APPENDICES

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English summary

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory lung disease, that causes severe respiratory symptoms, airflow limitation and a poor quality of life. According to the World Health Organization, COPD became the 3rd leading cause of global deaths in 2019 contributing to 3.23 million deaths. There are no effective pharmacological treatments available to slow the progression of COPD. COPD is characterized by airflow obstruction, an abnormal inflammatory response in the lungs and alterations in the extracellular matrix (ECM) in both the airways and parenchyma. Excessive ECM deposition in the airways contributes to airway wall fibrosis, whereas ECM degradation in the lung parenchyma contributes to the development of emphysema. Fibroblasts and airway smooth muscle cells (ASM), as key ECM producers, play a crucial role in maintaining normal ECM homeostasis. However, as the composition and biomechanical properties of ECM are altered in COPD, the behavior of fibroblasts and ASM residing in the ECM is also changed, leading to an alteration in matrix production. This indicates that changes in lung ECM can result in aberrant cellular behavior and vice versa, aberrant cellular behavior can contribute to an altered ECM environment. This thesis aimed to investigate the underlying mechanisms and implications of the disrupted fibroblast/ASM-ECM crosstalk in COPD lung tissue, with a specific focus on the small airways.

Chapter 2 provides a comprehensive overview of the current understanding regarding the roles played by mechanosensitive channels, transient receptor potential cation channel subfamily V member 4 (TRPV4) and Piezo, in the physiology of both healthy and diseased lungs. Special attention is given to exploring the interactions between these mechanosensitive channels located on cell surfaces and various local environmental factors, including immune cells, the ECM, and the cellular cytoskeleton. Additionally, this chapter discusses potential areas for future research. Building on this knowledge, Chapter 3 explores expression and functional patterns of the little-explored mechanosensitive channel Piezo in healthy and COPD small airways, with a particular focus on ASM. Immunohistochemical analysis revealed the presence and localization of both Piezo 1 and 2 channels within ASM bundles and in the small airway epithelial layer of lung tissue. There was higher Piezo expression in both of these compartments in patients with COPD IV compared to those without COPD. Activation of Piezo 1 channel in ASM provided information about the functional role of Piezo 1 in COPD. ASM derived from COPD stage II patients exhibited a lower response to Piezo 1 activation in terms of intracellular Ca\textsuperscript{2+} influx when compared to the COPD IV and non-COPD ASM cells. Furthermore, the activation of Piezo 1 led to the downregulation of ECM related genes. These findings indicate that there is altered expression and response to Piezo activation in COPD, influencing how these airways respond to mechanical stimuli such as stretching.
While Chapters 2 and 3 are dedicated to elucidating the mechanisms by which cells perceive their environment, Chapter 4 highlights alterations in ECM in COPD through an investigation of changes in the lysyl oxidase (LO) enzyme family. LOs play a pivotal role in orchestrating the formation of collagen cross-links, thereby modulating ECM organization and biomechanical properties. Smoking and the severity of COPD influenced the gene and protein expression of various members of the LO enzyme family in small airways. These findings underscore the potential distinct functions of LO enzymes within the lung and emphasize the necessity of examining the role of each protein individually. Inhibition of LOs results in loss of collagen cross-linking. Non-selective inhibition of LOs led to reduced tissue stiffness and greater contraction of airways. Therefore, COPD-associated changes in LOs, may be related to smoking and contribute to impaired airway function, providing potential novel targets for preventing or treating small airway changes in COPD. Chapter 5 builds on the foundation of Chapter 4 which demonstrated an increase in both gene and protein expression of lysyl oxidase like-1 (LOXL1) in COPD small airways. As ECM remodeling affects ECM-fibroblast crosstalk, the study examined how alterations driven by LOs/LOXL1 in the ECM affect fibroblast behavior, thereby contributing to further ECM remodeling in COPD. To investigate this, fibroblasts obtained from healthy donors were treated with inhibitors of LOs and LOXL1. The ECM produced by these cells was collected for the purpose of studying how healthy fibroblasts respond when seeded on these ECM matrices, serving as a model for examining fibroblast-ECM crosstalk. No differences in cell behavior were observed across these different matrices. Therefore, LOs and LOXL1 inhibition has no effect on healthy fibroblast-ECM crosstalk in this in vitro model but whether this differs in COPD fibroblasts has yet to be examined.

