Monitoring mechanical ventilation in lung transplantation and lung cancer surgery using particles in exhaled air

Preclinical and clinical implementation.

ELLEN BROBERG | FACULTY OF MEDICINE | LUND UNIVERSITY

Ellen Broberg graduated from Lund University Medical School in January 2007 and did her foundation years at Örnsköldsvik County Hospital. In September 2009 she started her speciality training in anaesthesia and intensive care at Nyköping County Hospital; 9 months later she continued at South General Hospital in Stockholm and finished her training at Newcastle Upon Tyne NHS Trust. In November 2013 she became qualified in anaesthesia and intensive care with the Swedish Board of Health. Medical work continued in Newcastle until December 2014 when she started working as a consultant in anaesthesia and intensive care at the department of cardiothoracic anaesthesia and intensive care at Skåne University hospital in Lund, Sweden. One year later in November 2015 she started her PhD studies.

This thesis addresses the safety, detection, measurement and analysis of particles in exhaled air in intubated mechanically ventilated patients. Issues of reproducibility and safety from pre-clinical studies to clinical trials have been achieved and several various factors related to mechanical ventilation have been addressed in different categories of patients. Exhaled particles in air have also been studied in lung transplantation from pre-clinical to clinical implementation and issues with both short-term and long-term signs of organ dysfunction and rejection of transplant have been evaluated.

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Abstract

Background

The aim of this dissertation was to examine the feasibility of the PExA device in conjunction with mechanical ventilation and to identify particle patterns and biomarkers in exhaled air in different ventilation modes and in different hospital settings. In addition, to explore the hypothesis of finding early signs of developing primary graft dysfunction (PGD) and the risk factors relating to the initial diagnosis and form of lung transplantation (LTx) and the effect that they have on the development of bronchiolitis obliterans syndrome (BOS).

Methods

In the two initial studies, particle flow during mechanical ventilation was studied in a pre-clinical setting. Each study explored pre-clinical feasiblity, volume-controlled ventilation (VCV) versus pressure-controlled ventilation (PCV) and the impact of recruitment manouvres (RM) both short-term and over 3 consecutive days along with ventilation during *ex vivo* lung perfusion (EVLP). Study III was a comparison of the impact of VCV and PCV along with RM in lungtransplantated patients in intensive care. In Paper IV intubated patients on mechanical ventilation with lung cancer during lung surgery were compared to normal breathing patients. Paper V was a retrospective review of journals of patients having LTx between 1990-2014 at Lund University Hospital.

Results

The PExA device has been shown to be safe to use in conjunction with mechanical ventilation in both pre-clincal and thereafter clinical setting. Particle flow increased stepwise as blood flow did likewise. In healthy lungs VCV resulted in a significantly lower particle count than PCV on the first day of ventilation. No impact on particle flow in RM was seen after 2 days. Patients who developed PGD had an significantly higher stepwise increase in particle flow, stayed significantly longer in mechanical ventilation and had higher inflammatory markers then those who did not develop PGD. Analysed markers showed significantly higher levels during *in vivo* compared to EVLP ventilation and significantly lower levels between mechnical ventilation during surgery and normal breathing patients. The retrospective study showed that double-LTx (DLTx) had a superior outcome compared with single-LTx (SLTx) despite a higher incidence of BOS.

Conclusion

For the first time exhaled particles in air have been measured and analysed in intubated mechanically ventilated in preclinical and clinical settings. This thesis has demostrated feasibility both in pre-clinical studies and clinical implementation. The studies have shown differences in particle flow and in analysed particles between the various groups studied. The thesis has demonstrated differences in particle flow between VCV and PCV which have shed light on the previously unknown impact of ventilation between different phases of *in vivo* and EVLP ventilation and between patients with and without PGD which may imply the usefulness of the technique in early detection of PGD. The studies have indicated that higher particle flow could be a sign of lung injury and therefore tidal volumes could be individually reduced to lower the risk for lung injury. Differences in analysed particles have been detected between *in vivo* and ELVP which might suggest a depletion of surfactant in longer EVLP evaluations. Long-term outcome has also been studied and demostrated that DLTx was associated with superior survival compared to SLTx, despite having a higher incidence of BOS.

Key words: Mechanical ventilation. Lung transplantation. Lung cancer. Exhaled particles.

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Dedicated to the patients who have participated in this thesis
and to my wonderful daughter Alice

Table of Contents

List of publications

Paper I

Broberg E, Wlosinska M, Algotsson L, Olin AC, Wagner D, Pierre L, Lindstedt S: A new way of monitoring mechanical ventilation by measurement of particle flow from the airways using PExA method in vivo and during ex vivo lung perfusion in DCD lung transplantation.
Intensive Care Medicine Experimental 2018 Jul 27; 6(1): 18.

Paper II

Broberg E, Pierre L, Fakhro M, Algotsson L, Malmsjo M, Hyllén S, Lindstedt S: Different particle flow patterns from the airways after recruitment manoeuvres using volume-controlled or pressure-controlled ventilation.
Intensive Care Medicine Experimental 2019 Mar 13; 7(1): 16.

Paper III

Broberg E, Hyllén S, Algotsson L, Wagner D, Lindstedt S: Particle flow profile from the airways measured by PExA differ in lung transplant recipients who develop primary graft dysfunction.
Experimental and Clinical Transplantion Accepted 11 July 2019.

Paper IV

Broberg E, Andreasson J, Fakhro M, Olin AC, Wagner D, Hyllén S, Lindstedt S: Mechanically ventilated patients exhibit decreased particle flow in exhaled breath as compared to normal breathing patients.
ERJ Open Research Accepted 17 October 2019.

Paper V

Fakhro M, Broberg E, Algotsson L, Hansson L, Koul B, Gustafsson R, Wierup P, Ingemansson R, Lindstedt S: Double lung, unlike single lung transplantation might provide a protective effect on mortality and bronchiolitis obliterans syndrome.
Journal of Cardiothoracic Surgery 2017 Nov 25; 12(1): 100.

Populärvetenskaplig sammanfattning (Summary in Swedish)

Denna avhandling är byggt på studier om partikelflöden i utandningsluften när andningshjälp i form av en respirator har använts. Mätningar av partikelflöden i utandningsluften hos de som behöver respirator har aldrig tidigare studerats.

Andningshjälp i form av behandling med respirator är en stödjande samt livsuppehållande behandling och för vissa en livräddande behandling. Det finns flera orsaker till respiratorbehandling såsom sövning inför och under kirurgi, livshotande tillstånd som allvarliga infektioner eller skador efter olycka samt som en del i behandlingen efter att en patient fått nya lungor så kallad lungtransplantation, för att nämna några. Idag finns det flera olika sätt till hands för en läkare att ställa in andningshjälpen i respiratorn. Behandlingen kan ställas in beroende på flera faktorer såsom önskad volym i varje andetag, så kallad volym-kontrollerad andning (VKA) eller vilket önskat tryck som ska användas för att få ner luft i lungorna, så kallad tryck-kontrollerad andning (TKA) eller en kombination av båda dessa sätt. Andra faktorer är antal andetag samt hur mycket patienten själv kan medverka i sin andning. Respiratorbehandling kan orsaka skada på lungvävnaden, både på kort sikt och i långa loppet. Det är ingen fullständig enighet om vilka behandlingssätt som är bäst lämpat för varje sjukdomstillstånd och hur resultatet av en behandling kan bedömas och utvärderas på ett tillfredsställande sätt. Att kunna minska risken för kvarstående lungskada genom att kunna ställa in den mest skonsamma behandlingen för varje enskild individ är en mycket fördelaktig väg att gå. Om risken för skador från respiratorbehandling skulle kunna minskas med ny kunskap genom att studera partikelflöden skulle en betydande grupp patienter kunna gynnas och särskilt de patienter med högst risk för lungskada.

Den här avhandlingen undersöker en metod att samla och analysera partiklar i utandningsluften, både i prekliniska och kliniska miljöer under pågående respiratorbehandling, något som aldrig tidigare har gjorts eller presenterats. Metoden kallas PExA och analyserar partiklar i utandningsluft. Vi har anpassat metoden för att på ett tillfredställande och säkert sätt kunna användas under respiratorbehandling, då denna teknik tidigare endast använts hos försökspersoner som andas själva.

Tekniken hade testats under flera olika situationer i prekliniska studier innan studier på patienter utfördes. Flera olika inställningar på respiratorn har undersökts och både enskilda och återkommande mätningar har gjorts för att säkerställa teknikens duglighet. I prekliniska studierna har mätningar gjorts både innan och efter döden för att likna förhållanden som uppstår i samband med lungtransplantation. Efter att det säkerställts att tekniken är skonsam, utan något extra intrång eller skada, har mätningarna på studiepatienter under operation och inom intensivvård genomförts.

I studierna har olika respiratorinställningar studerats och både enskilda samt återkommande mätningar har genomförts under flera dagar. Vid lungoperationer har mätningar gjorts under andning med båda lungorna samtidigt och även med endast en enskild lunga, så kallad lungseparation. Andning med bara en lunga är vanligt förekommande inom lungkirurgi för att underlätta för kirurgen att operera på den lunga som behöver kirurgi. Den här avhandlingen studerar partiklar från friska lungor och hos patienter med framförallt lungcancer samt hos patienter som genomgått en lungtransplantation.

Lungor består av flera olika typer av celler med olika uppbyggnad och uppgifter i lungan. Sammansättningen av lungans olika celler spelar en stor roll i sammansättningen av den vätska som täcker luftvägarna. Detta vätskeskikt är en komplex struktur som täcker luftvägarnas cellskikt och utsöndring av celler i olika delar av luftvägen kommer att återspeglas i skillnader i vätskeskiktets sammansättning. Några av partiklarna som finns i detta vätskeskikt har med denna teknik kunnat studeras genom biokemisk analys i ett laboratorie. Detta har genomförts för att kunna identifiera möjliga biomarkörer och har studerats i prekliniska studier innan kliniska studier.

Avhandlingen presenterar även en studie på lungtransplanterade patienter med fokus på långtidsresultat efter en transplantation. Studien undersöker faktorer som kan påverka utvecklingen av eventuell avstötning av nya lungor och hur patienternas grundsjukdom som ledde till lungtransplantation kan påverka deras överlevnad vid eventuell avstötning av de transplanterade lungorna.

Avhandlingen är baserad på 5 delarbeten och varje delarbete och dess slutsatser presenteras här var för sig.

Delarbete I

För första gången har vi uppmätt partikelflödet från luftvägarna under behandling i respirator. Studien gjordes prekliniskt med genomförde mätningar både i livet och efter uttag av lungorna, något som sker vid till exempel lungtransplantation. Resultaten visade att VKA jämfört med TKA gav ett lägre partikelflöde från luftvägarna under mätningar i livet. I både VKA och TKA gav stora andetag en ökning av partikelflödet jämfört med små andetag. Efter uttag av lungorna kan lungor utvärderas med en metod som kan ge luft till lungorna och cirkulera runt

blod i dess vävnad utanför kroppen, en teknik som ibland användas vid lungtransplantationer. Vid utvärdering av lungorna efter döden visade partikelflödet ha en stegvis ökning samtidigt som en stegvis ökning av blodflödet genom lungorna ökades. Biomarkörer i utandningsluften samlades in och analyserades efteråt i laboratoriet. Mängden partiklar som är viktiga för att lungans små luftblåsor ska hålla sig öppna, så kallad surfaktant, var mindre vid insamling av partiklar under respiratorbehandling när lungorna var utanför kroppen jämfört med tidigare när de var i kroppen. Detta skulle kunna bero på att produktionen av surfaktant upphör efter döden och det uppstår en brist på surfaktant när man gör värdering av lungorna utanför kroppen. Detta delarbete visade på att denna teknik är säker att använda i samband med respiratorbehandling i prekliniska studier.

Delarbete II

Denna prekliniska studie gjordes med mätningar av partikelflöde i utandningsluften under flera efterföljande dagar. Resultaten visade att olika respiratorinställningar, såsom VKA och TKA gav olika partikelflöde från luftvägarna under de olika dagarna. Under de tre på varandra följande dagarna sågs en stegvis minskning av partikelflödet. I denna studie gjordes även ett skonsamt försök att blåsa upp lungorna för att öppna upp lungvävnad som kan ha fallit ihop, en så kallad rekryteringsmanöver. Partikelflöde från VKA och TKA hade olika mönster under dessa försök att öppna upp tidigare sammanfallen lungvävnad. Denna studie har varit viktig för att visa att denna tekniken är säker att använda i samband med respiratorbehandling både vid engångsmätning men också vid upprepade mätningar under flera dagar.

Delarbete III

I delarbete III studerades lungtransplanterade patienter under tiden de behövde respiratorbehandling, från att de genomgått sin operation tills respiratorn togs bort. Denna studie har visat att denna teknik är säker att använda i samband med respiratorbehandling hos patienter inom intensivvården. De patienter som får nya lungor kan inom tre dygn direkt efter operationen utveckla en akut lungskada, så kallad primary graft dysfunction. De som utvecklade denna akuta lungskada hade ökat partikelflöde från luftvägarna jämfört med de som inte utvecklade denna akuta lungskada. Även i denna studie gjordes mätningar med VKA och TKA och resultaten visade att det fanns skillnader i partikelflödet mellan två olika inställningarna och att partikelflödet från luftvägarna förändrades under tiden patienterna behövde andningshjälp från respiratorn. Denna information om att olika respiratorinställningar ger olika partikelflöde från luftvägarna skulle möjligt i framtiden kunna utnyttjas till att individanpassa respiratorbehandlingen för varje enskild patient.

Delarbete IV

Denna studie har fastställt säkerheten med att genomföra mätningar med denna teknik under operationen för olika sjukdomstillstånd i lungorna, såsom lungcancer. Studien genomfördes på patienter som genomgick lungkirurgi och fick sin andningshjälp via en respirator och dessa jämfördes med patienter som andades själva. Denna studie visade på olika och mätbara skillnader i partikelflöde från patienter som fick hjälp med sin andning i en respirator jämfört med patienter som andades själva. I denna studien analyserades biomarkörer och resultaten visade på att olika partikelsammansättningar kan uppmätas mellan patienter med respiratorbehandling under lungkirurgi och med patienter som andas själva.

Delarbete V

I delarbete V samlades information från patientjournaler hos samtliga lungtransplanterade patienter mellan januari 1990 och juni 2014 på Lunds Universitetssjukhus. Studien visade att de patienter som fick båda sina lungor utbyta hade en bättre chans att överleva jämfört med de som fick en lunga utbyt trots utveckling av kronisk avstötning. Patienter med cystisk fibros, som är en ärftlig lungsjukdom och patienter med lungfibros där vävnad liknande ärrvävnad utvecklas på lungan hade bättre överlevnad efter sin transplantation trots att de utvecklade kronisk avstötning jämfört med andra patientgrupper som genomgick lungtransplantation.

Abbreviations

AAT1	Alpha-1-antitrypsin
ARDS	Acute respiratory distress syndrome
BMI	Body mass index
BOS	Bronchiolitis obliterans syndrome
CLAD	Chronic lung allograft dysfunction
COPD	Chronic obstructive pulmonary disease
CT	Computer tomography
CF	Cystic fibrosis
CO	Cardiac output
DBP	Diastolic blood pressure
DLCO	Diffusing capacity of carbon monoxide
DLT	Double-lumen tube
DLTx	Double lung transplantation
DLV	Double-lung ventilation
DCD	Donation after circulatory death
DPPC	Di-palmitoyl-phosphatidyl-choline
E	Elastance
ECC	Extracorporeal circulation
ECMO	Extracorporeal membrane oxygenation
ELISA	Enzyme-linked immunosorbent assay
EVLP	Ex vivo lung perfusion
FEV_1	Forced expiratory volume for 1 minute
FiO_2	Fraction of inspired oxygen
FRC	Functional residual capacity

GVHD	Graft-vs-host disease
HRCT	High-resolution computed tomography
HLA	Human leukocyte antigen
HLTx	Heart and lung transplantation
IS	Internal Standard
ISHLT	The International Society for Heart and Lung Transplantation
LAP	Left atrial pressure
MAP	Mean arterial pressure
MPAP	Mean pulmonary artery pressure
MRI	Magnetic resonance imaging
NB	Normal breathing
NPWT	Negative pressure wound therapy
NSCLC	Non-small-cell lung cancer
OLV	One-lung ventilation
P	Pressure
PAF	Pulmonary artery flow
PH	Pulmonary hypertension
PCV	Pressure-controlled ventilation
PCWP	Pulmonary capillary wedge pressure
PGD	Primary graft dysfunction
PEEP	Positive end-expiratory pressure
PET	Positron-emission tomography
PEx	Particles exhaled
PExA	Particles in exhaled air
PF	Pulmonary fibrosis
PFR	Particle flow rate
POPC	Palmitoyl-oleoyl-phosphocholine
PVR	Pulmonary vascular resistance
OPC	Optical particle counter

R	Resistance
RAS	Restrictive allograft syndrome
RTLF	Respiratory tract lining fluid
RM	Recruitment manoeuvres
SEM	Standard error of the mean
SBP	Systolic blood pressure
SD	Standard deviation
SLTx	Single lung transplantation
SP-A	Surfactant A
TNM	Tumour-node-metastasis
TV	Tidal volume
V	Volume
VCV	Volume-controlled ventilation
VILI	Ventilator induced lung injury
W	Work
WOB	Work of breathing

Introduction

Mechanical ventilation is a life-supporting and for some life-saving treatment, but it may come at a risk. There are several indications for mechanical ventilation, these include general anaesthesia for surgery, life-threatening conditions such as severe infections and as an initial treatment after lung transplantation, to mention a few. Several ventilation modes are available and there is no full consensus on which is the most appropriate in every condition and how the effect of various modes should best be assessed. The major risk with mechanical ventilation is injury to the lung, both short-term and long-term. If the risk could be reduced by new knowledge of the impact of mechanical ventilation it would benefit a substantial group of patients, especially for those patients with the highest risk of lung injury. The idea of following and optimising mechanical ventilation non-invasively in real time is a very tempting prospect and this thesis has explored a technique that could shed new light on the impact of mechanical ventilation.

Detection and analysis of particles in exhaled air has never before been investigated in mechanically ventilated patients. Healthy subjects and patients with inflammatory diseases have been studied during normal breathing circumstances but never under mechanical intubation. The device used for sampling and measuring particles in exhaled air has been custom built before being used in conjunction with mechanical ventilation. This thesis explores subjects with healthy lungs, lung-cancer affected lungs or other lung pathologies during surgery and recipients after lung transplantation. The technique has been tested under several different situations such as *in vivo*, post-mortem and *ex vivo* lung perfusion ventilation along with one-lung ventilation during surgery and over subsequent days in an intensive care setting. The technique described in this thesis has been tested in the pre-clinical setting in animal studies with different ventilation modes and settings taken into account before clinical implementation was performed successfully.

This thesis explores the detection and analysis of particles biochemically in exhaled air for potential biomarkers in intubated mechanically ventilated subjects, this has also been studied in the pre-clinical setting before clinical implementation.

The thesis also investigates the long-term development of chronic rejection in lung transplantation recipients in relation to the initial diagnosis and the correlation of developing rejection with single (SLTx) or double (DLTx) lung transplantation.

The respiratory system

Anatomy, physiology and morphology

The lung is in direct contact with the outside world, as compared to other vital organs and parts of the airway and lungs have different functions. The structure of the airways and the lung are highly efficient to achieve the end result of facilitating gas exchange between oxygen and carbon dioxide. The airways predominantly heat, humidify and protect the lungs by filtering the air from harmful substances. The lungs' gas exchange surface area is approximately 130 m^2 and made up of 300 g of tissue, and 200 ml of capillary blood volume is exchanged every second. The lungs have approximately a staggering 300-800 million alveoli [1-4].

