respiratory progenitor cells to decellularized trachea in vivo. Tissue Eng Part C Methods 2019;25(2):93—102.

[96] Doyle AD. Generation of 3D collagen gels with controlled diverse architectures. Curr Protoc Cell Biol 2016;72:10.20.1—10.20.16.

[97] Joannes A, et al. FGF9 and FGF18 in idiopathic pulmonary fibrosis promote survival and migration and inhibit myofibroblast differentiation of human lung fibroblasts in vitro. Am J Physiol Lung Cell Mol Physiol 2016;310(7):L615—29.

[98] Bourke JE, et al. Collagen remodelling by airway smooth muscle is resistant to steroids and beta(2)-agonists. Eur Respir J 2011;37(1):173—82.

[99] Su K, Wang C. Recent advances in the use of gelatin in biomedical research. Biotechnol Lett 2015;37 (11):2139—45.

[100] Xiao S, et al. Gelatin methacrylate (GelMA)-based hydrogels for cell transplantation: an effective strategy for tissue engineering. Stem Cell Rev Rep 2019;15 (5):664—79.

[101] Rodríguez-Rodríguez R, et al. Composite hydrogels based on gelatin, chitosan and polyvinyl alcohol to biomedical applications: a review. Int J Polym Mater Polym Biomater 2020;69(1):1—20.

[102] Xing Q, et al. Increasing mechanical strength of gelatin hydrogels by divalent metal ion removal. Sci Rep 2014;4(1):4706.

[103] Klotz BJ, et al. Gelatin-methacryloyl hydrogels: towards biofabrication-based tissue repair. Trends Biotechnol 2016;34(5):394—407.

[104] Melchels F et al. A dual crosslinking strategy for reinforcing gelatine-methacrylamide hydrogels for tissue repair; 2016.

[105] Bello AB, et al. Engineering and functionalization of gelatin biomaterials: from cell culture to medical applications. Tissue Eng Part B, Rev 2020;26(2):164—80.

[106] Jaipan P, Nguyen A, Narayan RJ. Gelatin-based hydrogels for biomedical applications. MRS Commun 2017;7 (3):416—26.

[107] Foox M, Zilberman M. Drug delivery from gelatin-based systems. Expert Opin Drug Deliv 2015;12(9):1547—63.

[108] Galliger Z, Vogt CD, Panoskaltsis-Mortari A. 3D bioprinting for lungs and hollow organs. Transl Res 2019;211:19—34.

[109] Mondal A, et al. Characterization and printability of Sodium alginate -Gelatin hydrogel for bioprinting NSCLC co-culture. Sci Rep 2019;9(1):19914.

[110] Wang X, et al. Tumor-like lung cancer model based on 3D bioprinting. 3 Biotech 2018;8(12):501.

[111] Ling T-Y, et al. Differentiation of lung stem/progenitor cells into alveolar pneumocytes and induction of angiogenesis within a 3D gelatin—microbubble scaffold. Biomaterials 2014;35(22):5660—9.

[112] Sun Y-J, et al. The preparation of cell-containing microbubble scaffolds to mimic alveoli structure as a 3D drug-screening system for lung cancer. Biofabrication 2020;12(2):25031.

[113] Bennet TJ et al. Airway-on-a-chip: development and in vitro validation of a microfluidic cell culture model for chronic obstructive pulmonary disease.

[114] Higuita-Castro N, et al. Using a novel microfabricated model of the alveolar-capillary barrier to investigate the effect of matrix structure on atelectrauma. Sci Rep 2017;7(1):11623.

[115] Lin C-H, et al. Surface modification of poly(ε-caprolactone) porous scaffolds using gelatin hydrogel as the tracheal replacement. J Tissue Eng Regen Med 2011;5(2):156—62.

[116] Park JH, et al. A novel tissue-engineered trachea with a mechanical behavior similar to native trachea. Biomaterials 2015;62:106—15.

[117] Aldana AA, et al. Fabrication of gelatin methacrylate (GelMA) scaffolds with nano- and micro-topographical and morphological features. Nanomaterials 2019;9(1).

[118] Erben A, et al. Precision 3D-printed cell scaffolds mimicking native tissue composition and mechanics. Adv Healthc Mater 2020;9(24):2000918.

[119] Chan HF, et al. Folding artificial mucosa with cell-laden hydrogels guided by mechanics models. Proc Natl Acad Sci 2018;115(29):7503 LP-7508.

[120] Amorim S, et al. Extracellular matrix mimics using hyaluronan-based biomaterials. Trends Biotechnol 2021;39(1):90—104.

[121] Trombino S, et al. Strategies for hyaluronic acid-based hydrogel design in drug delivery. Pharmaceutics 2019;11(8):407.