To investigate cell-ECM crosstalk in the context of COPD and/or other pulmonary diseases, various in vitro experimental models have been widely used. Numerous models have been used to elucidate cell behavior which are now evolving towards complex three-dimensional (3D) models incorporating ECM, aiming to replicate the cells' native environment. Little is known about the cellular changes that occur when moving from two-dimensional (2D) to 3D cell culture. Chapter 6 briefly outlines the composition of the lung ECM and the changes associated with chronic lung diseases. It also provides an overview of the progress and state-of-the-art research conducted using 3D ECM models, discusses the advantages and challenges related to these models and summarizes the properties of an ideal 3D model.

Multiple investigations have used 3D models to culture fibroblasts, examining the interplay between ECM and fibroblasts in various organ systems. Chapter 7 demonstrates the difference in gene expression profiles in primary human lung
fibroblasts cultured on 2D stiff collagen I-coated cell culture plastic with those cultured in 3D soft collagen I hydrogels to gain a better understanding of changes in cellular behavior between these two model systems and the potential consequences for fibroblast-ECM crosstalk. The transcriptional response of fibroblasts cultured in 3D indicated inhibition of proliferation, and alterations in Hippo and ECM pathways indicating a switch from proliferation to ECM remodeling.

Overall, this thesis provides evidence and insights into the disrupted crosstalk between fibroblasts/ASM and the ECM in the context of COPD, resulting in the remodeling of lung tissue. It also underscores the importance of considering the impact of 3D models on cell-ECM interactions, as these models better represent in vivo conditions. This thesis opens new avenues for further research into the cellular and molecular mechanisms underlying COPD pathophysiology, offering valuable insights for developing future therapeutic strategies.
Nederlandse samenvatting

Chronische obstructieve longziekte (COPD) is een progressieve inflammatoire longziekte die ernstige ademhalingsproblemen en een slechte levenskwaliteit veroorzaakt. Volgens de Wereldgezondheidsorganisatie was COPD in 2019 wereldwijd de op twee na belangrijkste doodsoorzaak en verantwoordelijk voor 3,23 miljoen sterfgevallen. Er zijn geen effectieve farmacologische behandelingen beschikbaar om de progressie van COPD te vertragen. COPD wordt gekenmerkt door een abnormale ontstekingsreactie in de longen die zorgt voor vernauwing en obstructie van de luchtwegen, schade aan de longblaasjes met emfyseem tot gevolg en veranderingen in de extracellulaire matrix (ECM) in zowel de luchtwegen als in de longblaasjes. Overmatige productie van ECM eiwitten in de luchtwegen draagt bij aan verdikking van de luchtwegwand met fibrose en vernauwing van de luchtwegen, terwijl er bij emfyseem juist sprake is van afbraak en verlies van de ECM. Fibroblasten en gladde spiercellen in de luchtwegen spelen als belangrijke ECM-producerende cellen een cruciale rol in het handhaven van een normale ECM-homeostase. Echter, als de samenstelling en biomechanische eigenschappen van de ECM veranderen bij COPD, verandert ook het gedrag van fibroblasten en gladde spiercellen die zich in de ECM bevinden, wat leidt tot een verandering in de ECM eiwit productie. Dit geeft aan dat er een belangrijke interactie plaatsvindt tussen de cellen en de ECM waarbij veranderingen in de ECM van de long kunnen leiden tot afwijkend celgedrag en vice versa, afwijkend celgedrag kan bijdragen aan een veranderde ECM omgeving. Het doel van dit proefschrift was om de onderliggende mechanismen en implicaties van de verstoorde interactie tussen fibroblasten en gladde spiercellen en de ECM in COPD longweefsel te onderzoeken, met een specifieke focus op de kleine luchtwegen.