The respiratory system can be divided into different segments depending on anatomy or physiological function.

Anatomy

Anatomically the respiratory system is divided into the proximal or upper respiratory tract and distal or lower respiratory tract. The proximal respiratory tract consists of organs outside the thoracic cage, which are the nose, the pharynx and the larynx. The nose (and its cavity) is divided into two separate nostrils by the nasal septum and lateral in both nostrils are three conchae. The pharynx starts where the nasal cavity ends and goes down to the cricoid cartilage and is approximately 13 cm long. It is divided into three separate parts: the nasopharynx, oropharynx and laryngopharynx. The larynx consists of nine separate cartilages that keep the larynx open and, of these, the arytenoid cartilages control the vocal cords. At the most superior part of the larynx lies the epiglottis, an elastic cartilage that moves freely up and down to direct air into the larynx and, for example, mucus, food and liquids into the oesophagus [5-7].

The distal respiratory tract consists of organs inside the thoracic cage, which are the trachea, the bronchi, the bronchioles, the alveolar duct and the alveoli. The trachea starts where the larynx ends at around the 6th cervical vertebrae and continues until the carina, which is at around the 5th thoracic vertebrae. At the carina the trachea divides into two separate bronchi, the left and right bronchi. The trachea has a tubular structure comprised of 16-20 cartilage rings which counteract collapse of this very important structure for breathing. It is approximately 11-13 cm long. The right and left bronchi each divide into smaller bronchi when entering the lung. The bronchi divide into three secondary bronchi for each lobe on the right side and into two secondary bronchi for each lobe on the left side. Individually these five secondary bronchi further divide into tertiary bronchi that will finally divide into bronchioles, which end with the terminal bronchioles. This

structure from the carina to the terminal bronchioles is termed the bronchial tree [5, 6].

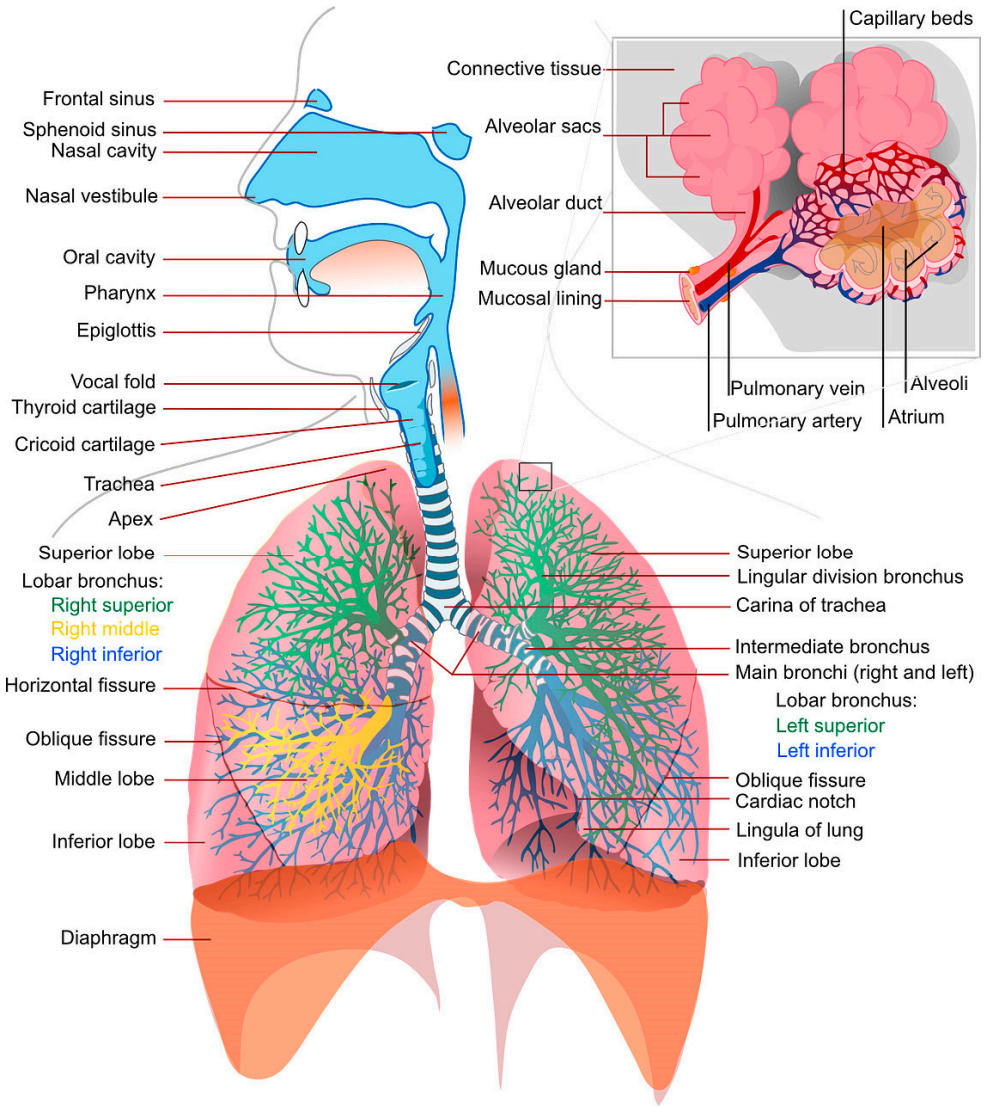

Figure 1 Anatomy of the respiratory system
Image by Clker-Free-Vector-Images from Pixabay

At the level of the terminal bronchioles the diameter is about 0.5 mm and there is very little difference in the diameter after this [8]. The division continues with the respiratory bronchioles and into the alveolar ducts; around the alveolar ducts lie the alveoli. Individually the lungs lie within the thoracic cavity and are protected

by the pleurae: the parietal pleura covers the chest wall and the visceral pleura covers the lungs. In between these two thin layers lies the pleural cavity containing a small amount of fluid to reduce friction during breathing [5, 6].

Physiology and morphology

The primary physiological function of the proximal airway is to humidify, heat and filter the air for the sensitive distal respiratory tract [5-7]. In 1963 the Weibel classification for the distal respiratory tract was developed and has thereafter been adopted widely. It describes the lung as a single unit with regular and symmetrical divisions, in total 23 generations. It divides the distal respiratory tract into three separate zones: the conducting zone, the transitional zone and the respiratory zone. The conducting zone starts from the trachea into the terminal bronchioles, generation 0-15. The transitional zone starts with the respiratory bronchioles and continues until the alveolar ducts, generation 16-19. The final zone, the respiratory zone, consists of the alveolar ducts and alveoli, generation 20-23 [1, 9, 10].

The lung consists of about 300-800 million alveoli [1, 5, 11, 12]. About 90% of the entire lung volume consists of the alveoli and the alveoli wall is comprised of type I and type II alveolar epithelial cells. Type I epithelial cells cover approximately 95% of the entire lining of the surface of the alveoli and are where gas exchange predominantly occurs. Scattered among the type I epithelial cells are type II epithelial cells that secrete alveolar fluid, containing surfactant. Surfactant consists of phospholipids and lipoproteins and keeps the alveolar open due to reduction of surface tension [5, 13].

Respiratory tract lining fluid

This section comprises an overview of the respiratory tract lining fluid (RTLF) while specific particles studied in this thesis will be explained later in the introduction.

The lung consists of both epithelial and inflammatory cells and its structure plays a significant role in the composition of the RTLF. The RTLF is a complex structure that overlies the epithelial cell layer of the airways. The epithelial cells' secretion in different parts of the airway will be reflected in regional variations of the RTLF's composition [5, 14]. Several other factors apart from particle production influence the composition of RTLF and contribute to its complexity, such as age, disease, breathing patterns and size and shape of the airways [15-21].

The airways epithelial layer consists of several different types of cells. The main clearance of particles, phlegm and fluid is performed predominantly by the ciliary cells, lining the airway from respiratory bronchioles to the glottis [5, 22, 23]. The mucus gel layer contributes greatly to the lungs' defence mechanisms with clearance along with the cilia cells and general immune homeostasis. The mucus

gel layer consists of two major groups: mucin glycoproteins (oligosaccharide and peptide) and proteoglycans (glycosaminoglycans) and these are particles of high molecular weight. They are secreted mostly from submucosal glands in the upper airway but also by goblet and serous cells [5, 22, 24-26].

In the RTLF several antioxidants exist and address the environmental load that may affect the lung in modern life, such as air pollutants and tobacco smoke. The antioxidants are of low molecular weight compared to the mucin layer [27-30]. The nasal cavity has been described as a potent scavenger for ozone among other air pollutants [29, 31]. In the RTLF other antioxidants such as glutathione, ascorbic acid and alpha-tocopherol exist and they have all been described with lower levels of antioxidants from the airway in diseases such as asthma and chronic obstructive pulmonary disease (COPD) which might indicate that these patient groups are at risk of, or affected by, increased oxidative stress [32-34]. Studies in both animals and humans have shown that lower levels of antioxidants increase the oxidative stress seen in subjects with asthma [33, 35, 36].

The RTLF contains several proteins of which albumin is probably the most well known but transferrin, bilirubin, immunoglobulins (A, G, M), alpha-2-macroglobulins, lysozyme, alpha-1-antitrypsin (AAT1) and Clara cells are also some of the components of the RTLF [28, 37-41]. Albumin is synthesised in the liver and is a major substance transporter in blood, has anti-oxidative qualities and plays a significant role in the distribution of fluid between bodily compartments [28, 42]. It reaches the RTLF by passive plasma transudation and the concentration in RTLF is not fully known but is substantially lower than in plasma [42, 43].

Surfactant is crucial for lung function and is an important component of RTLF. It is secreted by type II alveolar cells and reduces the surface tension within the alveoli. It was first discovered in 1929 by Kurt von Neergaard and has thereafter been studied extensively and has been a lifesaving discovery predominantly for neonatal infants [5, 44-46]. 90% of surfactant is of lipid form and 10% of protein form [47-49].

Blood flow through the lungs

The primary function of the pulmonary circulation is to participate in the gas exchange of oxygen and carbon dioxide in the surface connecting the alveoli and the blood. The lungs, like no other organ in the body, receive the whole of the cardiac output and the lungs' physiological capability to handle this large and changeable volume is due to its low resistance and high capacity [5, 50, 51].

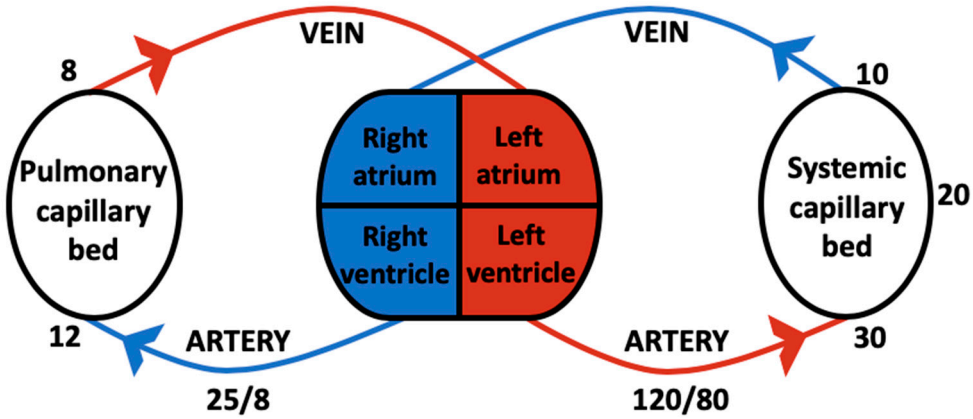

Figure 2 Overview of blood flow and pressures in the lung and systemic circulation
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The deoxygenated blood volume passing through the heart is pumped from the right ventricle through the pulmonary valve into the pulmonary trunk that divides into the right and left pulmonary artery. They follow the route of the bronchial tree before entering the lung and when they reach the level of the alveoli they comprise a compact net of capillaries. The pulmonary veins, containing the oxygenated blood volume, will follow the course of the pulmonary arteries up to bronchial level and from here on each pulmonary vein takes an individual route back to the left atrium of the heart [5, 50, 51].

The pulmonary blood flow and circulation is more or less a passive system that copes on a daily basis with alternating blood volumes depending on sleep or activity, everything from 3-30 l/min. The pulmonary circulation is a system of low resistance and high capacity. Its resistance is primarily in the micro vessels and capillaries as compared to the arterioles in the systemic circulation. Its high capacity is due to expansion of open capillaries and recruitment of unopened capillaries and the distensibility of larger vessels. [14, 52, 53].

The process of breathing

To generate air volume into the lungs a pressure gradient is required. This process follows the simple rules of physics and is described by Boyle's law, which states that volume is inversely proportional to pressure, so when the pressure is reduced the volume increases [5, 54, 55].

In spontaneous breathing the inspiratory muscles (diaphragm, external intercostal muscles and accessory muscles such as scalene, sternomastoid muscles and muscles of the neck and head) work to expand the lungs and create an inspiratory

airflow by causing a sub-atmospheric pressure in the alveoli. This sub-atmospheric pressure, more commonly known as transpulmonary pressure, is the alveolar pressure minus the intrapleural pressure [5, 54-57].

Expiration is different from inspiration since it is predominantly a passive process and the effect of Boyle's law is reversed. Expiration starts when the alveolar pressure is equal to the atmospheric pressure and the inspiratory muscles start to relax and thereby start to reduce the volume in the lung and the intrathoracic cavity. Boyle's law states that a reduction in volume will lead to an increased pressure. The lungs and the chest wall both have a natural tendency to return to their unstretched position at the end of expiration, this is called elastic recoil. The inward forces for elastic recoil depend on recoil of the lung's connective tissues, elastin fibres and collagen, and the forces of surface tension from alveolar fluid. The chest walls' inward forces are predominantly due to muscle tone [5, 54-57].

The volume left in the lung at the end of normal expiration is called the functional residual capacity (FRC) and at this point the inward forces and the outward forces are at equilibrium and the transpulmonary pressure is approximately 5 cm H_2O and intrapleural pressure is -5 cm H_2O. FRC is the body's oxygen reservoir [5, 54-56].

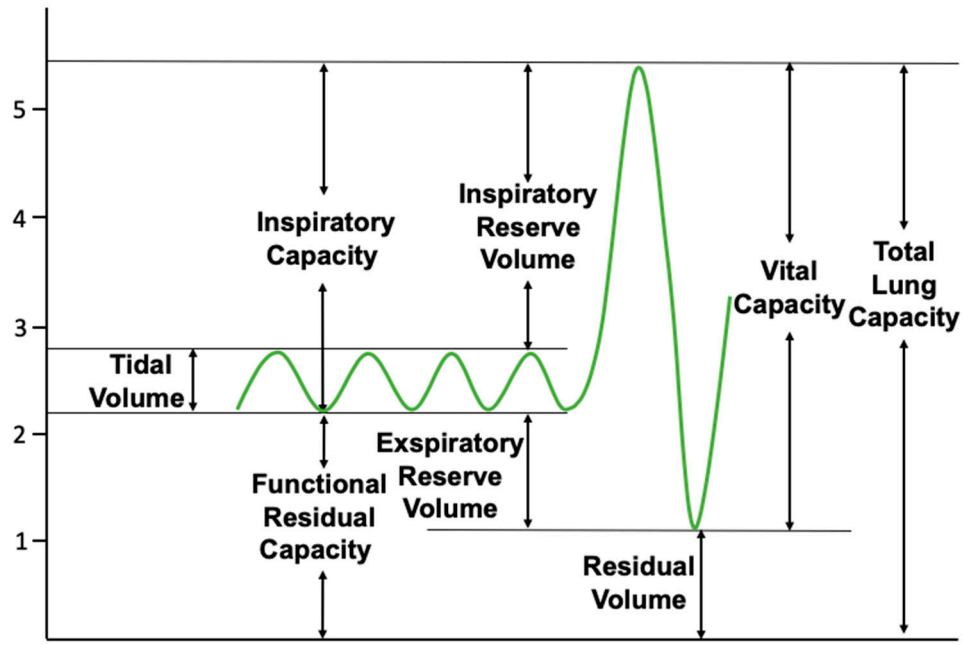

Figure 3 Lung volumes
The figure displays different lung volumes during tidal breathing and during maximum inspiration and expiration.

When there is no respiratory effort the lung will have an air volume equal to the FRC and to initiate inspiration the inspiratory muscles must overcome both airway resistance and compliance of the lung and chest wall. Airway resistance is equal to a pressure gradient (alveolar pressure minus atmospheric pressure) divided by airflow, as an analogy of Ohm's law of resistance. Airway compliance is defined as volume change per unit pressure change. Airway resistance mainly occurs in the bronchial part of the lung; in obstructive diseases, such as asthma, airway resistance is increased. Airway compliance depends on the lung and chest wall's elasticity and restrictive diseases such as pulmonary fibrosis worsen compliance [5, 54, 55].

Mechanical ventilation

For those who are in need of mechanical ventilation it is always life-supporting and life-sustaining and for some it is life-saving, but at the risk of lung injury [58]. The main purpose of mechanical ventilation is to facilitate gas exchange of oxygen and carbon dioxide and relieve the work of breathing.

In the 16th century Andreas Vesalius, an anatomy professor in Padua, Italy, described the first endotracheal intubation and assisted ventilation in animals [59, 60]. The first wider use of assisted ventilation came centuries later with the so-called iron lung which was developed in 1929 by Drinker and Shaw and it functions by negative pressure ventilation. The patient is confined to a box (i.e. iron lung) and by using sub-atmospheric pressure inside the iron lung it facilitates or replaces the work of breathing. It was a great step forward but caused grave difficulties in physically nursing the patient [61, 62]. Mechanical ventilation as of today, i.e. positive pressure ventilation, was triggered by the polio epidemic in Copenhagen in the beginning of 1950 which at its most had 50 patients a day admitted to hospital and a mortality rate above 80%. The anaesthetist Bjørn Aage Ibsen concluded that the terminal symptoms of polio, such as raised plasma CO_2, hypertension and severe sweating was not due to renal failure, which was the common belief at the time, but it was the result of respiratory failure. Ibsen recommended tracheostomy and assisted ventilation with positive pressure ventilation [63]. In corporation with the chief physician Alexander Lassen at Blegdam hospital, where they both worked, patients were ventilated with positive pressure ventilation and due to the lack of ventilators the majority were hand ventilated. The mortality dropped with a staggering efficacy, from 87% down to close to 40% This was just not the start of mechanical ventilation as we know it today, but also the beginning of the intensive care unit since the polio epidemic and the huge numbers of hand ventilated patients needed centralising for logistic reasons. [64, 65].

Respiratory failure is the main indication for mechanical ventilation [66]. Patients receive mechanical ventilation for other clinical conditions such as: altered conscious level (intoxication, trauma, all form of general anaesthesia), extended recovery after surgery, protection of the airway and exhaustion due to increased work of breathing that will eventually lead to respiratory failure.

Breathing during mechanical ventilation

When breathing spontaneously the respiratory muscles overcome the elastic recoil along with the resistance in the airways and air is drawn into the lungs because of altered pressure gradient as explained earlier. Expiration is predominantly passive [66].