[122] Spearman BS, et al. Tunable methacrylated hyaluronic acid-based hydrogels as scaffolds for soft tissue engineering applications. J Biomed Mater Res A 2020;108(2):279—91.

[123] Bermejo-Velasco D, et al. Modulating Thiol p Ka promotes disulfide formation at physiological pH: an elegant strategy to design disulfide cross-linked hyaluronic acid hydrogels. Biomacromolecules 2019;20(3):1412—20.

[124] Tam RY, et al. Rationally designed 3D hydrogels model invasive lung diseases enabling high-content drug screening. Adv Mater 2019;31(7):e1806214.

[125] Kumar P, et al. A composite Gelatin/hyaluronic acid hydrogel as an ECM mimic for developing mesenchymal stem cell-derived epithelial tissue patches. J Tissue Eng Regen Med 2020;14(1):45−57.

[126] Bahlmann LC, et al. Gelatin-hyaluronan click-crosslinked cryogels elucidate human macrophage invasion behavior. Adv Funct Mater 2021;2008400.

[127] Seidlits SK, et al. Fibronectin-hyaluronic acid composite hydrogels for three-dimensional endothelial cell culture. Acta Biomater 2011;7(6):2401−9.

[128] Ondeck MG, Engler AJ. Mechanical characterization of a dynamic and tunable methacrylated hyaluronic acid hydrogel. J Biomech Eng 2016;138(2):021003.

[129] Trujillo S, et al. Engineered 3D hydrogels with full-length fibronectin that sequester and present growth factors. Biomaterials 2020;252:120104 November 2019.

[130] Calitz C, et al. A Biomimetic model for liver cancer to study tumor-stroma interactions in a 3D environment with tunable bio-physical properties. J Vis Exp 2020;(162).

[131] Albers S, et al. Differentiation of respiratory epithelium in a 3-dimensional co-culture with fibroblasts embedded in fibrin gel. Multidiscip Respir Med 2015;11(1):6.

[132] Kreimendahl F, et al. Three-dimensional printing and angiogenesis: tailored agarose-type I collagen blends comprise three-dimensional printability and angiogenesis potential for tissue-engineered substitutes. Tissue Eng Part C Methods 2017;23(10):604−15.

[133] Kreimendahl F, et al. Combination of vascularization and cilia formation for three-dimensional airway tissue engineering. J Biomed Mater Res A 2019;107(9):2053−62.

[134] Bachmann B, et al. Stiffness matters: fine-tuned hydrogel elasticity alters chondrogenic redifferentiation. Front Bioeng Biotechnol 2020;8:373.

[135] Garreta E, et al. Tissue engineering by decellularization and 3D bioprinting. Mater Today 2017;20(4):166−78.

[136] Badylak SF, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. Annu Rev Biomed Eng 2011;13:27−53.

[137] Gilbert TW. Strategies for tissue and organ decellularization. J Cell Biochem 2012;113(7):2217−22.

[138] Song JJ, Ott HC. Organ engineering based on decellularized matrix scaffolds. Trends Mol Med 2011;17(8):424−32.

[139] Badylak SF. The extracellular matrix as a biologic scaffold material. Biomaterials 2007;28(25):3587−93.

[140] Cramer MC, Badylak SF. Extracellular matrix-based biomaterials and their influence upon cell behavior. Ann Biomed Eng 2020;48(7):2132−53.

[141] Keane TJ, Swinehart IT, Badylak SF. Methods of tissue decellularization used for preparation of biologic scaffolds and in vivo relevance. Methods 2015;84:25−34.

[142] Gilpin SE, Wagner DE. Acellular human lung scaffolds to model lung disease and tissue regeneration. Eur Respir Rev 2018;27(148):180021.

[143] Rosmark O, et al. Quantifying extracellular matrix turnover in human lung scaffold cultures. Sci Rep 2018;8(1):5409.

[144] Elowsson Rendin L, et al. Matrisome properties of scaffolds direct fibroblasts in idiopathic pulmonary fibrosis. Int J Mol Sci 2019;20(16).

[145] Sava P, et al. Human pericytes adopt myofibroblast properties in the microenvironment of the IPF lung. JCI Insight 2017;2(24).

[146] Hedström U, et al. Impaired differentiation of COPD bronchial epithelial cells grown on bronchial scaffolds. Am J Respir Cell Mol Biol 2021.

[147] Kruk DMLW, et al. Abnormalities in reparative function of lung-derived mesenchymal stromal cells in emphysema. Am J Physiol-Lung Cell Mol Physiol 2021;320(5):L832−44.