Hoofdstuk 2 gaat over de invloed van biomechanische veranderingen op de cellen en de ECM en geeft een uitgebreid overzicht van de huidige kennis over de rol van 3 specifieke mechanosensitieve kanalen, te weten ‘transient receptor potential cation channel subfamily V member 4 (TRPV4)’ en Piezo 1 en Piezo 2, in de fysiologie van zowel gezonde als zieke longen. Speciale aandacht wordt besteed aan het onderzoeken van de interacties tussen deze mechanosensitieve kanalen op het celoppervlak en immuuncellen, de ECM en het cellulaire cytoskelet. Daarnaast bespreekt dit hoofdstuk potentiële gebieden voor toekomstig onderzoek. Voortbouwend op deze kennis, hebben we in hoofdstuk 3 onderzoek gedaan naar de rol en aanwezigheid van Piezo 1 en Piezo 2 in de kleine luchtwegen van COPD patiënten in vergelijking met controle patiënten, met speciale aandacht voor de gladde spiercellen. Immunohistochemische analyse onthulde de aanwezigheid en lokalisatie van zowel Piezo 1 als 2 kanalen in de gladde spierbundels en in het bekledende luchtwegepitheel van de kleine luchtwegen.
Er was een hogere Piezo expressie in beide compartimenten bij patiënten met stadium IV COPD vergeleken met patiënten zonder COPD. Activering van het Piezo 1 kanaal in gladde spiercellen geeft informatie over de functionele verschillen van Piezo 1 in COPD. Gladde spiercellen afkomstig van COPD stadium II patiënten vertoonden een lagere respons op Piezo 1 activering in termen van intracellulaire Ca2+ influx in vergelijking met de COPD IV en niet-COPD spiercellen. Bovendien leidde de activering van Piezo 1 tot een verlaging van ECM-gerelateerde genen. Deze bevindingen geven aan dat er een veranderde aanwezigheid en respons is op Piezo activering bij COPD, en dit kan invloed hebben op hoe deze luchtwegen reageren op mechanische prikkels zoals bijvoorbeeld uittrekkende invloed van de ademhaling.

Terwijl de hoofdstukken 2 en 3 gewijd zijn aan het ophelderen van de mechanismen waarmee cellen hun omgeving waarnemen, belicht hoofdstuk 4 veranderingen in de ECM bij COPD door de familie van de lysyloxidase (LO) enzymen te onderzoeken. LO's spelen een centrale rol bij de vorming van collageen bundels en de interactie tussen de losse collageen strengten. Op deze manier beïnvloeden LO's de ECM-organisatie en de biomechanische eigenschappen daarvan. Onze resultaten laten zien dat roken en de ernst van COPD invloed heeft op de gen- en eiwitexpressie van verschillende leden van de LO-enzymfamilie in kleine luchtwegen. Deze bevindingen onderstrepen de mogelijk verschillende functies van LO-enzymen in de long en benadrukken de noodzaak om de rol van elk eiwit afzonderlijk verder te onderzoeken. Daarnaast laten onze resultaten zien dat het remmen van LO's resulteert in minder interacties tussen de collageen strengten, verminderde stijfheid van longweefsel en een sterkere samentrekking van de luchtwegen. Op basis hiervan concluderen wij dat de COPD-geassocieerde veranderingen in LO's gerelateerd zijn aan roken en bijdragen aan een verminderde functie van de luchtwegen. Deze bevindingen dragen bij aan potentiële nieuwe behandeldoelen gericht op de kleine luchtwegveranderingen bij COPD. Hoofdstuk 5 bouwt voort op de basis van hoofdstuk 4, waarin een toename werd aangetoond van zowel gen- als eiwitexpressie van lysyloxidase like-1 (LOXL1) in de kleine luchtwegen in COPD. Aangezien veranderingen in de ECM, de interactie tussen fibroblasten en de ECM kan beïnvloeden, hebben we in dit hoofdstuk onderzocht hoe veranderingen aangedreven door LO's/LOXL1 in de ECM het gedrag van fibroblasten beïnvloedt en zo bijdraagt aan verdere ECM remodulering in COPD. Om dit te onderzoeken werden fibroblasten van gezonde donoren behandeld met remmers van LO's en LOXL1. De ECM die door deze cellen werd geproduceerd, werd verzameld om te bestuderen hoe gezonde fibroblasten reageren als ze op deze ECM-matrices moesten groeien. Er werden geen verschillen in celgedrag waargenomen tussen de verschillende matrices. Remming van LO's en LOXL1 heeft dus geen effect op de interactie tussen gezonde
fibroblasten en de ECM in dit celkweek model. Of dit anders is bij COPD-fibroblasten moet nog worden onderzocht.