For simplicity one can start by looking at breathing both spontaneously and with mechanical ventilation as work (W) and W's relationship with volume (V) and pressure (P). The work of respiratory muscles (W_m) = elastic work (W_e) plus resistive work (W_r), which gives:

$$W_m = W_e + W_r$$

If V is constant the work of breathing can be an equation of P:

$$P_m = P_e + P_r$$

If these physical properties are applied to positive-pressure ventilation and P_{vent} is the ventilator pressure applied to the airway, the equation is as follows:

$$P_{vent} + P_m = P_e + P_r \text{ and without muscle work } P_{vent} = P_e + P_r$$

Since breathing is more complex than just pressure, the above equation can be extended, and the equation of motion can further explain the mechanics of breathing. The equation of motion is as follows:

$$P_{vent} = (E_{aw} * V_f) + (R_{aw} * V_f) + P_0$$

E_{aw}; elastance in the airway, which is the inverse of compliance (E_{aw} = 1/compliance), V_f; gas flow rate, R_{aw}; resistance in the airway, P_0; alveolar pressure at the beginning of inspiration, which can be either atmospheric pressure or any pressure above, i.e. positive end-expiratory pressure (PEEP) along with so-called intrinsic PEEP. Intrinsic PEEP is the pressure within the lung at the end of expiration which is higher than atmospheric pressure or a pre-set PEEP, and occurs predominantly in patients with airway obstruction such as asthma [60, 66].

Modes and settings

Ventilators of today have several modes that can be tailored to the patient's condition and needs. A patient with an obstructive lung illness will not benefit from the same ventilation as someone with restrictive lung illness or severe head injury.

First the use of positive end-expiratory pressure (PEEP) will be addressed, since it is of great importance in all forms of mechanical ventilation. PEEP is an increase in the end-expiratory pressure by using a resistance on the outflow of the respiratory circuit. The main function of PEEP is to keep the distal airways open, reduce the risk of collapse, increase FRC and reduce intrapleural shunt (blood flow through the lung that is not ventilated and, therefore, this blood will not be oxygenated) [66-69]. The use of moderate PEEP has been shown to be beneficial in all mechanical ventilation settings, but the optimal level of PEEP still requires further studies [70-75].

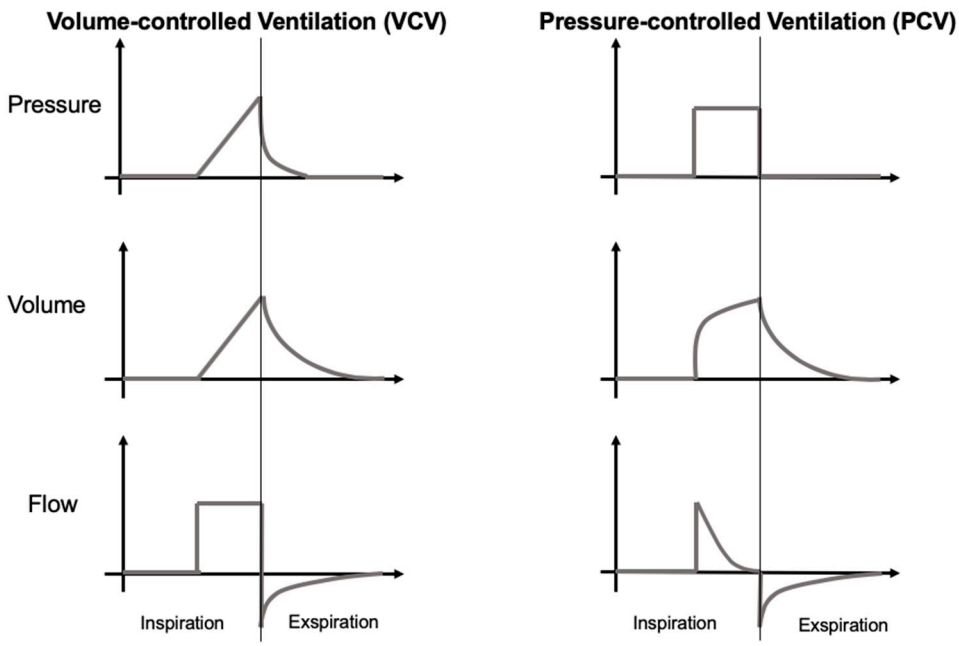

Figure 4 Volume-controlled ventilation (VCV) and pressure-controlled ventilation (PCV)
The Figure shows pressure, volume and flow curves during one breath for the two different modes used in Papers I-III. Copyright Ellen Broberg

Volume-controlled ventilation (VCV) is a ventilation mode with a target volume delivered by a constant flow and volume increase in a linear fashion until the desired tidal volume is reached, as seen in Figure 4. When using a pre-set tidal volume, the peak pressure is also dependent on airway resistance and lung compliance [66, 71, 76].

Pressure-controlled ventilation (PCV) is in contrast a ventilation mode with pressure as the target; each breath has the same pre-set inspiratory pressure delivered by a decelerating flow and volume increase until the pressure target is reached, as seen in Figure 4. When using a pre-set inspiratory pressure the tidal volume is also dependent on airway resistance and lung compliance [66, 71, 76].

Mechanical ventilation setting is predominantly up to tidal volume, inspiratory pressure and respiratory rate. Tidal volumes have been studied extensively and since a large clinical multicentre study, published in 2000 demonstrating the benefits of lower tidal volumes, i.e. 6-8 ml/kg in patients with acute lung injury (acute respiratory distress syndrome [ARDS]), lower tidal volumes have been the way forward [75, 77-79]. The use of protective ventilation by using lower tidal volumes has also been proven beneficial in patients without lung injury in an operating theatre or intensive care setting along with the use of moderate PEEP [72, 74, 80-85]. The next step in protective ventilation after reducing the tidal volumes has been to further individualise tidal volumes in relation to each patient's compliance instead of using total body weight as a guide [86, 87]. Using protective ventilation has become the norm after years of studies and evidence of its benefits for patients are seen not just in intensive care but also in the operating theatre. Postoperative lung complications could possibly be reduced by using protective ventilation in the operating theatre [72, 80, 82, 88]. Respiratory rate is predominantly used to reduce hypercapnia.

The aim of recruitment manoeuvre (RM) is to improve oxygenation by mainly increasing tidal volume and/or PEEP but this could be so at the cost of lung damage especially in the most vulnerable group of patients, such as those with ARDS [89-91]. The optimal RM during mechanical ventilation in terms of how, when and in whom does not have a conclusive answer and is still up for discussion [92, 93].

During lung surgery one-lung ventilation (OLV) and double-lung ventilation (DLV) are commonly used [70]. During OLV one or both lungs can be ventilated depending on need, usually a double-lumen tube (DLT) is used which has two separate lumen, one for each lung or a endotracheal tube and a bronchial blocker is used by inflating a balloon which blocks off a bronchi. There are several other indications for OLV such as: thoracic surgery, haemorrhages in one lung, pulmonary lavage and bronchopleural fistulae, split lung ventilation and to isolate an infected lung [94].

Lung injury during mechanical ventilation

Lung injury during mechanical ventilation is referred to as ventilator-induced lung injury (VILI). Several aspects of the mechanical forces such as pressure and volume can act on the lung tissue and cause VILI.

The triggers for developing VILI can be due to a single factor but more often a complex interaction of several factors occurs where volutrauma and atelectrauma are the most prominent. Volutrauma is alveolar overdistension either caused by high volumes and/or high pressures and atelectrauma is deformation of the epithelial wall by repeated re-opening of a previously closed lung [95-98].

Generally after surgery, no matter what its indication is, there are several complications that can affect the lung and cause tissue injury. The European Society of Anaesthesiology and European Society of Intensive Care Medicine has made a united statement about lung complications after surgery. Due to surgery and, more so, general anaesthesia, the lungs can be affected by: respiratory failure, pulmonary infection and aspiration, leading to pneumonitis, pleural effusion, pneumothorax, bronchospasm and atelectasis [99, 100].

Studies have shown that PCV might be preferable to VCV to reduce the risk of VILI, but the studies are not fully conclusive [77, 101-103]. It has been shown that using protective ventilation may reduce the release of cytokines. The release of systemic cytokines has previously been shown to have great impact on multiorgan failure and death [83, 85, 104-106].

It is important to limit the damage that mechanical ventilation can cause on the lung tissue either with or without prior surgery. Knowledge of the impact that mechanical forces have on the lung is not yet fully known.

Lung cancer

Lung cancer is the number one leading cause of death from cancer in the world and one-sixth of all cancer deaths are due to lung cancer [107, 108].

Non-small-cell lung cancer (NSCLC) accounts for about 85% of all cases of lung cancer and 15% of all cases of lung cancer are small-cell lung cancer. In men about 90% and in women about 80% have a history of smoking [107, 108]. Histologically NSCLC is divided into three major groups: adenocarcinoma, squamous-cell carcinoma and large-cell carcinoma [107, 109].

Symptoms of lung cancer are predominantly pulmonary, such as cough, dyspnoea, haemoptysis and pneumonia. Chest pain is a late symptom which suggests metastatic spread outside the lung parenchyma. Suspected tumours are usually found by computer tomography (CT) but since correct diagnosis is of the essence, a biopsy of the suspected tumour is essential, either by bronchoscopy, percutaneous fine-needle aspiration or by surgery such as mediastinoscopy [108, 110].

Staging of lung cancer is used to direct the physician towards the most appropriate treatment. It is divided into tumour-node-metastasis (TNM) and staging involving taking information gleaned from chest radiography, CT, magnetic resonance imaging (MRI) and positron-emission tomography (PET) along with histological findings [108, 110, 111].

Treatment of lung cancer depends on the stage, histology and the patient's general health. In general, lung cancer in an early stage is treated with surgery. The treatment options are tailored for each patient and may involve chemotherapy, radiotherapy, surgery and direct molecular target treatments [110, 112].

Lung transplantation

LTx is the final treatment option for patient with end-stage pulmonary disease.

The first LTx was performed in Jackson, Mississippi, USA in 1963 and the patient lived for 18 days and died due to kidney failure and malnourishment [113]. LTx continued to be performed, but it was not until the 1980s that a more successful outcome was seen. In 1981 the first heart and lung transplantation was performed in a patient with pulmonary hypertension (PH), in 1983 a patient with pulmonary fibrosis (PF) had a successful single-LTx and in 1986 the first successful double-LTx was carried out in a patient with emphysema [114-116]. The first lung transplantation in Sweden was in Lund in January 1990.

Over the years improvement in treatment has occurred in several fields, such as operative techniques, lung selection and preservation along with better poatoperative care and immunosuppressive medicines. As with all organ donation, the lack of a donor organ is by far the predominant limiting factor.

Several pulmonary diagnoses are considered for LTx. The most common diagnoses are: chronic obstructive pulmonary disease (COPD) and PF followed by cystic fibrosis (CF); other diagnoses are emphysema due to alpha-1 antitrypsine deficiency (AAT1), sarcoidosis, PH and bronchiectasis from causes other than CF [117-119].

Patients with advanced lung disease with the following criteria can be considered for LTx: >50% risk of death due to lung disease within 2 years, >80% likelihood of surviving at least 90 days after LTx and >80% likelihood of 5-year post-transplant survival from a general medical perspective provided there is adequate graft function [118, 120].

The main reason for LTx recipients' low median survival of a little more than 5 years is primary graft dysfunction (PGD) and chronic lung allograft dysfunction (CLAD) [121-124]. PGD develops within the first 72 hours after the transplant procedure and is a syndrome of acute lung injury similar to ARDS and is the major cause of death within the first 30 days [125, 126]. The major long-term cause of death is CLAD and it rarely develops within the first year after LTx but the rate increases rapidly with a cumulative incidence of 40% to 80% within 5 years after LTx surgery [123, 127-129]. CLAD has a large pathological spectrum but the two major syndromes are bronchiolitis obliterans syndrome (BOS) and restrictive allograft syndrome (RAS) [130, 131].

Table 1 Primary graft dysfunction
Abbreviations: PaO_2/FiO_2 ratio, ratio of arterial oxygen pressure to inspired oxygen concentration; PGD, primary graft dysfunction

PGD Grade	Pulmonary Edema on Chest Radiography	PaO_2/FiO_2 Ratio, mm Hg
Grade 0	Absent	> 300
Grade 1	Present	> 300
Grade 2	Present	200-300
Grade 3	Present	< 200

The initial clinical diagnosis of PGD is characterised by decreasing the ratio of arterial oxygen pressure to inspired oxygen concentration (PaO_2/FiO_2) and pulmonary infiltration on chest radiography, as seen in Table 1. It has an incidence of around 30% in its severest form and is associated with an increase in both short- and long-term mortality [132-136]. Postoperatively and for a few days after lung

transplant surgery, mechanical ventilation is used in the majority of patients and the duration of it may play a role in the onset of PGD [137-139]. An international survey demonstrated that in postoperative care after lung transplantation surgery different modes of mechanical ventilation were employed and PCV was used in 37% of patients, VCV in 35% of patients, and for 28% a mix of both PCV and VCV was applied [140].

BOS leads to advanced loss of pulmonary function and it is obliteration of the bronchioles by fibromyxoid granulation tissue; histologically it is found from biopsies [141, 142]. It is possible to miss BOS while taking biopsies since it often has an irregular spread, so a more clinical approach was developed in the 1990s by the International Society for Heart and Lung Transplantation (ISHLT) [130, 141, 143]. To diagnose BOS clinically a permanent 20% drop in the forced expiratory volume in one second (FEV_1) is required without any other ongoing pathologies [143]. The trigger of BOS is not fully clear, both immune activation such as donor-specific antigen for anti-human leukocyte antigen (HLA) and viral and bacterial infections may play a role [144-148]. RAS is a syndrome of a persistent decline in total vital capacity and with a decline in FEV_1 of 20% along with signs of pulmonary fibrosis as detected by high-resolution computed tomography (HRCT) [130, 149, 150].

A lung transplantation patient has a lower survival rate than any other organ transplantation. Between January 1990 and June 2014 LTx patients had a median survival of 5.8 years, with 89% survival rates at 3 months, 80% at 1-year, 65% at 3 years, 54% at 5 years and 32% at 10 years [151].

Donation after circulatory death

To address the global issue of the lack of donor organs for lung transplantation including the growing demand for re-transplantation over the year, donation after circulatory death (DCD) has been explored. DCD was previously both named donation after cardiac death and non-beating organ donation and DCD refers to organ retrieval for transplantation from patients with confirmed death due to circulatory criteria [152]. Circulatory criteria are defined as the absence of pupillary response to light, absence of corneal reflex, absence of any motor response to supra-orbital pressure and loss of capacity to breathe [153]. There are two forms of DCD: controlled and uncontrolled. Controlled DCD occurs in patients with anticipated cardiac arrest or cardiac arrest in a declared brain-dead donor and uncontrolled is in a patient who arrived dead to the hospital, was unsuccessfully resuscitated or suffered unexpected cardiac arrest in intensive care [152]. Due to organ shortage and mortality among patients waiting for lung transplantation DCD has a strong potential to fill a need for donor organs [154-157].

Ex vivo lung perfusion

Ex vivo lung perfusion (EVLP) has become a valuable method of evaluating initially rejected donor lungs due to poor blood gases [158-160]. For DCD, EVLP has also become a useful and fulfilling method to evaluate lung function in this group of possible donors [156, 161-163]. EVLP was developed by Professor Stig Steen at Lund University and at the turn of the millennium, the first six patients in the world successfully received lungs which had been reconditioned with the EVLP technique. A few years later the Toronto lung group successfully transplanted 20 patients using EVLP [156, 158, 159, 164]. In EVLP the lungs are placed in a sterile plastic hard-shell dome and perfusion is through a circuit with an oxygenator/deoxygenator, a leukocyte filter, a pump along with temperature and blood flow probes. The lungs are treated with a solution containing nutrients, protein and oxygen. During the process of EVLP which takes 3-4 hours in total, evaluation is done by monitoring blood gases and physiological parameters [156, 165]. EVLP will be described further in the Method section.

Particles in exhaled air

The non-invasive technique used for measuring and collecting biochemical particles in exhaled air in Papers I-IV will be described. There are several methods for collecting particles in exhaled air from breath and in this thesis the Particles in exhaled air (PExA) device has been used and customised to function in conjunction with mechanical ventilation.

Method of collection

The PExA technique involves taking samples of particles by impaction and count particles by means of an optical particle counter (OPC). Particles within the diameter range of 0,41-4,55 µm were counted by the PExA device in paper I-IV.

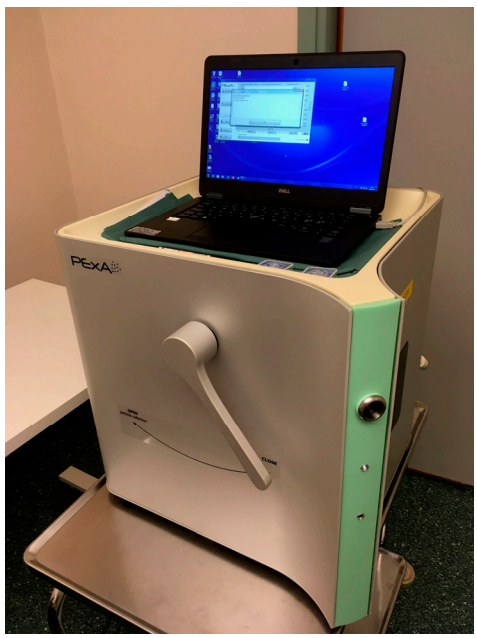

Figure 5 PExA device
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Impaction catagorises particles according to their mass by using the particles inertia, i.e. an objects tendency to stay in motion or stay at rest and its inherent strong resistance to change direction or velocity. The potential sampled biomarkers that hit the impaction plate can be analysed by biochemical methods. When both the mass of biomarkers and total mass of particles hitting the impaction plate are known individually the concentration of biomarkers among the particles

can be calculated. By knowing these two concentrations, the percentage of biomarkers in exhaled air can be established.

In this thesis the PExA device has been rebuilt to be used in conjunction with intubated subjects on mechanical ventilation. The rebuilt respiratory circuit has been tested for feasibility in pre-clinical settings before being implemented clinically. A non-rebreathing valve is used to stop rebreathing and reversed particle flow and it is possible to use a respiratory tube between the subject and the PExA device. In Figure 6A the respiratory circuit is displayed and in Figure 6B the non-rebreathing valve is shown. The technique has been tested in conjunction with mechanically ventilated subjects in relation to signs of rebreathing, altered pressure levels and any signs of altered haemodynamics before being implemented clinically.

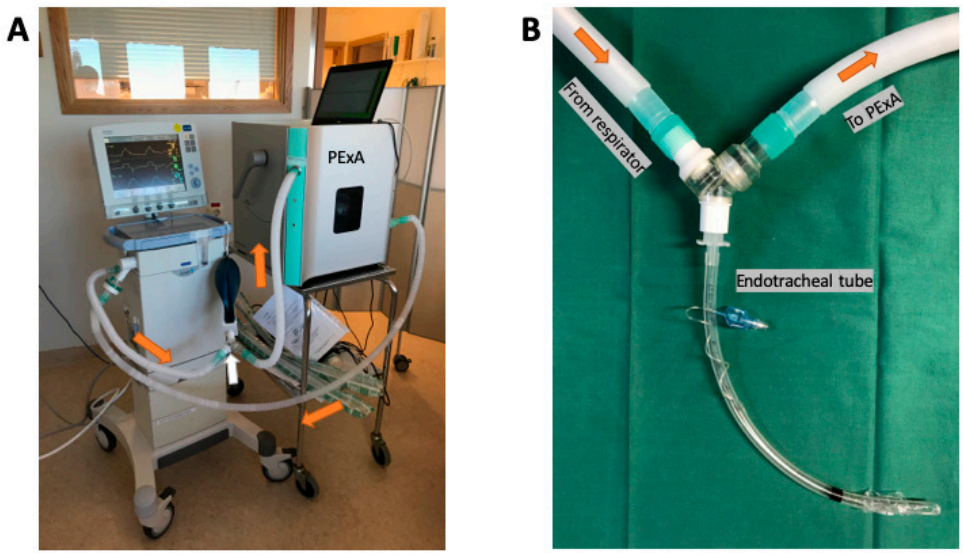

Figure 6 The ventilator in conjunction with the PExA device.
(A) Displays the respiratory circuit with the black balloon representing the patient and the optical particle counter PExA connected to the outflow tract of the circuit. The orange arrows show the direction of air flow and the white arrow shows the non-rebreathing valve. **(B)** Displays the non-rebreathing valve connection, with the orange arrows showing the direction of air flow. Copyright Ellen Broberg

Studied biomarkers

In these initial exploring studies of analysing biomarkers from exhaled air from subjects under mechanical ventilation we aimed in this first step to try to analyse the major parts of the RTLF that we hypothetically would be able to find. In the studies presented in this thesis we analysed albumin and components of surfactant. For surfactant we analysed phospholipids that constitute about 90% of surfactant and a specific protein, surfactant A. Surfactant is synthesised in type II alveoli cells and stored in so-called lamellar bodies, which are intracellular organelles [5, 45, 46, 49, 166]. The phospholipids constitute predominantly palmitoyl-oleoyl-phosphocholine (POPC or PC) and di-palmitoyl-phosphatidyl-choline (DPPC); we analysed these two substances, but smaller quantities of other phospholipids also exist (phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, sphingomyelin and phosphatidylserine) [167-170].