[148] Palma R, et al. Characterization of acellular lung scaffolds derived from equine asthma model. Eur Respir J 2019;54(Suppl. 63):PA599.

[149] Mishra DK, et al. Human lung cancer cells grown in an ex vivo 3D lung model produce matrix metalloproteinases not produced in 2D culture. PLoS One 2012;7(9):e45308.

[150] Xiong G, et al. Development of an ex vivo breast cancer lung colonization model utilizing a decellularized lung matrix. Integr Biol 2015;7(12):1518−25.

[151] Gilpin SE, et al. Regenerative potential of human airway stem cells in lung epithelial engineering. Biomaterials 2016;108:111−19.

[152] Gilpin SE, et al. Perfusion decellularization of human and porcine lungs: bringing the matrix to clinical scale. J Heart Lung Transp 2014;33(3):298−308.

[153] Godin LM, et al. Decreased laminin expression by human lung epithelial cells and fibroblasts cultured in acellular lung scaffolds from aged mice. PLoS One 2016;11(3):e0150966.

[154] Kitano K, et al. Orthotopic transplantation of human bioartificial lung grafts in a porcine model: a feasibility study. Semin Thorac Cardiovasc Surg 2021.

[155] Moser PT, et al. Creation of laryngeal grafts from primary human cells and decellularized laryngeal scaffolds. Tissue Eng Part A 2020;26(9−10):543−55.

[156] Ott HC, et al. Regeneration and orthotopic transplantation of a bioartificial lung. Nat Med 2010;16(8):927−33.

[157] Petersen TH, et al. Tissue-engineered lungs for in vivo implantation. Science 2010;329(5991):538−41.

[158] Price AP, et al. Development of a decellularized lung bioreactor system for bioengineering the lung: the matrix reloaded. Tissue Eng Part A 2010;16(8):2581−91.

[159] Sokocevic D, et al. The effect of age and emphysematous and fibrotic injury on the re-cellularization of de-cellularized lungs. Biomaterials 2013;34(13):3256−69.

[160] Zhou H, et al. Bioengineering human lung grafts on porcine matrix. Ann Surg 2018;267(3):590−8.

[161] Nichols JE, et al. Production and assessment of decellularized pig and human lung scaffolds. Tissue Eng Part A 2013;19(17−18):2045−62.

[162] Wagner DE, et al. Comparative decellularization and recellularization of normal vs emphysematous human lungs. Biomaterials 2014;35(10):3281−97.

[163] Pouliot RA, et al. Development and characterization of a naturally derived lung extracellular matrix hydrogel. J Biomed Mater Res A 2016;104(8):1922−35.

[164] Saldin LT, et al. Extracellular matrix hydrogels from decellularized tissues: structure and function. Acta Biomater 2017;49:1−15.

[165] Brightman AO, et al. Time-lapse confocal reflection microscopy of collagen fibrillogenesis and extracellular matrix assembly in vitro. Biopolymers 2000;54(3):222−34.

[166] Hussey GS, et al. Ultrasonic cavitation to prepare ECM hydrogels. Acta Biomater 2020;108:77−86.

[167] Pouliot RA, et al. Porcine lung-derived extracellular matrix hydrogel properties are dependent on pepsin digestion time. Tissue Eng Part C Methods 2020;26(6):332−46.

[168] Chaudhuri O. Viscoelastic hydrogels for 3D cell culture. Biomater Sci 2017;5(8):1480−90.

[169] Chaudhuri O, et al. Substrate stress relaxation regulates cell spreading. Nat Commun 2015;6:6364.

[170] Chaudhuri O, et al. Hydrogels with tunable stress relaxation regulate stem cell fate and activity. Nat Mater 2016;15(3):326−34.

[171] De Santis MM, et al. Extracellular-matrix-reinforced bioinks for 3D bioprinting human tissue. Adv Mater 2021;33(3):e2005476.

[172] Petrou CL, et al. Clickable decellularized extracellular matrix as a new tool for building hybrid-hydrogels to model chronic fibrotic diseases in vitro. J Mater Chem B 2020;8(31):6814−26.

[173] Zhou J, et al. Lung tissue extracellular matrix-derived hydrogels protect against radiation-induced lung injury by suppressing epithelial-mesenchymal transition. J Cell Physiol 2020;235(3):2377−88.

[174] Young BM, et al. Electrospun decellularized lung matrix scaffold for airway smooth muscle culture. ACS Biomater Sci Eng 2017;3(12):3480−92.

[175] Hillebrandt KH, et al. Strategies based on organ decellularization and recellularization. Transpl Int 2019.

[176] Baiguera S, et al. Long-term changes to in vitro preserved bioengineered human trachea and their implications for decellularized tissues. Biomaterials 2012;33(14):3662.