Om de cel-ECM interactie in de context van COPD en/of andere longziekten te onderzoeken, zijn verschillende in vitro experimentele modellen op grote schaal gebruikt. Er zijn talloze tweedimensionale (2D) modellen gebruikt om het gedrag van cellen op te helderen, die nu evolueren naar complexe driedimensionale (3D) modellen met ECM, met als doel de natuurlijke omgeving van de cellen zo goed mogelijk na te bootsen. Er is echter weinig bekend over de cellulaire veranderingen die optreden bij de overgang van een 2D naar een 3D-celkweek. **Hoofdstuk 6** geeft een kort overzicht van de samenstelling van de ECM van de long en de veranderingen die geassocieerd zijn met chronische longziekten. Het geeft ook een overzicht van de vooruitgang en het state-of-the-art onderzoek met 3D ECM modellen, bespreekt de voordelen en uitdagingen van deze modellen en vat de eigenschappen van een ideaal 3D model samen.

Verschillende onderzoeken hebben 3D-modellen gebruikt om fibroblasten te kweken, waarbij de wisselwerking tussen ECM en fibroblasten in verschillende orgaansystemen is onderzocht. **Hoofdstuk 7** laat het verschil zien in genexpressieprofielen in primaire menselijke longfibroblasten gekweekt op 2D stijf collageen I-gecoat celkweekplastic met die gekweekt in 3D zachte collageen I-hydrogels om een beter begrip te krijgen van veranderingen in cellulair gedrag tussen deze twee modellen en de mogelijke gevolgen voor de interactie tussen fibroblasten en ECM. De veranderingen in gen expressie patronen van fibroblasten gekweekt in 3D wees op remming van de celgroei en veranderingen in ECM genen en genen betrokken by signalering via Hippo. Tezamen suggereren deze bevindingen dat er een omschakeling plaatsvindt van proliferatie naar actieve remodulering van de ECM.

Samenvattend levert dit proefschrift bewijs en inzicht in de verstoorde interactie tussen fibroblasten en gladde spiercellen en de ECM in de context van COPD, wat resulteert in de remodulering van longweefsel. Het onderstreept ook het belang van het gebruik van 3D modellen voor het bestuderen van cel-ECM interacties, omdat deze modellen beter in vivo condities repliceren. Dit proefschrift opent nieuwe wegen voor verder onderzoek naar de cellulaire en moleculaire mechanismen die ten grondslag liggen aan de pathofysiologie van COPD en biedt waardevolle inzichten voor de ontwikkeling van toekomstige therapeutische strategieën.
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And I have a special word for my cat **Misha** “Mee-eh”.
Curriculum Vitae

Nataliya Migulina, born on 16 August 1996 in Moscow, Russia, commenced her academic journey by earning her bachelor’s degree in Human Physiology with high honors from Lomonosov Moscow State University (MSU) in 2017. Nataliya was awarded a full Orange Tulip scholarship in the same year, allowing her to enroll in the Master’s program in Medical and Pharmaceutical Drug Innovation (MPDI) at the University of Groningen. In 2017 she conducted a research project at the Department of Pathology and Medical Biology in University Medical Center Groningen (UMCG) aimed to understand extracellular matrix assembly disruption in chronic obstructive pulmonary disease. In 2018, Nataliya delved into further research during her Master's program at the Faculty of Science and Engineering, University of Groningen. Her Master's thesis investigated the targeting of the Tumor Necrosis Factor signaling pathway in Multiple Sclerosis.

In March 2020, Nataliya embarked on a collaborative PhD project that spanned UMCG, the Experimental Pulmonology and Inflammation Research (EXPIRE) group, and Mayo Clinic's Department of Anesthesiology in Rochester, USA. Specializing in chronic lung diseases, her work focused on pre-clinical studies aimed at discovering and developing new targets and therapies. Additionally, she contributed to the bioengineering of the extracellular matrix environment for lung model development, with implications in fibrosis. Throughout Nataliya's PhD trajectory, her research findings on potential targets for the treatment of chronic obstructive pulmonary disease were published in high-impact journals such as FASEB and Comprehensive Physiology. She showcased her work at numerous national and international scientific conferences. Collaborating with top professors and doctors in the field, she was part of an outstanding international team focused on advancing novel therapies for treating lung diseases. After successfully concluding her PhD, Nataliya transitioned to a new chapter in her career, entering the life science venture capital industry.
List of publications