Albumin

In plasma 50% of all protein is albumin. It has several vital functions in plasma but also in the rest of the body. It is a small protein comprised of 585 amino acids and has a tertiary structure and has a three homologous domain, named I-III. These domains individually contain two sub-units, named A and B which in turn are made up of four to six alpha-helices. The sub-units are flexible loops which facilitate the ability of albumin to bind to multiple different substances [42, 168].

Albumin is synthesised primarily in the polysomes in hepatocytes in the liver and the majority of the production is released into plasma and a small portion is stored in the liver. Albumin circulates from the plasma into other bodily departments (interstitial fluid, RTLF) and back to plasma via the lymphatic system [42, 168, 171].

Albumin has several functions within the body. It works as a ligand-binding structure and binds to fatty acids, metal ions, metabolites and a numerous amount of drugs. It has antioxidant properties by reducing the possibility of oxidation of several oxidizable substances, both endogenous and exogenous [28, 42, 168]. Its most well-known function is its major role in the colloid oncotic pressure. Negative charges surrounding albumin attract sodium and thereby water and albumin plays a major role in the distribution of fluid between the compartments of the body [42, 168]. Albumin is a small protein and permeability over the epithelial wall into the RTLF is related to size with increased permeability for small proteins compared to larger proteins [43, 172, 173].

Albumin and thereby fluid leakage into the respiratory system, primarily the alveoli, could alter the function of surfactant and thereby a possible increase in inflammation seen in for example patients with asthma or COPD patients [172, 174, 175]. In asthma patients after they were induced with a pulmonary allergy reaction, lower levels of albumin in particles in exhaled air were found, which

could be a sign of increased inflammation in the small airways [18]. In patients with COPD, lower levels of oxidised albumin have been seen in comparison to patients with normal lung function [176].

POPC and DPPC

The phospholipids are by far the major constituent of the surfactant structure. Free fatty acids, cholesterol and triglycerides are the predominant lipids within surfactant, but the major fraction is in the form of phospholipids, where POPC along with DPPC are in abundance but a few other phospholipids also exist [166, 169].

The phospholipids are produced by type II alveoli cells [46, 48]. DPPC is either produced in about 40-70% by desaturation of POPC or by direct synthesis by the type II alveoli cells and plays an essential role in the important function of reducing surface tension [47, 166, 169, 177]. POPC is rate limited in its synthesis by enzyme control (choline-phosphate cytidylyltransferase) and deletion or mutation in this enzyme results in neonatal respiratory distress syndrome and death within minutes of birth [46, 178]. Studies in ARDS have shown decreased levels of phospholipids including POPC, which has an effect on the surface tension properties of surfactant with reduced function [179-181]. A decreased level of phospholipids has been seen in conditions such as asthma which could be a sign of enhanced inflammation [182]. The same occurs in smokers who have shown a decrease in the phospholipid content of RTLF which might result in decreased function of surface tension [183].

Surfactant A

Surfactant-associated proteins (A, B, C and D) constitute 10% of surfactant and are synthesised by type II alveoli cells [5, 45, 49, 184]. Surfactant A (SP-A) is an glycoprotein and within in a group called collectins. In humans SP-A consist of two different polypeptide, SP-A1 and SP-A2 and they have individual gene transcription, each chain has one SP-A1 and two SP-A2 chains. SP-A has six polypeptide chains which makes a total of 18 chains or 248 amino acids connected by disulphide bonds. The binding capacity of SP-A is Ca^{2+} dependent [45, 184, 185]. SP-A is predominantly found in the alveoli and to a lesser extent in the proximal part of the airways [186]. SP-A has both anti-inflammatory and pro-inflammatory properties depending on the need to reduce inflammation and tissue damage or to limit the effects of infection [49, 187, 188]. SP-A has shown lower levels in both COPD patients and patients developing BOS after LTx which can indicate a relationship between SP-A levels and COPD and BOS [17, 19]. Studies in animals have also shown that SP-A may play a role in modulating allergic sensitisation by inhibiting allergen-specific IgE and as a result of which, it has been suggested that SP-A is involved in the initiation of allergic reactions [189, 190].

Aims

Paper I

To explore the feasibility and hypothesis that non-invasive particle flow measurements, using a customised PExA device, during mechanical ventilation in pre-clinical settings may show differences depending on the ventilation mode and tidal volumes *in vivo*, post-mortem and during EVLP. To explore if collected particles can be detected and analysed *in vivo* and under EVLP.

Paper II

To explore the hypothesis that non-invasive particle flow measured, using a customised PExA device, during mechanical ventilation may show differences depending on ventilation mode, the duration of ventilation and the influence of recruitment manoeuvres in a pre-clinical setting. To evaluate the feasibility of using a non-invasive technique to repeatedly measure particle flow in exhaled air during mechanical ventilation.

Paper III

To explore the hypothesis whether it was safe to use a customised PExA device in conjunction with mechanical ventilation in clinical intensive care settings in lung transplant recipients. To explore whether patients who developed PGD had different particle flow patterns from the airways compared to patients who did not develop PGD in two different ventilation modes. To explore if it is possible to detect differences in particle flow pattern during daily recruitment manoeuvres in two different ventilation modes.

Paper IV

To evaluate the feasibility and safety of using a customised PExA device during surgery on intubated mechanically ventilated patients. To explore the hypothesis that differences in particle flow and composition of exhaled particles can be detected between mechanically ventilated patients and normal breathing patients.

Paper V

To explore in lung transplant recipients if the end-points of BOS and death are influenced by SLTx or DLTx or by the initial diagnosis for receiving a lung transplantation.

Material and methods

Ex vivo lung perfusion

EVLP uses an artificial perfusion circuit after the lungs have been taken out of the donor. Worldwide there are to date predominantly three protocols: Lund, Toronto and the Organ care system. The difference between the protocols lies in modifications of several aspects such as pressure within the pulmonary artery and left atrium, ventilatory settings and target flow [191]. In this thesis emphasis will be on the Lund protocol since this one was used in Paper I.

Before harvesting the lungs are perfused with Perfadex (XVIVO Perfusion AB, Gothenburg, Sweden) a preserving solution and are cooled for a minimum of 1 hour. The EVLP perfusion circuit consists of a sterile plastic hard-shell dome, a centrifugal pump, a membrane oxygenator/deoxygenator with a heat exchanger, a leukocyte filter and temperature and flow probes. The pulmonary artery is cannulated with a 28-French cannula and the perfusion pressure is measured with a small catheter. The perfusion solution is delivered through the cannula and flows directly out into the hard-shell dome through the left atrium which is open. Priming of the system is with a predefined buffered priming solution with electrolytes, albumin and dextran. The purpose of the solution is to maintain pressure and flow by keeping a sufficient colloid osmotic pressure to avoid pulmonary oedema. Mixed with the priming solution is also O-compatible erythrocyte concentrate (pre-treated with irradiation, leukocyte filtered and washed) [159].

Reconditioning can be divided into two steps: in the first step the lungs are not ventilated and during the second step the lungs are ventilated. Evaluation is performed after the two reconditioning steps when the lung is fully ventilated and perfused [159].

In the main pulmonary artery, the tip of a 28-French cannula is placed, and a small catheter is placed in the pulmonary artery to measure perfusion pressure. The perfusion solution flows directly out from the left atrium into the hard-shell dome so the pressure within the left atrium is zero. Before any perfusion through the lungs is started the pulmonary artery cannula is connected to the extracorporeal circuit and any air is removed. The EVLP circuit is displayed is Figure 7 [159].

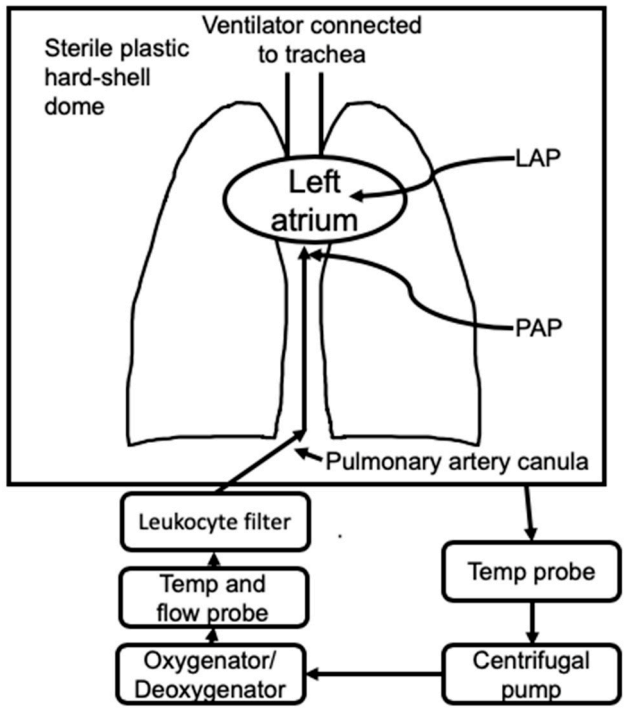

Figure 7 EVLP circuit
EVLP circuit. LAP; Left atrial pressure, PAP; Pulmonary artery pressure. Copyright Ellen Broberg

During step 1 initially air is removed from the pulmonary artery and perfusate blood is oxygenated and perfused through the circuit with perfusion pressure ≤ 20 mmHg and a starting temperature of 25ºC and a flow of 50-100 ml/h. The temperature is gradually increased in the perfusate to 32ºC and the left atrium pressure is kept at 0 mmHg [159].

During step 2 the lung ventilation is commenced and the perfusate blood is oxygenated. When the temperature is stable at 32ºC, ventilation is carefully started with low minute ventilation (1 l/min) to reduce the risk of lung membrane injury due to ventilation of cold lungs. Every increase by 1ºC in the perfusate leaving the lung ventilation is increased by 1 l/min and at 37ºC ventilation of 100 ml/kg/min is used. FiO_2 is kept at 100%, and pCO_2 is kept between 4.5-5 kPa by altering the gases in the oxygenator. The PEEP target is 5 cm H_2O at 37ºC and it is gradually increased during step 2. Perfusion flow is stepwise increased until 5-6 l/min is reached while keeping the pulmonary pressure ≤ 20 mmHg [159].

When the lungs are fully ventilated and perfused the lung evaluation phase can begin. Deoxygenation of the perfusate blood is performed with a mixture of 93% nitrogen and 7% CO_2. The purpose for deoxygenation is to have similar venous

blood in the pulmonary artery as under normal conditions for more accurate evaluation. PEEP is kept at 5 cm H_2O and, in order to reduce atelectasis, PEEP is shortly increased to 8 cm H_2O and pCO_2 is kept between 4.5-5 kPa. Venous and arterial blood gases are routinely measured after altering FiO_2 levels for 5 minutes at 100%, 50% and 21%. In the last part of the evaluation a test of global lung atelectasis is performed by disconnecting the endotracheal tube from the ventilator. If the lungs are adequate for transplant, they should pass the test by being totally deflated and have a pCO_2 of 50 kPa at FiO_2 100%. For the Lund model the time for reconditioning steps 1 and 2 along with evaluation takes between 1-2 hours After these three steps and the lungs are accepted for transplantation they will be preserved and cooled until the transplantation procedure. [159].

The PExA device is connected to the ventilator with the rebreathing valve in place and a respiratory tube is placed between the ELVP and the PExA device, as seen in Figure 8.

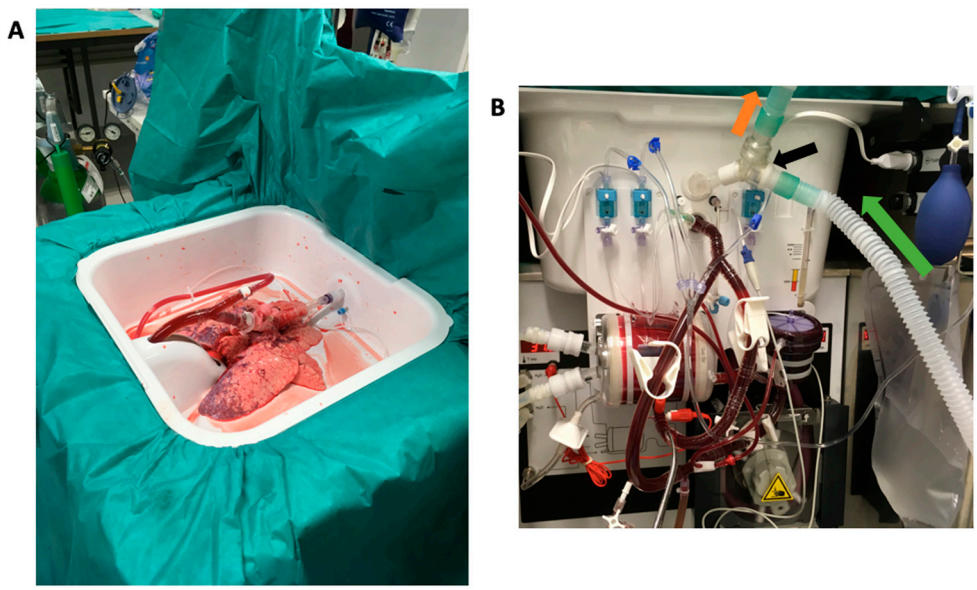

Figure 8 EVLP in conjunction with the PExA device.
(A) Lungs connected to the EVLP machine. **(B)** The green arrow display the direction of airflow from the ventilator into the lungs and the orange arrow display the direction of airflow from the lungs towards the PExA device and the black arrow display the non-rebreathing valve. Copyright Ellen Broberg

Collection of particles in exhaled air

PEx

Particles measured by the PExA device are defined as particles exhaled (PEx) and collected particles are referred to as PEx sample. The size range for PEx used in Papers I-IV is 0.41–4.55 μm.

Method of collection

The PExA device measures and collects particles in exhaled air and measures particles by the OPC and samples particles by impaction. Here, these two functions of the PExA device will be described and in Figure 9 a schematic illustration of components of the PExA device is shown.

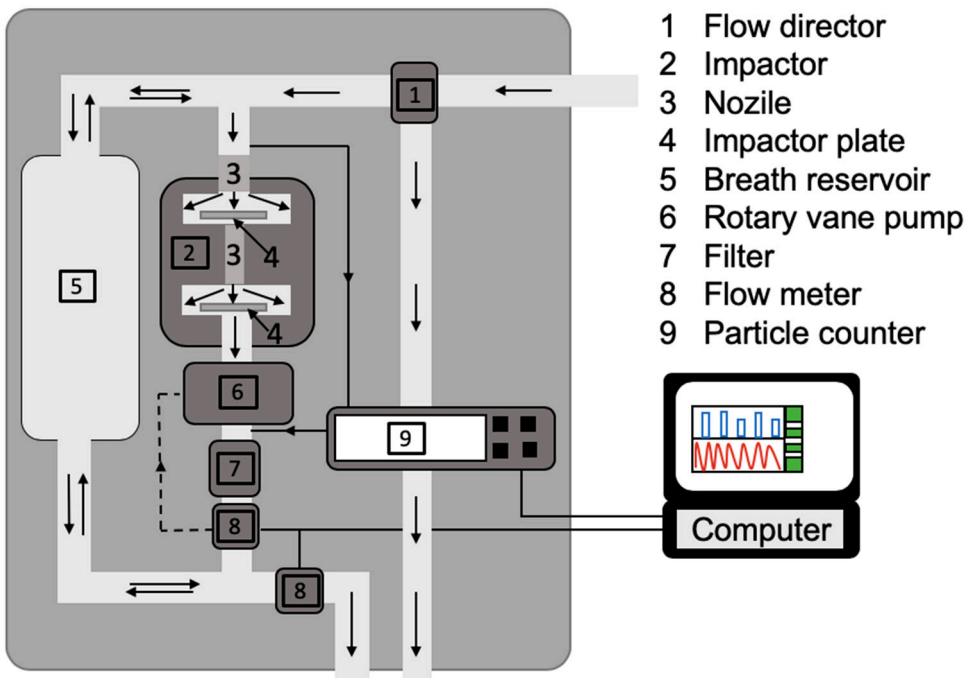

Figure 9 Schematic illustration of components of PExA 2.0
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Optical particle counter

Measurement of particle size and particle number concentration in exhaled air is done by the OPC. Particle size and particle number concentration can be defined based on light scattering. When particles are illuminated by a laser beam light scattering occurs. This OPC determines size dependent on the light scattering in eight size intervals, also called bins, and also total count of particles is possible. The size bins are 0.41–0.55, 0.55–0.70, 0.70–0.92, 0.92–1.14, 1.14–1.44, 0.44–2.36, 2.36–2.98 and 2.98–4.55 µm. The bins' mean diameter are: particle size 1, 0.48 µm; particle size 2, 0.59 µm; particle size 3, 0.75 µm; particle size 4, 0.98 µm; particle size 5, 1.22 µm; particle size 6, 1.67 µm; particle size 7, 2.52 µm; and particle size 8, 3.37 µm. [192].

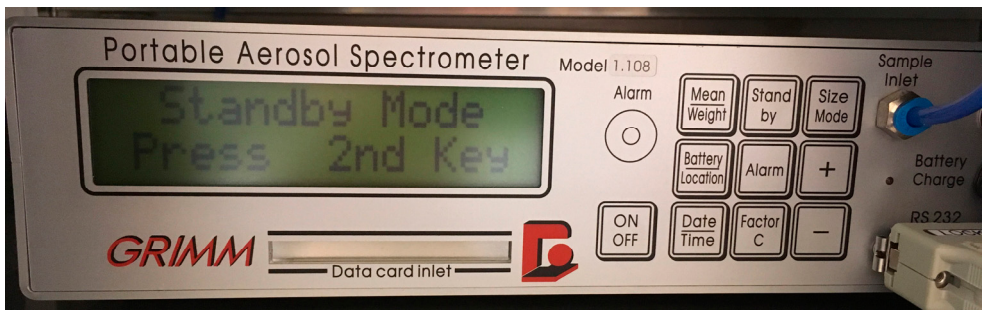

Figure 10 Optical particle counter
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Sampling by impactor

Exhaled flow and volume is measured by a flow meter and particles are drawn with the help of a vacuum pump through a two-level impactor for sampling particles. In the impactor the exhaled air is forced to a 90 degree bend hereby the particles that are too heavy to follow the stream will impact on a sample substrate. Assuming the particles as spheres, particle mass for those particles that hit the impaction plate can be calculated from measurements made with the OPC. In the PExA device in paper I-IV particles were collected with diameters between 0.5-7,0 µm. Cut-off sizes are mathematically calculated and in bin 1 it is calculated that 36% of particles are sampled since the cut-off is 0.5 µm, for the other bins 100% of particles are sampled. The sampling of particles have been validated in previous studies [17, 192].

