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Imaging the pulmonary extracellular matrix

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The pulmonary extracellular matrix (ECM) plays an important role in the structure and function of the lung. In many respiratory diseases the profile of the ECM reflects pathological changes. The capacity to visualize the ECM and its alterations is of considerable importance to facilitate a better understanding of pulmonary diseases and eventually augment therapeutic solutions. This short review summarizes the current and novel possibilities for imaging the pulmonary ECM by the use of computed tomography (CT), optical coherence tomography (OCT), confocal laser endomicroscopy (CLE) and molecular imaging. While not all these techniques are as yet implemented in standard clinical practice, we address their main features along with the key possibilities for the future.

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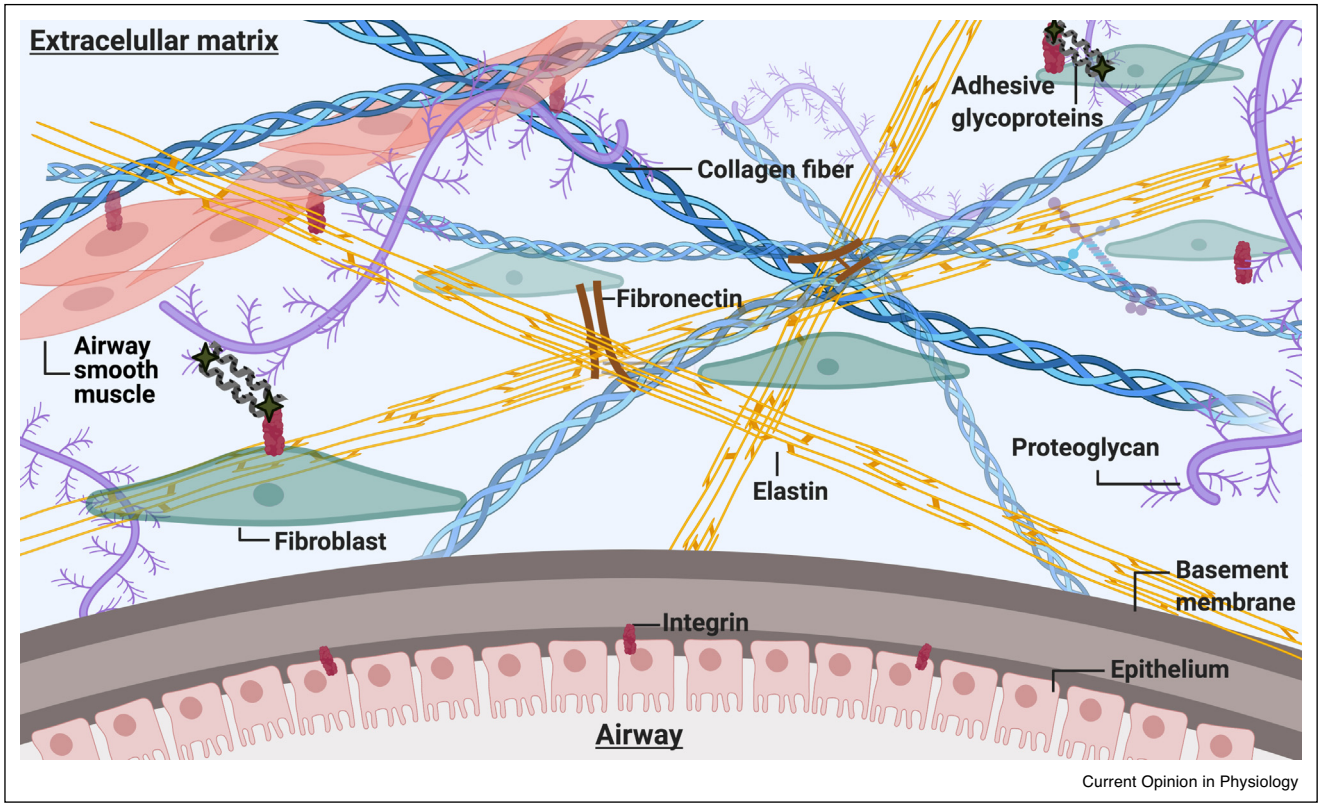
Introduction