Figure 11 Impactor
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Filter/substrate and chemical analysis

Filter/substrate used for analysis in Paper I and Paper IV was LCR Membrane Filter (Hydrophobic Polytetrafluoroethylene, Millipore FHLC02500). Analysis was performed through the Department of Public Health and Community Medicine, Sahlgrenska University Hospital/Sahlgrenska Academy.

For quantification of DPPC and POPC, selected reaction monitoring was used. Samples were extracted using 160 µL of a solvent consisting of methanol, chloroform, and 40 mM ammonium acetate after the addition of Internal Standard (IS). From the extracted sample, 20 µL was injected using a flow gradient injection method with an isocratic mobile phase of methanol, chloroform, and 40 mM ammonium acetate. Standard samples were prepared in cryotubes containing PEx sampling membranes spiked with IS before adding a known amount of DPPC and POPC (Avanti lipids Alabaster, AL, USA). From standards, a linear regression model was constructed and used for calculating amounts in unknown samples. Measured DPPC and POPC concentrations in exhaled particles were expressed as weight percent, wt%.

For albumin and SP-A an extraction buffer with 10 mM phosphate buffered saline containing 1% bovine serum albumin w/v and 0.05% TWEEN-20 (Thermo Scientific, Rockford, IL, USA) was prepared. 140 µl of extraction buffer was pipetted onto the PEx filter followed by 60 min of shaking at 400 rpm and 37°C

(eppendorf Thermomixer comfort, Eppendorf AG, Hamburg, Germany). The sample volume was split: 40 µl for SP-A and albumin respectively, the rest as a backup. The extracted PEx samples were stored frozen at -20°C until analysis. SP-A was analysed the day after and albumin the following day. The samples were quantified by enzyme-linked immunosorbent assay (ELISA). The manufacturer's instructions for SP-A ELISA (BioVendor, Brno, Czech Republic) and a human albumin ELISA kit from Immunology Consultants Laboratory, Inc. (Portland, OR, USA) were used with minor modifications. Prior to analysis, 80 µl assay dilution buffer was added to the samples. To match the sample matrix, extraction buffer: assay dilution buffer, in a 1:2 ratio, was prepared and the final buffer composition was the same for PEx samples, controls and standard samples. Incubation time for SP-A was 2 h, at 37°C with vibration 300 rpm and for albumin 1 h, room temperature and at 300 rpm. Finally, a reaction time of 9 minutes was allowed for each assay.

Mass spectrometry

In Papers I and IV triple quadrupole mass spectrometry was used to analyse DPPC and POPC, previously these have been analysed with this technique in patients breathing spontaneously [193, 194]. A description of the functionality of a mass spectrometry is described below.

Mass spectrometry measures ions according to their mass-to-charge ratio (m/z). In general, a mass spectrometry device consists of an ion source, a mass analyser (or spectrometry) and a detector. Basically quadrupole mass spectrometry works by having four parallel metal rods and it uses the stability of the trajectories in an oscillating electric field to separate ions according to their m/z ratios down the parallel rods. A radio frequency voltage with a direct current offset voltage is applied between one pair of rods and the other. Ions that will reach the detector have a certain m/z ratio at a known ratio of voltages; other ions have unstable trajectories and will collide with the rods and not reach the detector [195]. A triple quadrupole mass spectrometer is more selective, has a higher accuracy and reproducibility than a simpler ones such as single quadrupole mass spectrometer. In a triple quadrupole mass spectrometer, it uses the first and third quadrupoles as a mass filter and the second through interaction of a colliding gas causing fragmentation of the substance that is to be analysed [196].

Enzyme-linked immunosorbent assay

In Paper IV an ELISA was used to analyse albumin and SP-A. A description of the functionality of the ELISA technique is described below.

ELISA is based on the immunological fact that an antibody binds an antigen. It is a useful technique because it has the possibility of detecting small quantities of antigens, such as proteins, peptides and hormones or by detecting the amount of antibodies bound to the antigen [197]. As the name describes it uses enzyme-linked antigens and antibodies to detect the analysed material and, in our studies, albumin and SP-A [18, 198]. Most commonly ELISA is performed in antigen-coated polystyrene plates and the antibody is allowed to bind to the antigen. Subsequently this antibody is then detected by another enzyme-linked antibody. The presence of the antibody bound to the antigen is indicated by the use of a chromogenic substrate specific for the enzyme used and it renders a colour change or fluorescence for the specific enzyme-linked antibody [197].

Subjects and study design

Paper I

Six Swedish landrace pigs were anaesthetised according to local protocol and given mechanical ventilation; PExA measurements were performed according to the timeline in Figure 12.

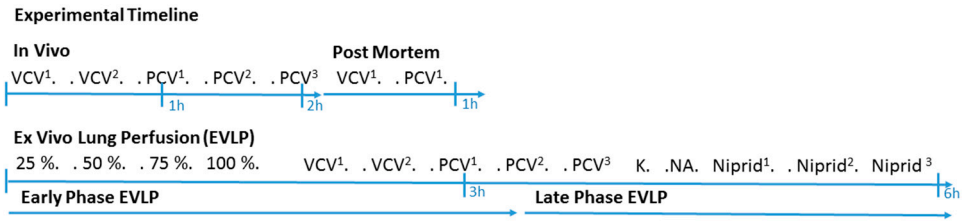

Figure 12 Experimental timeline in Paper I
VCV; volume-controlled ventilation, PCV; pressure-controlled ventilation, K; potassium, NA; norepinephrine

Five different ventilation modes were used, all had an inspiratory:expiratory (I:E) ratio of 1:2 for all subjects:

1. VCV with small tidal volumes 6-8 ml/kg, breathing frequency at 16, and PEEP at 2 (VCV_1).

2. VCV with large tidal volumes 10-12 ml/kg, breathing frequency at 16, and PEEP at 2 (VCV_2).

3. PCV with small tidal volumes 6-8 ml/kg, breathing frequency at 16, and PEEP at 2 (PCV_1).

4. PCV with large tidal volumes 10-12 ml/kg, breathing frequency at 16, and PEEP at 2 (PCV_2).

5. PCV with large tidal volumes 10-12 ml/kg, breathing frequency at 16, and PEEP at 10 (PCV_3).

Each ventilation mode was analysed during a time-span of 15 minutes with periods of restoration in between the settings. The total accumulated mass (ng) and the total accumulated number of particles (count) from the airways was measured continuously by the PExA device. The PExA device was connected to the respiratory circuit on the expiratory line and its purpose was to keep the circuit closed; to counteract rebreathing a non-rebreathing valve was used.

After *in vivo* PExA measurements were performed according to the timeline seen in Figure 12, a median sternotomy was carried out and ventricular fibrillation was induced electrically. When circulatory arrest was confirmed, the tracheal tube was left open to the air and a temporary closure of the sternotomy and the skin were performed, and no manipulation of the animal was done. After 1 hour after declaration of death ventilation was re-started. PExA measurements were performed post-mortem according to the timeline in Figure 12. After 30 min after re-start of ventilation the sternotomy was reopened and cannulation with a 28 French cannula of the pulmonary artery was performed and the left atrium and inferior vena cava were opened. The lungs were perfused in an antegrade manner with Perfadex (XVIVO Perfusion AB, Gothenburg, Sweden) and the cannula was removed from the pulmonary artery. The lungs were harvested *en bloc* in a standard fashion and weighted. During the retrieval, a segment (~ 8 cm) of the descending aorta was also excised. The lungs were immersed in cold Perfadex with the aortic segment and put in cold storage at 8ºC for 1 hour. EVLP was then performed as described in detail in the method section. PExA measurements were performed during different pulmonary flow rates, during the five different ventilation modes and at the end during which different drugs were administered, as seen in Figure 12. During drug administration VCV_1 was used as the ventilation mode.

Chemical analysis of the collected particles and quantification for DPPC and POPC were performed using mass spectrometry, as described previously in the method section.

Blood gases were analysed in between the different ventilation modes *in vivo* and *ex vivo* during the entire experiments and haemodynamic parameters were monitored continuously.

Paper II

Six Swedish landrace pigs were anaesthetised according to local protocol and given mechanical ventilation; PExA measurements were performed on a daily basis for 3 days.

All pigs had a laparotomy to mimic a clinical situation in an intensive care unit with mechanical ventilation on subjects with no previous lung injury, and all animals had a secondary temporary closure with negative pressure wound therapy (NPWT) in a standard manner. A 30 cm long midline incision was performed on each pig and the V.A.C.® Abdominal Dressing (KCI®, Inc., San Antonio, TX, USA) was used.

PExA measurements were performed each day during two different ventilation modes: VCV and PCV, with 1 hour measurements in one mode followed by an equilibrium period of 30 minutes and then 1 hour measurement in the other mode. Three animals had VCV before PCV and three had PCV before VCV. Ventilator settings were set to a tidal volume of 6 ml/kg, positive end-expiratory pressure of 5 cm H_2O and end-inspiratory pressures < 25 cm H_2O with an I:E of 1:2. These settings remained unchanged during the study period. Mechanical ventilator settings were named as followed: VCV day 1 (VCV_1), VCV day 2 (VCV_2), VCV day 3 (VCV_3), PCV day 1 (PCV_1), PCV day 2 (PCV_2) and PCV day 3 (PCV_3).

Twice daily a recruitment manoeuvre was performed in VCV and PCV for 60 s at PEEP 10, four breaths/min, and I:E ratio of 2:1. Measurements were carried out 3 min before the RM, during the RM and 3 min after the RM.

The total accumulated number of particles from the airways was measured continuously by the PExA device.

All animals had a central venous catheter, standard monitoring and haemodynamic parameters were recorded continuously in a standard manner.

Paper III

From 2017 and 2018, 13 patients who underwent lung transplant were included in our study. One patient, who developed severe PGD stage 3, was excluded from further analysis due to an intervention with the administration of an inhaled drug.

All patients arrived in the ICU post-transplant with a 7.5 mm tracheal tube. The ventilator settings were made according to local guidelines: (tidal volume of 6 ml/kg, PEEP of 5 cm H_2O, end-inspiratory pressure of < 25 cm H_2O, and target CO_2 levels of 4.6-6 kPa. Inspiratory-to-expiratory ratios of 1:2 were used in all patients. The type of ventilator used for all patients was the Maquet SERVO-I (Getinge Group, Solna, Sweden). These settings remained unchanged during the study period. The PExA device was connected to the outflow tract of the respiratory circuit with the use of a non-rebreathing valve as seen in Figure 6A and B and measurements were performed daily until extubation. The total accumulated number of particles (count) was measured continuously by the PExA device.

Six patients received VCV before PVC and six received PCV before VCV. Each patient was monitored daily for 1 hour during VCV and 1 hour during PCV until extubation. Before the collection period began for the second ventilation mode, there was an equilibration period of 30 min with the second ventilation mode.

A recruitment manoeuvre was performed twice daily in the same fashion as in Paper II.

All patients had a central venous catheter and an arterial line. The blood gases and haemodynamic parameters were recorded continuously in a standard manner.

Paper IV

A total of 32 patients were included: 26 patients were included in the mechanical ventilation cohort and were subsequently divided into two cohorts: 17 patients with NSCLC (MV-NSCLC) and nine patients without NSCLC (MV-C). Another group of six patients with NSCLC who were not intubated was also included, they had normal breathing (NB). A flow chart is shown in Figure 13 and demographic data are shown in Table 2.

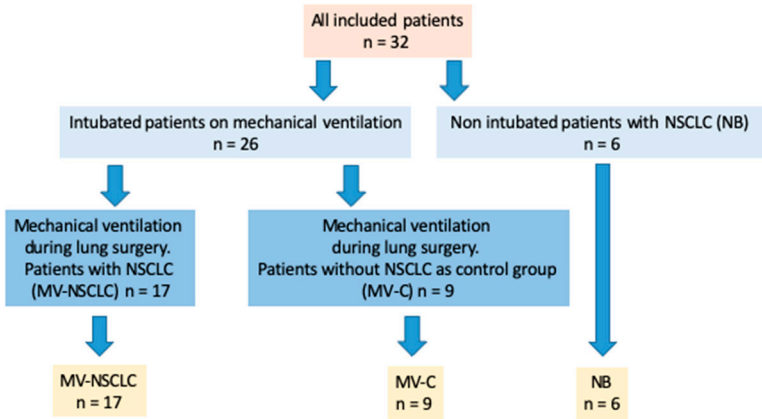

Figure 13 Flow chart in Paper IV

Mechanical ventilation during lung surgery divided into mechanical ventilation - non-small cell-lung cancer (MV-NSCLC) and mechanical ventilation-control (MV-C). The third group is none- intubated, normal breathing (NB) patients.

Table 2 Demographics for Paper IV

Demographics for the three different groups. Mechanical ventilation in non-small-cell lung cancer (MV-NSCLC). Mechanical ventilation in control (MV-C) and normal breathing patients (NB). Body mass index (BMI). Chronic obstructive pulmonary disease (COPD). Tidal volume (TV). Forced expiratory volume for 1 minute (FEV_1). Diffusing capacity of lung for carbon monoxide (DLCO).

	MV-NSCLC	***MV-C***	***NB***
Age	68 ± 2	60 ± 5	71 ± 3
Gender (Female)	9 (50%)	5 (56%)	2 (33%)
BMI (kg/m^2)	27 ± 1.5	27 ± 2.4	27 ± 2.4
Smoking	14 (78%)	3 (33%)	4 (67%)
COPD	2 (11%)	0	2 (33%)
TV (Litre)	3.42 ± 0.18	3.86 ± 0.25	3.20 ± 0.37
TV (%)	85 ± 5	84 ± 3	79 ± 8
FEV_1 (Litre)	2.45 ± 0.16	2.15 ± 0.18	2.18 ± 0.27
FEV_1 (%)	86 ± 5	74 ± 4	74 ± 8
DLCO (%)	84 ± 4	80 ± 6	72 ± 5

Anaesthesia during surgery was performed according to standard procedure with target-controlled infusion. Muscle relaxant was used and reverse of muscle relaxation effect was performed at the end of surgery. Oral intubation was performed using a double-lumen endotracheal tube and mechanical ventilation was performed using volume-controlled pressure-support. The ventilator settings were according to local guidelines: tidal volume of 6-8 ml/kg, minimum PEEP of 5 cmH_2O, end-inspiratory pressures < 25 cmH_2O, and target CO_2 levels of 4.6 to 6 kPa. Fluid loss was compensated for by continuous infusion with Ringer's acetate.

The device was connected to the outflow air of the mechanical respiratory circuit, as seen in Figure 14. During surgery, the PExA device measured the number of particles (count) and total accumulated mass (ng) of particles from the airways. The number of particles is described as particles per minute, i.e. named particle flow rate (PFR). Particles were collected onto a membrane for biochemical analysis and referred to as PEx.

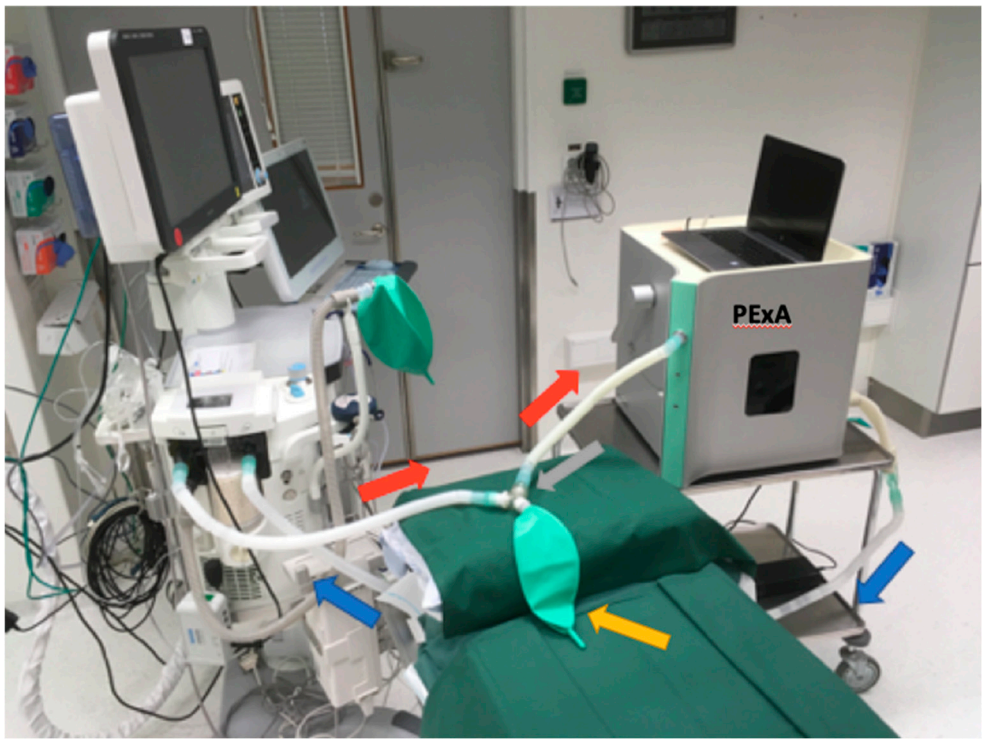

Figure 14 The ventilator in conjunction with the PExA device.
The figure displays the respiratory circuit. The yellow arrow shows the balloon representing the patient and the grey arrow the non-rebreathing valve. The red arrows shows the direction of air flow from the mechanical ventilator to the balloon representing the patient and further on to the PExA device. The blue arrow shows the direction of air flow from the PExA device back to the ventilator.

During lung surgery, the affected lung was disconnected from the mechanical ventilation to optimise the removal of affected lung parenchyma. Measurements were first performed during OLV. Then, for nine patients in total from both MV-NCSLC and MV-C, an additional collection period of 5 min occurred at the end of surgery as the patients transitioned from OLV to DLV.

PEx were collected in the NB group using a validated PExA method with a breathing manoeuvre for patients breathing spontaneously [17-19, 198, 199].

Mass spectrometry was used to quantify the DPPC and POPC. Albumin and SP-A were analysed by ELISA. Both techniques and preparations are described previously in the Method section.

Out of the 26 mechanically ventilated patients only 15 patients reached a collected mass of PEx of ≥ 50 ng. Only membranes with a collected mass of ≥ 50 ng were analysed. Due to the low collected mass the rest of the membranes were not analysed. In all NB patients the PEx collection stopped when a total of 100 ng was reached whereas the surgery time was a limiting factor among the mechanically ventilated patients. Membranes containing a total collected mass above 100 ng were divided into two and were sent for both phospholipid and protein analysis. The remaining membranes were randomised into either phospholipid or protein analysis. Among the mechanically ventilated patients 13 samples were analysed for DPPC and POPC. All of the samples reached the detection level. Eleven samples from the mechanically ventilated patients were analysed for albumin where nine of the samples reached the detection level. Furthermore 11 samples were analysed for SP-A of which eight samples reached the detection levels. Among NB patients six samples were analysed for DPPC and POPC and six samples were analysed for albumin and SP-A. All samples from the NB patients reached detection levels.

All intubated and mechanically ventilated patients had an arterial line. The blood gases and haemodynamic parameters were recorded continuously in a standard way.

Paper V

Between January 1990 and June 2014, 278 patients underwent lung transplantation at Skåne University Hospital, Lund University.

DLTx was performed in 172 patients, SLTx in 97 patients, and heart and lung transplantation (HLTx) in nine patients. Of these, 129 were male and 149 were female. Re-lung transplantation (Re-LTx) was performed in 15 patients. Among the Re-LTx recipients, of whom five were female and 10 were male, seven recipients had a DLTx and eight had an SLTx. In the present study the median age was 51 years with a range of 12–71 years. The major indications were defined as COPD (n = 67), CF (n = 54), AAT1 (n = 55), PF (n = 38), PH (n = 39), and a group deemed as 'other' (n = 25), which included bronchiectasis, sarcoidosis, bronchioalveolar cancer, silicosis, and graft-vs-host disease (GVHD). HLTx was performed via median sternotomy in seven patients and via a clamshell (bilateral anterolateral thoracotomy with a transverse sternotomy) incision in the 4th intercostal space in two patients. SLTx and DLTx were performed in standard fashion. SLTx was performed through a posterolateral thoracotomy in 86 patients, via clamshell in seven patients, and via median sternotomy in four patients. DLTx was performed through a clamshell-incision in 146 patients, via median sternotomy in 17 patients, and via anterolateral thoracotomy in nine patients. Preoperative respiratory support was used in 13 operations (CF four, PF five, Re-LTx three and PH one). Preoperative extracorporeal membrane oxygenation (ECMO) (extracorporeal membrane oxygenation) support was used in 12 operations (CF six, PF three, ARDS one, PH one and Re-LTx one). Intraoperative circulatory support in the form of extracorporeal circulation (ECC) was used in 105 cases, and intraoperative ECMO was used in 73 cases. Intraoperative circulatory support was not used in 115 cases. Recipient characteristics are shown in Table 3.

Table 3
Recipient characteristics in Paper V

Baseline Characteristics of the 278 Patients	
Variable	Median (Range) or No. (%)
Recipient age, year	51 (12–71)
Recipient primary disease	
Cystic fibrosis	54
Pulmonary fibrosis	38
Chronic obstructive pulmonary disease (COPD)	67
α1-antitrypsin deficiency (AAT1)	55
Pulmonary hypertension (PH)	39
Other	25
Transplant type	
DLTx	172
SLTx	97
HLTx	9
Re-LTx	15
DLTx	7
SLTx	8
Gender	
Female	149
Male	129
Transplant year	
1990–2002	126
2003–2014	167
Preoperative ventilator	13
Preoperative ECMO	12
Perioperative ECC	105
Perioperative ECMO	73
Postoperative ECMO	30

According to ISHLT guidelines, BOS is defined as a more than 20% decline in FEV_1 from the highest obtained baseline, [130, 143] and is characterised by perivascular and interstitial mononuclear cell infiltrates or chronic rejection characterised by dense scarring and eosinophilic infiltrates. If rapid deterioration of pulmonary function was detected as a sign of CLAD, bronchoscopies with transbronchial biopsy were conducted and anti-rejection treatment was initiated with pulsed methylprednisolone often together with tacrolimus or everolimus as a replacement for cyclosporine. In this study, patients with BOS grade ≥ 2 were included and chosen for analysis.

Statistical analysis

Paper I

All statistical analyses were performed, using Graph Pad Prism Software Version 7 (La Jolla, CA, USA). Significance was defined as $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), and $p > 0.05$ (not significant, n.s.).

The results are presented for the different parameters divided into the different groups. Statistically significant difference between the groups was tested with repeated measurement ANOVA. Descriptive statistics, in the form of the number of experimental animals, mean, and the standard error of the mean (SEM) for the different parameters were analysed. DPPC and PC results are shown as mean, and standard deviation (SD).

Paper II

All statistical analyses were performed, using GraphPad Prism Software, Version 7 (La Jolla, CA, USA). Significance was defined as $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), and $p > 0.05$ (not significant, n.s.).

Descriptive statistics, number of patients, mean and SEM for the different parameters were analysed. The results are presented for the different parameters divided into the different groups. Statistically significant differences between the groups were tested using a paired *t*-test.

Paper III

All statistical analyses were performed using GraphPad Prism Software Version 7 (La Jolla, CA, USA). Significance was defined as $p < 0.05$.

Descriptive statistics, including number of patients and mean and SEM, for the different parameters were analysed, with results are presented for the two different groups. A paired *t*-test was used to compare the two groups.

Paper IV

All statistical analysis was performed using Graf Pad Prism Version 8 (La Jolla, CA, USA). Significance was defined as $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), and $p > 0.05$ (not significant, n.s.). A power calculation was performed based on biochemical analysis results in prior studies on normal breathing patients [18, 199-201].

Descriptive statistics, in the form of the number of patients, mean, and SEM for the different haemodynamic parameters were analysed. Results of the analysed particles in exhaled air are shown as median with confidence interval (5–95%). Statistically significant differences between the different groups were tested with

Mann-Whitney and differences within the groups were tested with the Wilcoxon signed-rank test.

Paper V

The statistical calculations were performed using SPSS Version 19.0. (IBM Corp, Armonk, NY). All statistical calculations were performed by Sidesoft AB, Malmö, Sweden. For all statistical analyses, a p-value of less than 0.05 was considered to be significant.

Primary stratification of the material was made into two sets of cohorts. The first cohort was based on the main indication for LTx, with the following indicator cohorts: COPD, AAT1, CF, PH, and PF. The second set divided the material based on type of LTx: DLTx or SLTx. The aim of this study was to analyse the occurrence of BOS (grade ≥ 2) after primary LTx. In this analysis, death acted as a competing risk event to BOS. In a competing-risks model, we analysed incidence of BOS grade ≥ 2 and death as two separate outcomes. Specifically, we estimated and compared the cumulative incidence functions for BOS grade ≥ 2 and death using Gray's test [202]. All calculations regarding competing risks were performed using R with the CMPRSK package (available at http://www.r-project.org).

Results

Paper I

Study Groups

Pre-operative partial pressure of oxygen in arterial blood (PaO_2) at a FiO_2 of 0.5 was 30.9 ± 0.7 kPa.

No anatomical anomalies, signs of infection, or malignancy were found in any of the animals at autopsy.

Ventilation *in vivo*

Accumulated particles (ng) – total accumulates mass

The accumulated particle mass from the airways was measured continuously by the PExA device during different ventilation modes. The accumulated particle masses were as follows: VCV_1 2.23 ± 0.79 ng, VCV_2 3.92 ± 1.04 ng, PCV_1 11.75 ± 3.76, PCV_2 19.23 ± 8.25, and during PCV_3 15.32 ± 7.62. Comparing the different groups, the accumulated particle mass in VCV_1 was significantly lower than VCV_2 (p = 0, 0186), and the accumulated particle mass was significantly higher in PCV_1 than in the VCV_1 (p = 0.0322). All other comparisons between the groups were found to not be statistically significant. The results are shown in Figure 15A.

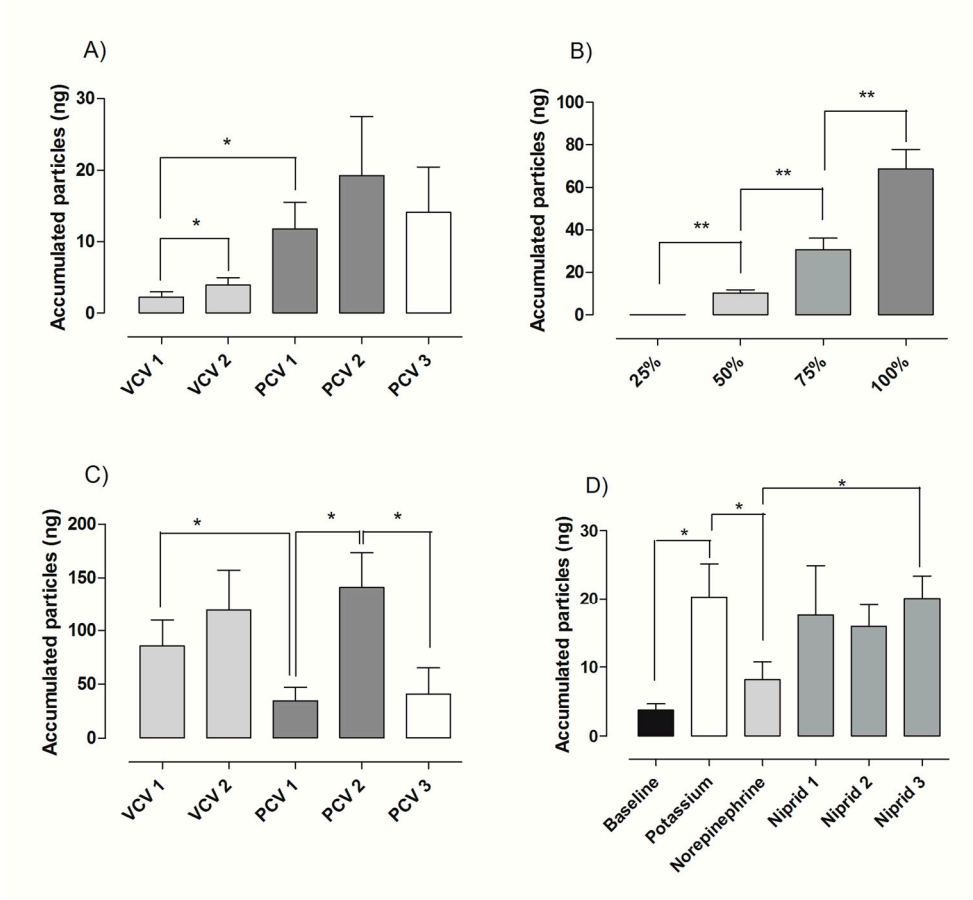

Figure 15 Total accumulated particle mass during the different phases in Paper I

Total accumulated particle mass (ng) measured by PExA **(A)** *In vivo*, **(B)** During different pulmonary flow i.e. percent of cardiac output in *ex vivo* lung perfusion (EVLP), **(C)** During different ventilation settings in EVLP, **(D)** During exposure to different drugs injected into the EVLP circuit. Volume-controlled ventilation (VCV) with small tidal volumes 6-8 ml/kg, breathing frequency at 16, and PEEP at 2 (VCV_1), VCV with large tidal volumes 10-12 ml/kg, breathing frequency at 16, and PEEP at 2 (VCV_2), Pressure-controlled ventilation (PCV) with small tidal volumes 6-8 ml/kg, breathing frequency at 16, and PEEP at 2 (PCV_1), PCV with large tidal volumes 10-12 ml/kg, breathing frequency at 16, and PEEP at 2 (PCV_2), PCV with large tidal volumes 10-12 ml/kg, breathing frequency at 16, and PEEP at 10 (PCV_3).

Accumulated particles (count)

The accumulated particle mass from the airways was then divided into eight different groups according to particle size where particle size 1 is the smallest and particle size 8 is the largest. The results are shown in Table 4.

Table 4
Shows total particle count for the different particle sizes from 1 to 8 during *in vivo* ventilation

	In vivo							
	VCV versus PVC			Small tidal volumes versus large tidal volumes		Low PEEP versus high PEEP		
	VCV[1]	PCV[1]	*p* value	VCV[2]	*p* value	PCV[2]	PCV[3]	*p* value
Particle 1	1650 ± 277	11,543 ± 3044	0.02*	6300 ± 1197	0.003**	18,428 ± 3044	15,387 ± 2350	n.s.
Particle 2	992 ± 208	7521 ± 2099	0.03*	4238 ± 793	0.002**	12,580 ± 1288	10,743 ± 1983	n.s.
Particle 3	1683 ± 286	10,455 ± 2481	0.06	4948 ± 918	0.009**	15,125 ± 2069	15,245 ± 1937	n.s.
Particle 4	1233 ± 159	7208 ± 651	0.01*	4130 ± 955	0.019*	6066 ± 1299	10,003 ± 2331	n.s.
Particle 5	138 ± 28	3025 ± 437	0.02*	1345 ± 390	0.029*	3890 ± 1944	3025 ± 1151	n.s.
Particle 6	350 ± 32	3766 ± 476	0.01*	1552 ± 370	0.028*	4245 ± 1461	3570 ± 1029	n.s.
Particle 7	175 ± 56	2018 ± 259	0.01*	1255 ± 451	0.046*	2137 ± 762	1723 ± 735	0.008*
Particle 8	92 ± 20	2293 ± 376	0.02*	1053 ± 485	n.s.	2133 ± 1059	1426 ± 687	n.s.

VCV1 volume-controlled ventilation with small tidal volumes and PEEP at 2, *VCV2* volume-controlled ventilation with large tidal volumes and PEEP at 2, *PCV1* pressure-controlled ventilation with small tidal volumes and PEEP at 2, *PCV2* pressure-controlled ventilation with large tidal volumes and PEEP at 2, *PCV3* pressure-controlled ventilation with big tidal volumes and PEEP at 10
Significance was defined as: $p < 0.01$ (**), $p < 0.05$ (*), and $p > 0.05$ (not significant, n.s.)

The same particle size distribution was seen in VCV and PCV during *in vivo* ventilation but with different total amount of particles, as seen in Figure 16.

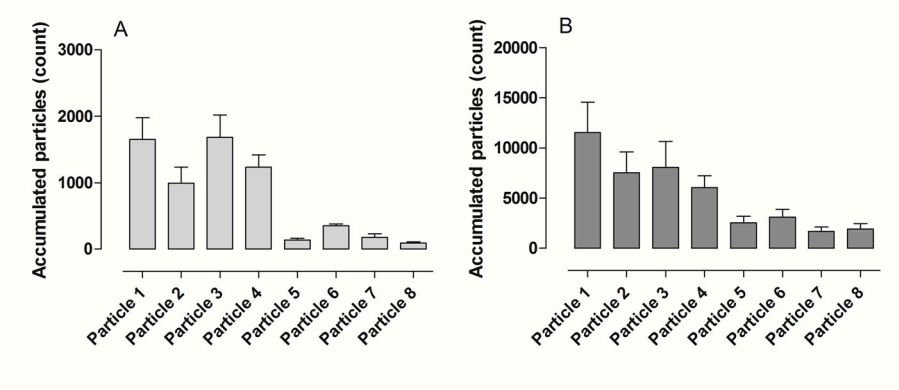

Figure 16 Distribution of different particle size during different ventilation modes *in vivo*.
(A): Volume-controlled ventilation (VCV) and **(B)**: Pressure-controlled ventilation (PCV).

Ventilation post-mortem

Accumulated particles (ng) – total accumulated mass

The accumulated particle mass from the airways was again measured continuously by the PExA instrument during different ventilation modes post-mortem. The number of particles was as follows: VCV_1 0.50 ± 0.22 ng, and at VCV_2 0.67 ± 0.21 ng (p = n.s.).

***Ex vivo* lung perfusion at different pulmonary flow**

Accumulated particles (ng) – total accumulated mass

The number of particles from the airways of *ex vivo* lungs was measured continuously by the PExA device. The total accumulated mass was measured during different pulmonary flows. The ventilation was kept at VCV with a small tidal volumes 6–8 l/kg, breathing frequency at 16, and PEEP at 2 (VCV_1). The total accumulated mass was as follows: 25% of the total pulmonary flow was 0 ± 0 ng, 50% 10.33 ± 1.53, 75% 30.67 ± 5.36, and at 100% 68.67 ± 9.24. Comparing the different groups, the total accumulated mass at 50% pulmonary flow was significantly higher than at 25% pulmonary flow (p = 0.0013), and the total accumulated mass at 75% pulmonary flow was significantly higher than at 50% pulmonary flow (p = 0.0089); furthermore the total accumulated mass at 100% pulmonary flow was significantly higher than at 75% pulmonary flow (p = 0. 0039) as seen in Figure 15B.

EVLP at different ventilation mode

Accumulated particles (ng) – total accumulated mass

The accumulated particle mass from the airways of *ex vivo* lungs was measured continuously by the PExA device. The accumulated particle mass was assessed during different ventilation modes. The number of particles was as follows: VCV_1 85.53 ± 24.22 ng, VCV_2 119.17 ± 38.03 ng, PCV1 34.67 ± 12.37 ng, PCV2 140.87 ± 32.54 ng, and at PCV_3 17.00 ± 5.83 ng. Comparing the different groups, the accumulated particle mass in VCV_1 was significantly higher than PCV_1 (p = 0.0371), the accumulated particle mass was significantly higher in PCV_2 than in the PCV_1 (p = 0.0127) and the accumulated particle mass was significantly higher in PCV_2 than in the PCV_3 (p = 0.0499). All other comparisons between the groups were found to be not statistically significant. The results are shown in Figure 15C.

Accumulated particles (count)

The accumulated particle mass from the airways was then divided into eight different groups according to particle size where particle 1 is the smallest and particle 8 is the biggest. The results are shown in Table 5.

Table 5
Shows total particle count for the different particle sizes from 1 to 8 during *ex vivo* ventilation.

	Ex vivo lung perfusion							
	VCV versus PVC			Small tidal volumes versus large tidal volumes		Low PEEP versus high PEEP		
	VCV[1]	PCV[1]	*p* value	VCV[2]	*p* value	PCV[2]	PCV[3]	*p* value
Particle 1	141,087 ± 36,170	51,135 ± 31,907	0.020*	224,313 ± 94,259	0.018*	177,117 ± 41,998	23,258 ± 5060	0.020*
Particle 2	150,750 ± 208	57,818 ± 33,823	0.022*	239,288 ± 95,435	0.026*	222,225 ± 56,596	23,913 ± 5575	0.025*
Particle 3	192,577 ± 46,145	76,725 ± 41,409	0.014*	316,550 ± 120,963	0.028*	308,437 ± 80,941	34,092 ± 8124	0.027*
Particle 4	106,700 ± 35,066	43,818 ± 21,247	0.021*	168,932 ± 63,215	0.028*	186,240 ± 49,551	19,900 ± 4730	0.029*
Particle 5	35,373 ± 12,136	14,056 ± 6778	0.020*	54,517 ± 21,202	0.034*	60,478 ± 19,729	8052 ± 2514	0.032*
Particle 6	23,542 ± 8709	7657 ± 3532	0.034*	33,600 ± 12,443	0.048*	35,550 ± 12,188	5486 ± 1543	0.038*
Particle 7	7070 ± 2883	2388 ± 1097	n.s.	10,107 ± 3723	n.s.	8497 ± 2997	2018 ± 538	0.536
Particle 8	3340 ± 1282	722 ± 222	n.s.	3757 ± 1354	n.s.	3000 ± 977	790 ± 262	0.045*

VCV1 volume-controlled ventilation with small tidal volumes and PEEP at 2, *VCV2* volume-controlled ventilation with large tidal volumes and PEEP at 2, *PCV1* pressure-controlled ventilation with small tidal volumes and PEEP at 2, *PCV2* pressure controlled ventilation with large tidal volumes and PEEP at 2, *PCV3* pressure-controlled ventilation with big tidal volumes and PEEP at 10
Significance was defined as: $p < 0.05$ (*), and $p > 0.05$ (not significant, n.s.)

The same particle size distribution was seen in VCV and PCV during EVLP ventilation but with different total amount of particles, as seen in Figure 17.

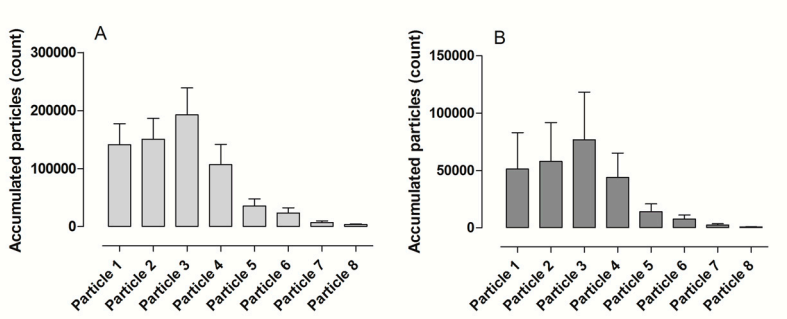

Figure 17 Distribution of particle sizes during different ventilation modes in *ex vivo* lung perfusion (EVLP). **(A)**: Volume-controlled ventilation (VCV), and **(B)**: Pressure-controlled ventilation (PCV).