The pulmonary extracellular matrix (ECM) provides the essential tissue architecture of the lung. It affords mechanical stability and elastic recoil, thereby facilitating physiological lung function. The ECM is composed of a diverse group of proteins and glycoproteins, adhesion proteins, glycosaminoglycans and proteoglycans (Figure 1) [1,2]. Changes in ECM occur in a diverse range of pulmonary diseases, where the composition, structural arrangement and function of the ECM can become markedly deranged [2,3]. Examples of pulmonary diseases in which structural and biomechanical changes in ECM occur are pulmonary emphysema characterized by destruction of elastic fibers and remodeling of collagen within the alveolar walls; whereas in chronic bronchitis there is increased deposition and reorganization of ECM in the thickened airway walls [4]. In asthma, next to airway smooth muscle (ASM) thickening, accumulation of ECM contributes to the narrowing of the airways; while in interstitial lung disease with fibrosis aberrant deposition of ECM in the interstitium impedes gas exchange in the alveolar regions and contributes to parenchyma stiffness [3,5]. In vascular diseases including pulmonary arterial hypertension, increased collagen deposition in the pulmonary artery wall and degradation of elastin fibers have an important role in the pathogenesis and leads to vascular rigidity [6]. The tissue structural and ECM changes that occur in pulmonary diseases have been covered in several comprehensive reviews [2,4]. The capacity for detecting and monitoring the dynamic changes in the ECM is a crucial aspect for comprehending the clinical significance of these events in lung diseases. To examine and evaluate ECM changes, histology using biopsies or resected tissue is the current gold standard. The applicability of extracted tissue is limited due to its invasiveness, and the required elaborate histological processing. Moreover, the information gained is limited to one specific sampling site. Alternative minimally invasive imaging techniques to detect the pulmonary ECM and distinguish its different components and organization are becoming more accurate, safe and feasible. Imaging of the ECM is currently explored at two levels: organ-scale (>100 μm) and cellular scale (<100 μm) depending on the approach taken [7•]. Organ scale imaging can visualize ECM changes as bulk features (areas of fibrosis or remodeling) rather than as individual ECM protein characteristics. Cellular scale imaging strives to visualize individual ECM components within a tissue but requires an imaging probe located near the area of focus. Cellular scale imaging within the pulmonary system provides unique

Figure 1



Components of the pulmonary ECM.
Schematic representation of the main components of the pulmonary ECM including a meshwork of collagen, proteoglycans, and adhesive glycoproteins. Both epithelial and mesenchymal cells (e.g. fibroblasts and ASM) interact with ECM via integrins. Not all ECM components (e.g. fibrillin, hyaluronan, and syndecan) are shown. *Created using* BioRender.com.

possibilities for the development of specialized probes for *in vivo* imaging that will facilitate the resolution of <100 μm that will be necessary for visualizing the pulmonary ECM. In this short review we present an overview of the available and emerging imaging techniques for pulmonary ECM (Table 1, Figure 2), highlight the potential and impact thereof in several exemplifier pulmonary diseases (not including lung oncology due to limited space within this review format) and provide a set of recommendations for research priorities in this field.

Computed tomography

High Resolution Computed Tomography (HRCT) scanning is an often applied and non-invasive method to image the pulmonary parenchyma and airway walls (Figure 2, image 3A). It is used in the diagnoses of many pulmonary diseases, and provides a spatial resolution of about 1 mm [8]. Improvement in spatial resolution up to 0.23–0.35 mm (230–350 μm) has been described [43], and a spatial resolution up to 0.12 mm (120 μm) is possible when Ultra High Resolution Computed Tomography (UHRCT) scanning is used, where a smaller detector element and X-ray tube focus size are used compared to standard HRCT [43,44]. UHRCT is not yet widely available [45].

Since the necessary spatial resolution to distinguish the ECM and individual ECM components is smaller than both UHRCT and HRCT provide, these techniques cannot be used to directly visualize distinct ECM components. HRCT and UHRCT can provide information about visible airway changes or bronchial wall thickening or dilation (i.e. bronchi(ol)ectasis) which is an important pathophysiological phenomenon seen in many pulmonary diseases. Increased airway wall thickness on HRCT has been reported in asthma, chronic obstructive pulmonary disease (COPD), various interstitial lung diseases [10,12] and is also seen in cigarette smokers with or without COPD [14,46]. In asthma, a thickened airway wall is associated with an increase of ECM components including reticular basement membrane (on HRCT) and ASM mass [11,47]. In COPD, changes observable on CT scanning include emphysema and an increased airway wall thickness. The latter is associated with an increased

Table 1

Overview ECM in different imaging techniques

Technique	Resolution	Pulmonary disease	ECM changes	Clinical (future) implication	Limitations
HRCT [8,9]	~1000 μm (230–350 μm possible)	Obstructive lung diseases (e.g. asthma, COPD) [10,11].	Airway wall [11,14] (e.g. bronchiectasis, bronchial wall thickening)	Degree and distribution of emphysema pivotal for decisions on lung volume reduction interventions	Ionizing radiation
		Interstitial lung diseases (e.g. IPF) [12]	Alveolar compartment [12] (e.g. Fibrosis)	Bronchiectasis detection in airways disease	High cost procedure
CT-Angiography		Vascular diseases (e.g. CETPH) [13]	Vascular compartment	Pattern of fibrosis essential for classification and treatment decisions of ILDs	Requires specialist optics expertise
Micro CT	16 μm	Model systems	Airway wall [15••] Alveolar compartment [9] (e.g. Fibrosis [16,17])	Currently only in scientific studies	Only applicable *ex vivo* or in small animals
(PS-) OCT	~10 μm with imaging depth of 2–3 mm	Obstructive lung diseases (e.g. asthma, COPD) [18,19]	Airway wall [22,23,24••,25]	Real time non-invasive histologic imaging	High cost equipment
		Interstitial lung diseases (e.g. IPF) [20]	Alveolar compartment [26] Vascular compartment [21]	Detection of airway wall components and remodeling	Limited availability of automatic software for in-vivo analysis
		Vascular diseases (e.g. CTEPH) [21]	For example ASM [27–29]	Detection of alveolar components and remodeling incl. fibrosis	Requires specialist optics expertise
			For example ECM structural changes [24••]	Detection of vascular remodeling	
CLE	3.5 μm with imaging depth of 70 μm	Obstructive lung diseases (e.g. asthma, COPD) [30•,31,32]	Airway structure [30•,34]	Real time non-invasive microscopic imaging	High cost equipment
			Alveolar network structure [34,35]	Airway and alveolar detection of ECM including elastin fibers	Limited availability of automatic software for in-vivo analysis
		Interstitial lung diseases (e.g. IPF) [33]	For example, ECM structural changes [34]	Detection of inflammatory cells	Requires specialist optics expertise
					Indicative need for intravenous or topical fluorescent contrast agents/ fluorescently labelled agents
MRI	1500–5000 μm	Interstitial lung diseases (e.g. fibrosis, IPF) [36,37]	For example, ECM structural changes [38••,39]	Potential for monitoring disease activity or progression longitudinally	Long time window for imaging is impacted by respiratory motion, limited signal-to-noise ratio in the lung
					High cost procedure
			Research-based detection of collagen, elastin, fibrin, matrix metalloproteinases		Not widely available due to need for specialized equipment
					Requires specialist optics expertise
PET	~5000 μm	Interstitial lung diseases (e.g. fibrosis, IPF) [36,40•]	Fibrotic deposits, active integrin expressing cells [41,42••]		Radiation exposure
					High cost equipment
					The need for intravenous fluorescently labelled agents
					Requires specialist optics expertise

CLE = confocal laser endomicroscopy, (HR)CT = (high resolution) computed tomography, IPF = idiopathic pulmonary fibrosis, MRI = magnetic resonance imaging, (PS)-OCT = (polarization sensitive) optical coherence tomography, CTEPH = chronic thromboembolic pulmonary hypertension, PET = position emission tomography.

collagen deposition and ASM in the (small) airway walls and a decrease of elastin fibers in airway walls and alveoli [10,48].

A relatively novel development is the imaging of the pulmonary parenchyma with micro-CT, which can provide a spatial resolution of up to 1 μm (Figure 2, image 3B). The use of this technique for humans is limited to the study of *ex vivo* material, due to scanner size limitations and the very high radiation doses required [9]. In mice, micro-CT has been used to study bleomycin-induced lung fibrosis [16] and rheumatoid arthritis associated interstitial lung disease [15••]. Bell *et al.* showed a correlation between tissue volume measured by micro-CT and histomorphic tissue area, which implies micro-CT can be used to assess ECM deposition [15••]. In human *ex vivo* idiopathic pulmonary fibrosis (IPF) affected lungs, micro-CT has been shown to be an extra tool to study parenchymal changes. Examples are fibrosis, number of terminal bronchioles and thickness of small airway walls [17,49,50].