EVLP – influence of different drugs

Accumulated particles (ng) – total accumulated mass

The accumulated particle mass from the airways was measured continuously by the PExA device during administration of different medications. The accumulated particle mass was measured during different ventilation modes. The amount of particles was as follows: baseline 3.80 ± 0.90 ng, potassium (K) 20.33 ± 4.85 ng, after norepinephrine (NA) 8.17 ± 2.60 ng, after Niprid 1 (50 µg) 17.61 ± 7.31 ng, after Niprid 2 (100 µg) 15.96 ± 3.16 ng and after Niprid 3 (150 µg) 20.08 ± 3.33 ng. The accumulated particle mass was significantly higher following administration of K as compared to baseline ($p = 0.0268$); the accumulated particle mass was significantly lower after administration of NA than after administration of K ($p = 0.0285$) and the accumulated particle mass was significantly higher after administration of 150 µg Niprid than after administration of NA ($p = 0.0349$). All other comparisons between the groups were found to be not statistically significant. The results are shown in Figure 15D.

Accumulated particles (count)

The accumulated particle mass from the airways was then divided into eight different groups according to particle size where particle 1 is the smallest and particle 8 the largest. The results are shown in Table 6.

Table 6

Shows total particle count for the different particle sizes from 1 to 8 during ex vivo lung perfusion ventilation during exposure to different drugs.

	Exposure to different drugs during ex vivo lung perfusion						
	Baseline	Potassium	*p* value	Norephineprine	*p* value	Niprid[3]	*p* value
Particle 1	3116 ± 501	34,651 ± 14,996	0.031*	26,477 ± 10,248	n.s.	51,112 ± 33,163	n.s.
Particle 2	3402 ± 699	43,478 ± 18,622	0.028*	30,545 ± 13,340	n.s.	70,152 ± 49,852	n.s.
Particle 3	5367 ± 1033	60,670 ± 24,601	0.047*	42,213 ± 16,477	n.s.	107,173 ± 74,085	n.s.
Particle 4	4649 ± 1140	37,785 ± 15,403	0.018*	25,788 ± 10,395	n.s.	71,795 ± 47,225	n.s.
Particle 5	1541 ± 502	13,298 ± 5487	n.s.	8900 ± 3438	n.s.	27,202 ± 17,516	n.s.
Particle 6	1112 ± 354	8500 ± 2967	n.s.	5643 ± 1982	n.s.	17,453 ± 11,027	n.s.
Particle 7	238 ± 87	2655 ± 895	0.048*	1757 ± 584	n.s.	5118 ± 3260	n.s.
Particle 8	221 ± 68	1182 ± 437	n.s.	908 ± 368	n.s.	2373 ± 1539	n.s.

Significance was defined as: $p < 0.05$ (*), and $p > 0.05$ (not significant, n.s.)

Differences in the total amount of particle count in the different settings were detected as well as similar particle size distribution in all the settings, as seen in Figure 18.

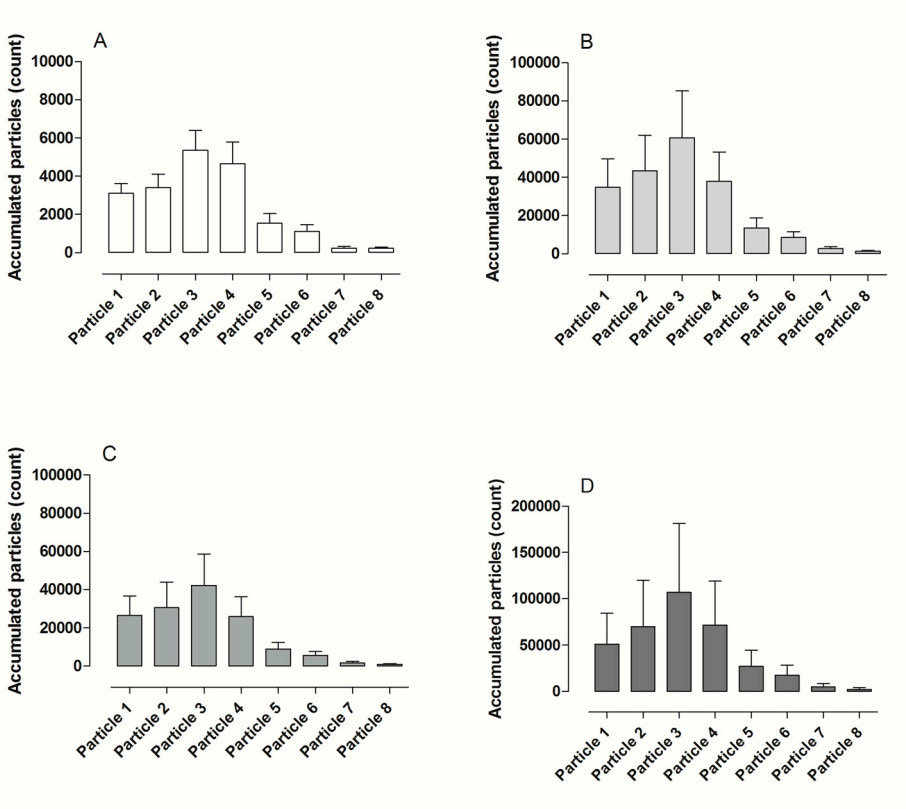

Figure 18
Different drugs were injected into the ex vivo lung perfusion (EVLP) circuit. Distribution of different particle size at **(A)** baseline, and after injection of **(B)** potassium (K), **(C)** norepinephrine (NA), and **(D)** Niprid. Volume-controlled ventilation (VCV) with small tidal volumes 6-8 ml/kg, breathing frequency at 16, and PEEP at 2 (VCV_1) was used during all settings.

Pulmonary gas function

The pulmonary gas function, blood gases, was analysed between every different mode: *in vivo*: baseline, VCV_1, VCV_2, PCV_1, PCV_2 and PCV_3, and during *ex vivo* lung perfusion: baseline, VCV_1, VCV_2, PCV_1, PCV_2 and PCV_3. The pulmonary gas function, blood gases, was also analysed between every different mode in *ex vivo* lung perfusion between exposure to different drugs: baseline, K, NA, Niprid 50µg, Niprid 100µg, and Niprid 150µg. All lungs had excellent blood gases during the whole experiments. No significant differences were found between the different settings.

Haemodynamic data during EVLP

Pulmonary artery flow (PAF) and mean pulmonary artery pressure (MPAP)

PAF i.e. cardiac output (CO) in the *ex vivo* model and MPAP was measured continuously. The PAF was not allowed to exceed 4.0 l/min, and the MPAP was not allowed to exceed 20 mmHg.

Pulmonary vascular resistance (PVR)

PVR (dyne x s/cm^5) was calculated using the formula where pulmonary capillary wedge pressure (PCWP) is equivalent to left atrial pressure (LAP) and CO is equivalent to PAF in the EVLP method:

$$PVR = 80 * ((MPAP - PCWP)/CO))$$

Haemodynamics and blood gases *in vivo* and *ex vivo* are shown in Table 7.

Table 7
Shows the haemodynamics and blood gases during *in vivo* early and late phase, and during *ex vivo* lung perfusion (EVLP) in the start and end of early and late phase.

	In vivo Early phase	In vivo Late phase	EVLP Early phase start	EVLP Early phase end	EVLP Late phase start	EVLP Late phase end
Heart rate (bmp)	91 ± 21	92 ± 18				
Systolic blood pressure (mmHg)	89 ± 18	88 ± 19				
Diastolic blood pressure (mmHg)	65 ± 16	67 ± 15				
Temperature (℃)	36.8 ± 0.9	36.8 ± 0.9	37 ± 0.1	37 ± 0.1	37 ± 0.1	37 ± 0.1
SpO_2 (%)	99 ± 1.0	99 ± 1.0	99 ± 1	99 ± 1	99 ± 1	99 ± 1
pH	7.5 ± 0.2	7.5 ± 0.2	7.5 ± 0.1	7.5 ± 0.2	7.5 ± 0.1	7.5 ± 0.2
P_{o2} (mmHg)	30.9 ± 0.7	31.2 ± 0.9	64.8 ± 6.0	65.6 ± 5.0	67.7 ± 1.8	63.3 ± 2.3
P_{co2} (mmHg)	3.87 ± 0.23	3.86 ± 0.24	2.9 ± 0.3	3.0 ± 0.4	3.2 ± 0.6	3.1 ± 0.9
FiO_2	0.5	0.5	1.0	1.0	1.0	1.0
Flowrate (L/min), i.e., cardiac output (CO)			3.5 ± 0.1	3.5 ± 0.1	3.4 ± 0.5	2.5 ± 0.5
Pulmonary pressure (mmHg)			12 ± 0.5	11 ± 0.4	15 ± 1.5	19 ± 1.0
Left atrium pressure (mmHg)			0 ± 0	0 ± 0	0 ± 0	0 ± 0
Pulmonary vascular resistance (PVR)			273 ± 12	251 ± 2	361 ± 23	623 ± 36

DPPC and PC concentration in PEx

DPPC and PC concentrations were measured in exhaled particles of four animals and were expressed as weight percent, wt%. The amount DPPC in percent (wt%) of total mass of the PEx sample is shown in Figure 19A. Note the significant increase in DPPC in EVLP late phase as compared to *in vivo* (p = 0.04). No differences were observed in the PC in wt% of total PEx sample, as shown in Figure 19B.

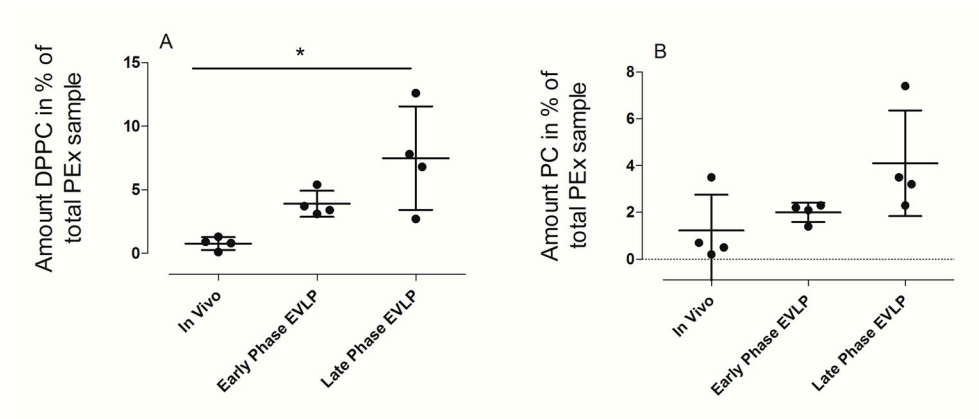

Figure 19 Di-palmitoyl-phosphatidyl-choline (DPPC) and palmitoyl-oleoyl-phosphocholine (PC) concentrations were measured in exhaled particles in four of the animals and were expressed as weight percent, wt%.
(A) The amount DPPC in percent (wt%) of total PEx. Note the significant increase in DPPC in EVLP late phase as compared to *in vivo* (p = 0.04). **(B)** No differences were observed in the PC wt% of the total PEx sample.

Paper II

Animals

Pre-operative venous oxygen saturation (SvO_2) at a FiO_2 of 0.5 was 60.5 ± 6 kPa with a saturation of 98 ± 1%. Baseline mean blood pressure and pulse was 81±3 mmHg and 79±12 beats per minute, respectively. No anatomical anomalies, signs of infection or malignancy were found in any of the animals at autopsy. One animal developed ARDS and was excluded and its case will be presented separately.

Feasibility of the PExA method used in conjugation with mechanical ventilation

This study has been performed as a feasibility study. No adverse events (mild, moderate or severe) as airway leakage, signs of rebreathing, altered pressure levels and haemodynamic interferences were seen. Ventilator peak pressures and mean pressures along with FiO_2 levels, venous blood gases, blood pressure, saturation

and pulse were measured continuously in a standard manner. We did not detect any statistically significant or clinically significant changes during the 3 days.

Effects of mechanical ventilation on total particle count from the airways

VCV and PCV were measured on a daily basis from day 1 until day 3 in all animals. On day 1 the total particle count was 45,063 ± 8775, on day 2 the total particle count was 30,749 ± 6033 and on day 3 the total particle count was 18,409 ± 3693. Comparing days 1 and 2, a significant difference was found (p = 0. 0274). A significant decrease in particle flow was seen on day 3 compared to day 2 (p = 0.0246), as seen in Figure 20A. One animal was excluded due to the development of clinical signs of ARDS, and the particle count from the airways in this animal was much higher than in the other animals.

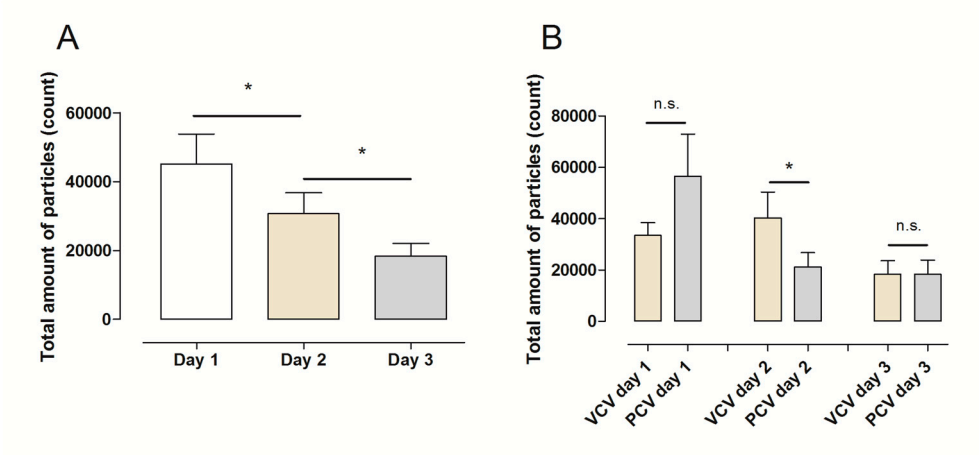

Figure 20 Daily total accumulated particle count and divided by ventilation mode
(A). The total accumulated particle count measured by the PExA device during volume-controlled ventilation (VCV) and pressure-controlled ventilation (PCV) during three consecutive days (n = 5). **(B)** VCV and PCV are divided into separate groups.

Effects of VCV and PCV

VCV was compared to PCV from day 1 until day 3. On day 1 the total particle count was 33,562 ± 4951 during VCV_1 and 56,564 ± 16,468 during PCV_1 (p = 0.1779), on day 2 the total particle count was 40,260 ± 10,097 during VCV_2 and 21,238 ± 5625 during PCV_2 (p = 0.0184) and on day 3 the total particle count was 18,343 ± 5347 during VCV_3 and 18,497 ± 5418 during PCV_3 (p = 0.5977), shown in Figure 20B.

Particle distribution during VCV and PCV

The total particle count from the airways are divided by the optical particle counter into eight different size distributions according to particle size where particle size 1 was the smallest and particle size 8 was the largest. The results of particle distribution from VCV and PCV modes during the three subsequent days are shown in Figure 21A. One animal developed severe ARDS on day 3 and had a different particle pattern from the airways from day 1 with significantly increased particle size 6. Interestingly, on day 3 when the animal developed ARDS, particle size 6 decreased. The animal that developed clinical signs of ARDS was excluded and its case is presented separately, as seen in Figure 21B.

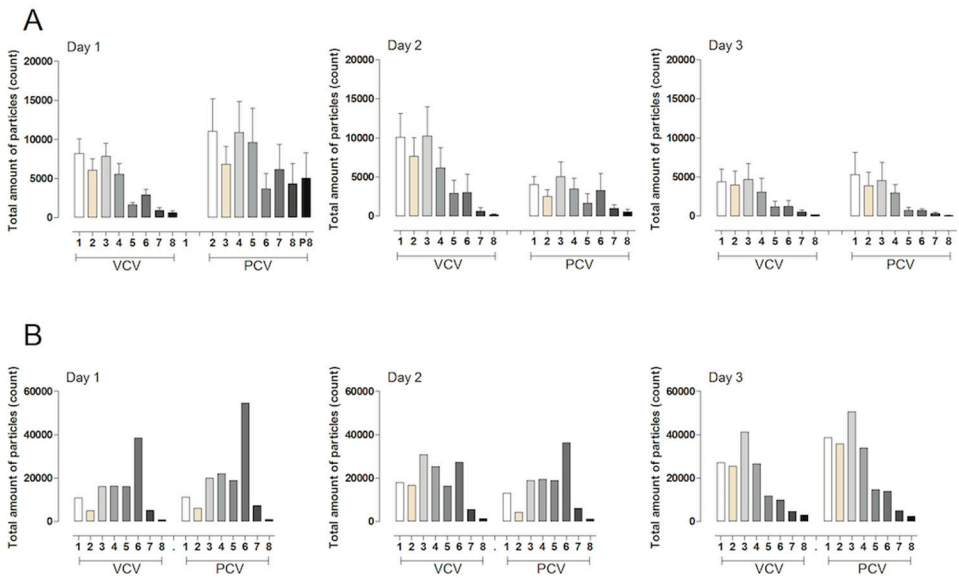

Figure 21 Size distrubution of particles between VCV and PCV

(A) Displays different particle size distributions during volume-controlled ventilation (VCV) and pressure-controlled ventilation (PCV) in five animals during three consecutive days. **(B)** One animal developed acute respiratory distress syndrome (ARDS) on day 3. Note how particles within size 6 were increased during days 1–2. On day 3 when the animal developed clinical signs of ARDS, a similar particle size pattern to that of the other five animals was seen.

Recruitment manoeuvres

The total particle count was measured 3 min before the RM, during the RM for 1 min, and 3 min after the RM. An RM was performed daily using VCV mode resulting in a total particle count of 379 ± 106 before, 1303 ± 427 during, and 873 ± 103 after recruitment day 1. Comparing the particle flow before and after RM, a significant difference was observed ($p = 0.0001$). Comparing the particle flow before and during RM ($p = 0.0746$), no significant difference was found. The total particle count was 918 ± 281 before, 2652 ± 6 during and 2692 ± 995 after recruitment day 2. Comparing the particle flow before and after RM, a significant

difference was observed (p = 0.0476). Comparing the particle flow before and during RM, a significant increase could also be observed (p = 0.0161). On day 3 the total particle count was 1357 ± 366 before, 1309 ± 251 during and 3359 ± 1252 after recruitment day 3. Comparing the particle flow before and after RM, no significant difference was observed (p = 0.0920). Comparing the particle flow before and during RM, no significant difference was observed (p = 0.8577) as seen in Figure 22.