Optical coherence tomography

Recently optical coherence tomography (OCT) has found clinical application in retinal and cardiovascular disease (e.g. coronary artery disease, atherosclerosis). In pulmonary disease OCT is investigated in clinical studies in obstructive (airway, parenchyma), interstitial and vascular (pulmonary artery) diseases [19–21] and shows promise to provide minimally invasive exploration of these compartments including ECM and ASM content [51]. This novel imaging technique is easily and safely combined with conventional bronchoscopy, through the introduction of the OCT probe in the working channel of the bronchoscope. Circumferential images are generated with the use of near-infrared light and have a resolution of ~10 μm and imaging depth of 2–3 mm. Light backscattering by structures is measured and compared with a reference beam using principles of optical interferometry. This enables capturing of real-time, depth resolved cross-sectional images by manual or automated pullback which enables 3D volumetric reconstruction of, for example, airway segments up to 5 cm length (Figure 2, image 4A.1, 5A.1, 6A and 7A).

Collagen, the major abundant fibrous protein family in the ECM [2], is detectable by OCT. A correlation between collagen deposition and OCT images has been reported in other research fields [52]. For the pulmonary ECM, Carpaij *et al.* were the first to show a correlation between OCT intensity-based area segmentation and ECM components in the airway wall [24••]. By using ex-vivo material, this study showed ECM structures detected using histology correlated with OCT data in airway wall cross-sectional images. Moreover, the potential of an automated analysis process for quantifying ECM structures, by using a light scattering-based intensity threshold was illustrated. To the best of our knowledge, the application of pulmonary automated analyses has not been reported previously. Future developments in such technologies will be essential for progressing the field of pulmonary ECM imaging.

OCT reveals anatomical segmentation of airway wall layers and enables identification and quantification of sublayers [22,23,51]. Furthermore, by use of birefringence features in polarization sensitive (PS)-OCT, tissue-specific contrast, and thereby automatic identification and quantification of the ASM is achievable and other ECM airway components become more distinguishable (Figure 2, image 4A.2 and 5A.2) [27,29]. In pulmonary vascular diseases, OCT enables measurement of the pulmonary artery wall thickness and visualization of remodeling (e.g. webs in chronic thromboembolic pulmonary hypertension) of the vascular wall [21].

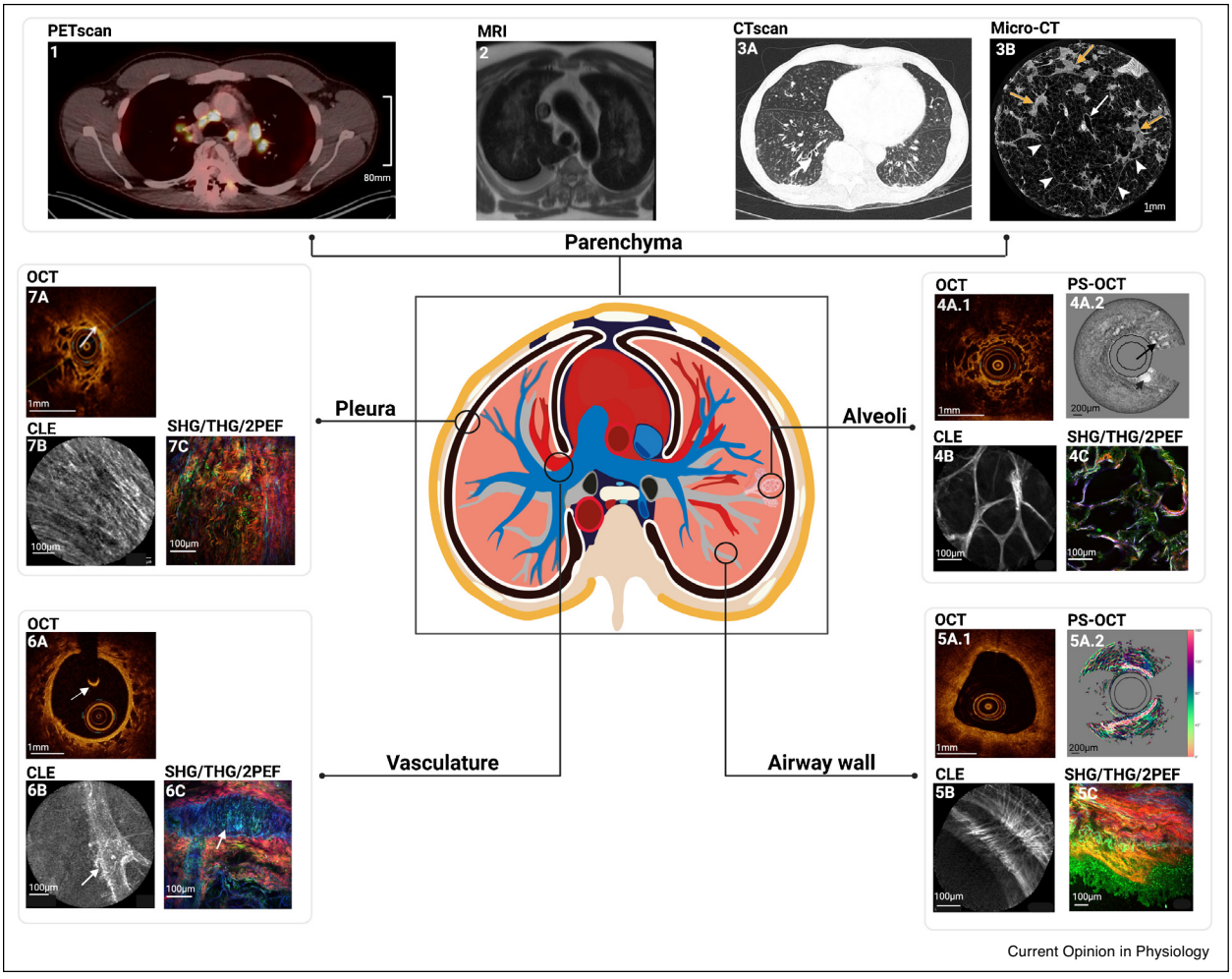
Besides the airway wall components, on which the majority of OCT studies to date have focused, research has also explored assessment of the alveolar compartment (Figure 2, image 4A.1) [26]. Thickening of alveolar septae and loss of alveolar structure were identified, and detection of pulmonary fibrosis by OCT, without tissue removal, has been reported [20].

Recently, the use of fluorescence imaging, integrated in the OCT acquisition device has shown the possibility to combine structural and molecular contrast [53]. Additionally, OCT has the potential to be combined with multiphoton microscopy, including second and third harmonic generation (SHG and THG), and two-photon excited autofluorescence imaging (2PEF), which allows for subcellular resolution imaging of ECM and cellular components without the use of exogenous contrast agents [54]. This multiphoton microscopy is a promising technique for non-invasive instant histopathology, visualizing, for example, collagen and elastin fibers, and epithelial cells (Figure 2, image 4C, 5C, 6C and 7C) [55].

Confocal laser endomicroscopy

Confocal laser endomicroscopy (CLE) is an endoscopic imaging modality developed to examine the microscopic structure of pulmonary tissue *in vivo* during a bronchoscopic procedure. It provides real-time images with a resolution up to 3.5 μm, with a maximum depth of 70 μm and a maximum field of view of 600 μm^2 [56]. The principle of this technique is based on tissue reflectance and fluorescence. A fiber-optic probe is advanced through the working channel of a bronchoscope and illuminates tissue with laser light. Reflected light is redirected back through a pinhole. Only the light which is exactly in focus passes through and results in high-resolution images (Figure 2, image 4B, 5B, 6B and 7B) [56,57].

Figure 2



Representative outputs from current imaging techniques that inform about ECM in the pulmonary space.
CT = computed tomography, CLE = confocal laser endomicroscopy, FDG = fluorodeoxyglucose, MRI = magnetic resonance imaging, (PS-)OCT = (polarization sensitive) optical coherence tomography, PET = position emission tomography, SHG = second harmonic generation, THG = third harmonic generation, 2PEF = two-photon excited autofluorescence.
1. Axial slice of FDG-PET examination in a patient with sarcoidosis, showing bilateral hilar and mediastinal lymphadenopathy. Increased metabolic activity is represented by high FDG uptake (i.e. high signal intensity).
2. Axial slice of MRI with overview of the lung.
3. (A) Axial slice of a CT scan with an overview of a chronic airway disease patient showing airway wall thickening (arrow). (B) Micro-CT images of a core obtained in a peripheral IPF lung, showing the outline of secondary pulmonary lobule (white arrowheads), areas of increased attenuation (yellow arrows), terminal bronchiole and blood vessel (white arrow).
4. Alveolar compartment. (A1) OCT cross sectional view of alveolar compartment with network of alveolar septae. The middle circle is the visible probe. (A2) PS-OCT image with low scattering areas showing the alveoli (arrow) and the white round area (arrow head) showing a small bronchus. The black circles represent the inner and outer edges of the catheter sheath. (B) CLE image showing alveolar septae with rectangular airspace. (C) SHG/THG/2PEF image of the alveolar structures with alveolar septae including collagen (red, SHG), cellular structures (green, THG) and elastin (blue, 2PEF).
5. Airway wall (A1) OCT cross-sectional view of an airway wall with visible airway wall layers. The middle circle is the visible probe. (A2) PS-OCT image with ASM layer (in pink) and cartilage structures surrounded by birefringent connective tissue (in green). The black circles represent the inner and outer edges of the plastic catheter sheath.
(B) CLE image showing a typical ring-like pattern of bronchiole. (C) SHG/THG/2PEF image of the airway wall with epithelial cells (green, THG), collagen (red, SHG) and elastin fibers (blue, 2PEF).
6. Vasculature (A) OCT cross sectional of a normal pulmonary artery (surrounded by alveoli tissue, the guide wire (arrow) and the middle circle represents the visible probe). (B) CLE image of a microvessel as seen in the alveolar compartment. (C) SHG/THG/2PEF image of a pulmonary vessel (arrow), collagen tissue (red, SHG) and elastin fibers (blue, 2PEF).
7. Pleura (A) OCT cross sectional of subpleural area (arrow) and probe (B) CLE with lamellar organized fibers. (C) SHG/THG/2PEF image of the pleura with organized collagen fibers (red, SHG) and elastin fibers (blue, 2PEF).
Figure 3B is reproduced with permission Mai et al. [17].
Created using biorender.com.

Altered organization of the pulmonary ECM occurs in obstructive lung diseases, including disruption and fragmentation of elastic fibers in asthma [30•]. The elastic fiber pattern can be identified with CLE. Using the auto- fluorescent signal from elastin components, CLE produces clear microscopic images of the bronchial elastic fibers and subepithelial lamina reticularis of the bronchial and bronchiolar walls [57]. CLE makes it feasible to compare elastin fibers of healthy and obstructive airways in a minimally invasive manner [34]. Furthermore, assessment of the alveolar compartment for interstitial and other parenchymal lung diseases with CLE has been described, where an increase and distortion of fibers in fibrotic lung is detected [31,32,35]. The histological pattern of fibers in the peripheral lung tissues are compatible with CLE images derived from the same tissues. CLE has promise to serve as a real-time minimally invasive tool for 'smart probe' tissue diagnosis and monitoring of biological processes, and enhancing capacity and accuracy when acting as a guidance tool for cryobiopsies in ILD [33]. Larger *in vivo* validation studies are needed before CLE can be implemented in clinical practice; the clarity with which elastin fibers are visuable is exciting and highly promising.

Molecular imaging of the ECM

Through the use of molecular imaging, such as magnetic resonance imaging (MRI) and position emission tomography (PET), there exists the potential to enable visualization of ongoing biological processes in the lungs, in a non-invasive manner. Molecular imaging uses the application of various chemical or biological agents to identify specific gross structures or regions within tissues, and applications for identification of components of the ECM are emerging.

Magnetic resonance imaging (MRI)

MRI uses a powerful magnetic field and a radiofrequency to stimulate, and then detect, a transfer of energy from protons within cells in tissue to facilitate construction of an image of the structures within that tissue, with a resolution of 1500–5000 μm depending on the field strength (Figure 2, image 2).

Recent advances, introducing vigorous image acquisition and post-processing approaches, have highlighted the applicability of MRI for identification of aberrant ECM deposition (fibrosis) in IPF [37]. Benlala *et al.* reported an association between interstitial lung disease signal-intensity volume (measured using MRI T2-weighted images) and the composite physiological index (a representation of the degree of fibrosis in the lungs) [58]. These data reflect an earlier study that showed that the lung volume measured using MRI reflected the increased ECM content in bleomycin treated mice [59].

MRI signals are enhanced through the addition of a (usually) metal-based probe; those currently approved for clinical use incorporate paramagnetic complexes, for example, gadolinium (Gd)-based substances or iron oxide nanoparticles. ECM targeting moieties, binding to collagen, elastin, fibrin or matrix metalloproteinases, have been coupled to MRI probes and explored in a research context for imaging in other organ systems (cardiovascular disease) [38••,39], however while promising for detecting pulmonary ECM, these have yet to be explored in lung diseases.

Position emission tomography (PET)

PET imaging visualises the location of a radiotracer that targets a particular molecule or substrate within a tissue, with a relatively poor resolution of approximately 5 mm. Coupling of PET with CT imaging makes interpretation easier (Figure 2, image 1).

Radiolabeled platelet glycoprotein VI-based ECM-targeting fusion protein (GPVI-Fc) has been used to investigate fibrotic tissues at sites of chronic inflammation. Strikingly the radiolabeled GPVI-Fc colocalized, in particular, with deposits of collagen III and fibronectin suggesting the potential for identifying specific ECM protein deposition [40•].

For directly imaging fibrotic responses in the lungs targeting of integrins has been explored. Integrins are cell surface receptors, important for cellular communication with the ECM. Defined classes of integrins are known to be expressed on endothelial cells or fibroblasts that are drivers of the pathological process in fibrotic lung diseases. The arginine-glycine-aspartic acid peptide ^{18}F-Fluciclatide binds to integrins αvβ3 and αvβ5 and has been shown to be taken up with higher efficacy in fibrotic, compared to nonfibrotic, volunteers' lungs [41]. Another integrin targeting molecule the αvβ6-selective [18F]FB-A20FMDV2 PET ligand has also been shown recently to be uptaken to a greater extent in the lungs of patients with fibrosis than those without [42••]. Similarly the collagen-targeted PET probe ^{68}Ga-CBP has shown strong specificity for uptake in fibrotic tissues in bleomycin mouse models and IPF patients compared to non-diseased controls [60,61•]. These studies suggest the potential for these ligands for future diagnosis and monitoring of ECM deposition (fibrosis) in the lungs.

Discussion

The potential for directly imaging pulmonary ECM is emerging, with possibilities within and outside the scope of pulmonary research suggesting this capability will expand in coming years. HRCT is yet the most widely implemented of the discussed imaging techniques, but provides the least functionality for directly imaging the pulmonary ECM. Currently, pulmonary OCT and CLE have been explored in research trials (Table 1) [62] but are yet to be implemented in standard clinical care practices. Whereas MRI and PET imaging are widely

used in both oncology and for physiological assessment of the lungs, their use for imaging the ECM has not been trialed.

An important advance that will be necessary for the implementation of any imaging possibilities in routine clinical practice will be the development of automated analysis processes and software.

The unknowns that are currently confronting the pulmonary field with respect to COVID-19 infection and long term outcomes highlight the need for non-invasive monitoring of pulmonary ECM. Little is known about how the ECM responds to the injury and inflammatory response seen in COVID-19 infection. Using a chest x-ray, Townsend and colleagues reported 4% of patients had persistent structural changes in their lungs at follow-up (median 75days post infection) [63]. CLE is being explored to visualize pulmonary fibrosis in SARS-CoV-2 infection [personal communication]. Refined imaging of the specific ECM would advance our understanding of the repair responses induced in patients. The described imaging techniques open exciting prospects for pulmonary disease detection and monitoring over time, visualization of biological processes including specific ECM changes and guidance during interventional (e.g. bronchoscopic and endoscopic) procedures.

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Conflict of interest statement

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CRediT authorship contribution statement

Pieta C Wijsman: Conceptualization, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Lisa H van Smoorenburg:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Daniël M de Bruin:** Writing - original draft, Writing - review & editing. **Jouke T Annema:** Writing - review & editing. **Huib AM Kerstjens:** Writing - review & editing. **Onno M Mets:** Writing - review & editing. **Maarten van den Berge:** Writing - review & editing. **Peter I Bonta:** Conceptualization, Writing - review & editing. **Janette K Burgess:** Conceptualization, Investigation, Supervision, Writing - original draft, Writing - review & editing.

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