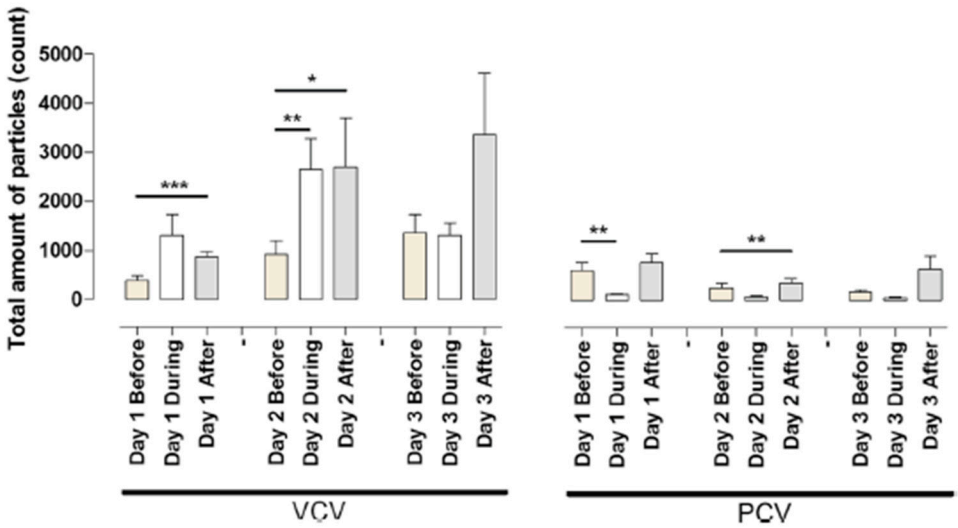

Figure 22 Total amount of particles during recruitment manoeuvres
All animals underwent recruitment manoeuvre (RM) twice daily starting on day 1 until day 3. The total accumulated particle count was measured by PExA 3 min before RM, during the RM (1 min) and 3 min after RM. The Figure shows the total particle count before, during and after RM for three consecutive days using volume-controlled ventilation (VCV) and pressure-controlled ventilation (PCV).

An RM was performed daily using PCV mode, and the total particle count was 737 ± 186 before, 146 ± 22 during and 923 ± 212 after recruitment day 1. Comparing the particle flow before and after RM, no significant difference was observed (p = 0.0563). Comparing the particle flow before and during RM, a significant decrease was observed (p = 0.0083). The total particle count was 321 ± 105 before, 104 ± 22 during and 433 ± 117 after recruitment day 2. Comparing the particle flow before and after RM, a significant increase was observed (p = 0.0157). Comparing the particle flow before and during RM, no significant difference could be observed (p = 0.0520). On day 3 the total particle count was 208 ± 59 before, 73 ± 21 during, and 771 ± 312 after recruitment. Comparing the particle flow before and after RM, no significant difference was observed (p = 0.0565). Comparing the particle flow before and during RM (p = 0.0824), no significant difference was observed, as seen in Figure 22.

Comparing particle flow for VCV with PCV before and after RM, a significant difference was found on day 1 ($p = 0.0185$) but not on day 2 ($p = 0.0584$) or day 3 ($p = 0.3013$). Particle flow during RM showed significant differences on all three days: day 1 VCV versus day 1 PCV ($p = 0.0232$), day 2 VCV versus day 2 PCV ($p = 0.0016$) and day 3 VCV versus day 3 PCV ($p = 0.0112$), as seen in Figure 23.

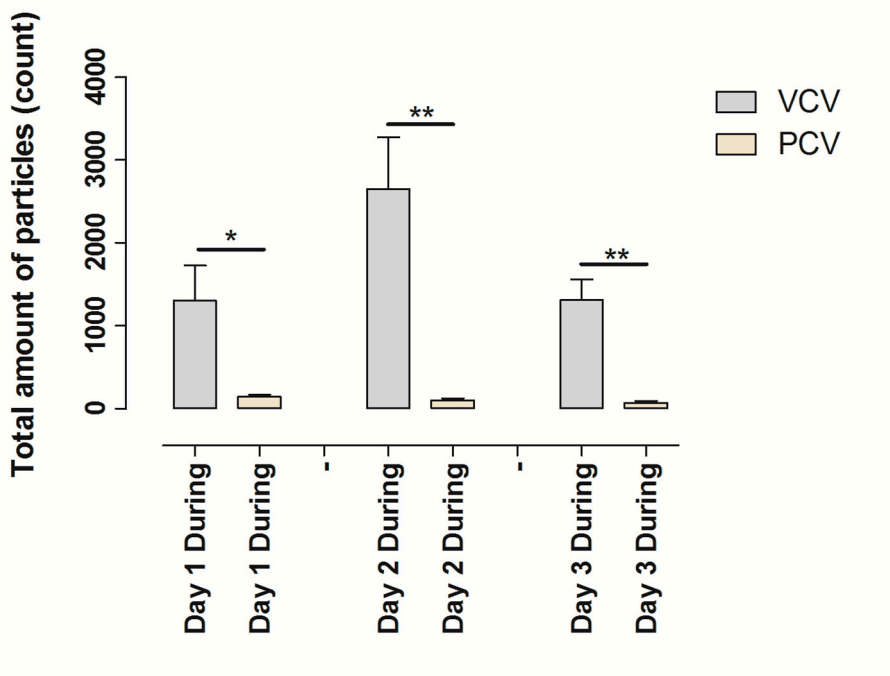

Figure 23 Total amount of particles during daily recruitment manoeuvres
A recruitment manoeuvre (RM) was performed on all animals twice daily starting on day 1 until day 3. The total accumulated particle count was measured by PExA during the RM for 1 min. The Figure shows the total particle count during RM during three consecutive days comparing volume-controlled ventilation (VCV) and pressure-controlled ventilation (PCV).

Fluid balance

The fluid balance was measured daily. On day 1 the fluid balance was 0 ± 0 ml, on day 2 the fluid balance was 968 ± 193 ml and on day 3 the fluid balance was 2498 ± 275 ml. Statistical significance was found comparing days 1 and 2 ($p = 0.0014$), and days 2 and 3 ($p = 0.0042$), as seen in Figure 24.

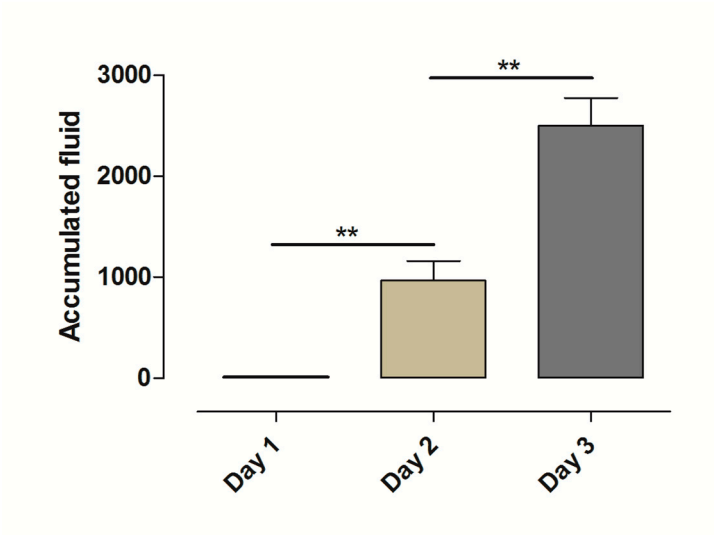

Figure 24 Fluid balance
Shows the animals' total accumulated fluid balance during three consecutive days.

Blood gases, haemodynamics and mechanical ventilation settings

Blood gases, haemodynamics and mechanical ventilation settings were taken at the start and at the end of each ventilation mode. During RM the same parameters were taken 3 min before RM and 3 min after RM. Fluid was given to maintain vital signs and an adequate urine output. All animals were stable during all measurements, and no significant changes in blood gases, haemodynamics or in mechanical ventilation settings could be found.

Paper III

Demographic characteristics of the patients

The mean age of the recipients was 56 ± 3 years. Five of the recipients were women and five were men.

Of the 12 patients, three patients underwent single lung transplant. All three of these patients were re-transplant patients who underwent a single lung transplant due to BOS. Primary transplant in two of these patients was due to CF, with the other patient having primary transplant due to PF.

The remaining nine of 12 patients had double lung transplants: one had COPD, four had PF, two had CF, and two had COPD due to AAT1.

Feasibility of PExA 2.0 used in conjunction with mechanical ventilation

The purpose of this study was to test the feasibility of PExA 2.0 used in conjunction with mechanical ventilation. No mild, moderate, or severe adverse events, such as airway leakage, signs of rebreathing, altered pressure levels and haemodynamic interferences were observed in our patients. Ventilator peak pressures and mean pressures along with FiO_2 levels, blood gases, blood pressure, saturation, and pulse were recorded continuously in a standard manner. Interestingly, we did not detect any statistically significant or clinically significant changes during any days or measurements.

Effects of volume-controlled versus pressure-controlled ventilation on total particle count

The total particle count during the two ventilation modes (VCV and PCV) was analysed at every time point, with VCV compared with PCV from day 0 until extubation. At day 0, the average total particle count was 10,299 ± 3420 during VCV and 11,678 ± 3593 during PCV (P = .6288); on day 1, the total particle count was 27,117 ± 13,508 during VCV and 15,238 ± 3918 during PCV (p = 0.3106); on day 2, the total particle count was 61,461 ± 42,060 during VCV and 80,373 ± 61,017 during PCV (p = 0.3604); and, on day 3, the total particle count was 143,585 ± 60,920 during VCV and 190,497 ± 74,156 during PCV (p = 0.0328), as seen in Figure 25A. Thus, we only observed significant differences between VCV and PCV on day 3.

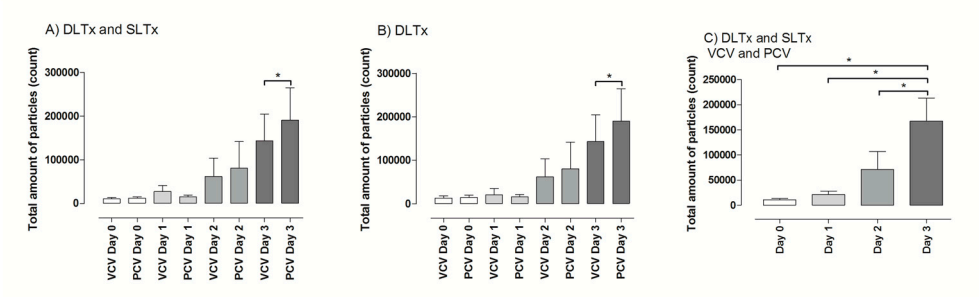

Figure 25 Total accumulated particle count measured by PExA 2.0
(A) Results for all lung transplant recipients (n = 12) showing volume-controlled ventilation (VCV) versus pressure-controlled ventilation (PCV) during daily measurements from day 0 until extubation at day 3. **(B)** Double lung transplant (DLTx) results (n = 9) showing VCV versus PCV during daily measurements from day 0 until extubation at day 3. **(C)** Mean VCV and PCV PExA 2.0 measurements from day 0 until extubation at day 3 for DLTx and single lung transplant (SLTx).

To exclude effects of SLTx versus DLTx, we analysed the same data but excluded patients who had received only SLTx, as seen in Figure 25B. At day 0, the total particle count was 13,023 ± 4855 during VCV and 14,442 ± 5102 during PCV ($p = 0.7506$); on day 1, the total particle count was 20,698 ± 13,933 during VCV and 15,877 ± 4991 during PCV ($p = 0.6702$); on day 2, the total particle count was 61,461 ± 42,060 during VCV and 80,373 ± 61,017 during PCV ($p = 0.3604$); and, on day 3, the total particle count was 143,585 ± 60,920 during VCV and 190,497 ± 74,156 during PCV ($p = 0.0328$). Thus, the same pattern was observed with only a significant difference between PCV and VCV on day 3.

Total particle count over time until extubation

Total particle count (all VCV and PCV measurements) from day 0 until extubation was measured in all 12 transplant patients. At day 0, the total particle count was 10,988 ± 2412; on day 1, the total particle count was 21,178 ± 6989; on day 2, the total particle count was 70,917 ± 35,881; and, on day 3, the total particle count was 167,041 ± 46,296, as seen in Figure 25C. We observed a significant increase in total particle count from the airways on day 0 versus day 3 ($p = 0.0146$), on day 1 versus day 3 ($p = 0.0128$), and on day 2 versus day 3 ($p = 0.0105$)

Primary graft dysfunction

During the initial 72 hours after transplant, six patients developed PGD and the other six did not. Four patients developed stage 1 PGD, one patient developed stage 2 PGD, and one patient developed stage 3 PGD. We observed no significant differences in total particle counts between VCV and PCV with regard to disease stage during the different days. When we compared the total daily particle count, patients with PGD were more prone to stay in mechanical ventilation for a longer time and showed a stepwise and significant increase in particle count over time, as

seen in Figure 26. These results were independent of the mode of ventilation that was used. In the PGD group, the total particle count on day 0 was 14,539 ± 3981; on day 1, it was 21,040 ± 10,601; on day 2, it was 91,782 ± 46,751; and, on day 3, it was 199,349 ± 49,473. There was a significant difference in total particle count between day 0 and day 1 compared with day 3 ($p = 0.0065$ and $p = 0.0082$, respectively). In the non-PGD group, the total particle count was 6650 ± 1125 on day 0, 21,315 ± 9585 on day 1, 8323 ± 1432 on day 2, and 5500 ± 1900 on day 3.

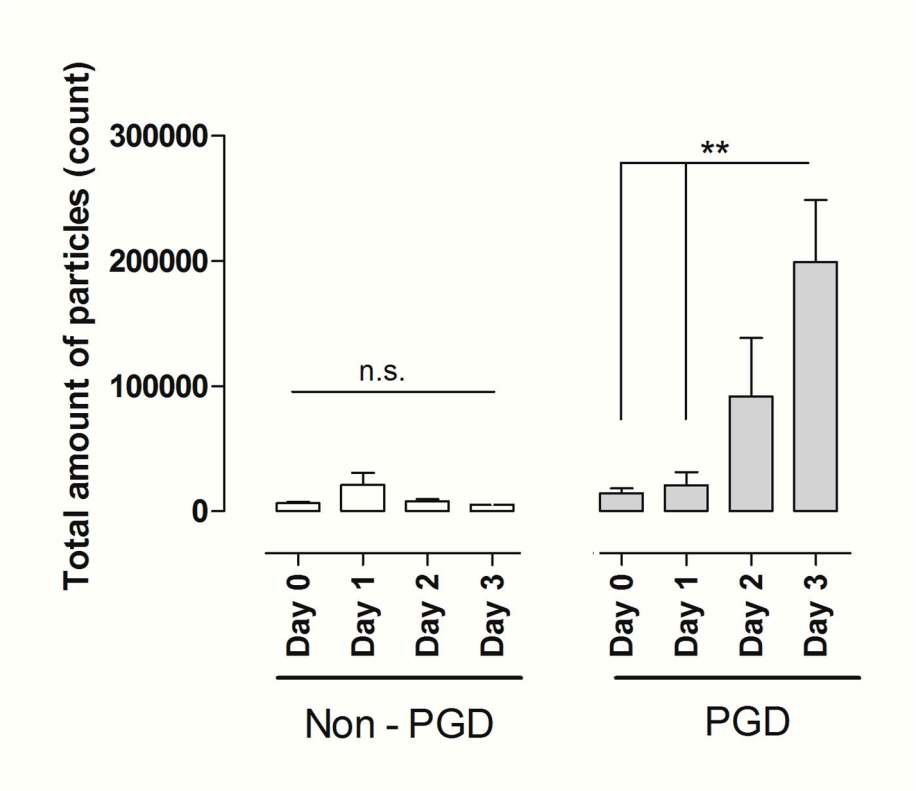

Figure 26 Total particle counts in patients who did and did not develop primary graft dysfunction.
Patients who developed primary graft dysfunction (PGD) showed a stepwise increase in total particle flow from the airways measured by PExA 2.0. However, the same pattern could not be observed among the patients who did not develop PGD. **p < .01.

Patients who developed PGD had a significantly longer treatment time in mechanical ventilation (2.3 ± 0.2 days) compared with patients who did not develop PGD (1.5 ± 0.3 days) ($p = 0.0041$).

Recruitment manoeuvres

During VCV, total particle count was 277 ± 107 before and 492 ± 134 after RMs on day 0, with a significant difference observed in particle flow before and after RM ($p = 0.0063$). The total particle count differed significantly before and after RMs on day 1 versus day 2, showing 313 ± 83 before RM and 1749 ± 852 after RM on day 1 ($p = 0.0495$) and 192 ± 70 before and 319 ± 100 after RM on day 2 ($p = 0.0303$). The total particle count was not significantly different ($p = 0.1724$) on day 3 before and after RMs, which showed 205 ± 121 before and 259 ± 108 after RM, as seen in Figure 27.

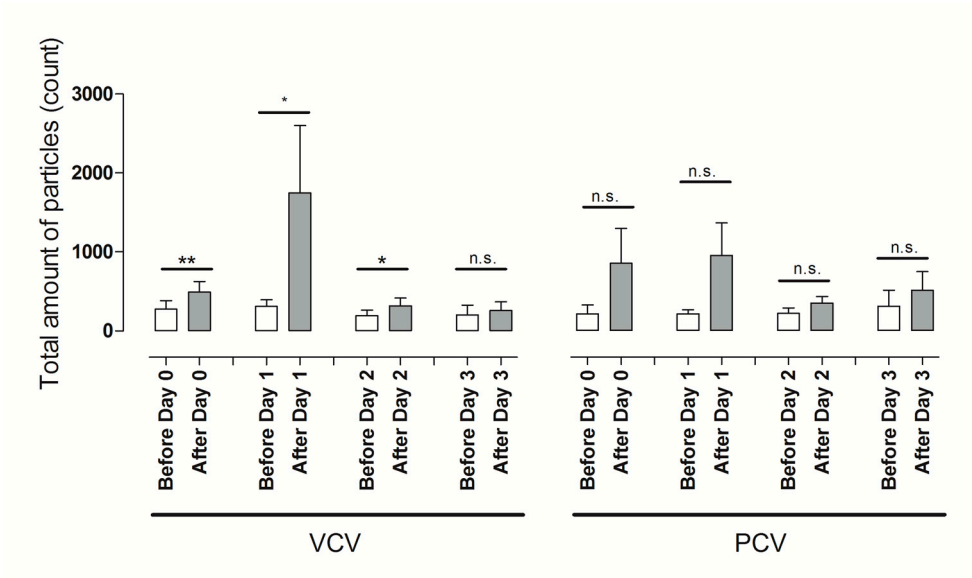

Figure 27 Total particle counts before and after recruitment manoeuvres

All patients underwent recruitment manoeuvres (RM) twice daily, starting at day 0 post-transplant until extubation (day 3 post-transplant). Total particle count was measured before and after RM on day 0, day 1, day 2, and day 3 post-transplant during volume-controlled ventilation (VCV) and pressure-controlled ventilation (PCV). Note that particle flow increased significantly after RM during the first 48 hours with VCV but not with PCV (*$p < 0.05$; **$p < 0.01$).

During PCV, no significant differences were observed before or after the RMs. The total particle count was 215 ± 112 before and 854 ± 443 after RM on day 0 ($p = 0.1118$). On day 1, the total particle count was 215 ± 50 before and 954 ± 413 after RM ($p = 0.0818$), whereas the total particle count was 220 ± 69 before and 350 ± 81 after RM on day 2 ($p = 0.0645$). On day 3, the total particle count was 312 ± 203 before and 511 ± 236 after RM ($p = 0.2406$), as seen in Figure 27.

Inflammatory biomarkers

We next investigated whether we could correlate early biomarkers with the onset of PGD. Interestingly, patients who developed PGD had significantly higher C-reactive protein (CRP) levels directly after transplant on day 0 (120 ± 31 mg/l) compared with patients who did not develop PGD (50 ± 7 mg/l) ($p = 0.0420$), as seen in Figure 28. During the remaining postoperative measurement days, no significant differences were observed between the two groups. In the PGD, group, CRP was 184 ± 20 mg/l on day 2 and 188 ± 16 mg/l on day 3. Among patients who did not develop PGD, the CRP levels were 143 ± 12 mg/l on day 2 and 151 ± 21 mg/l on day 3.

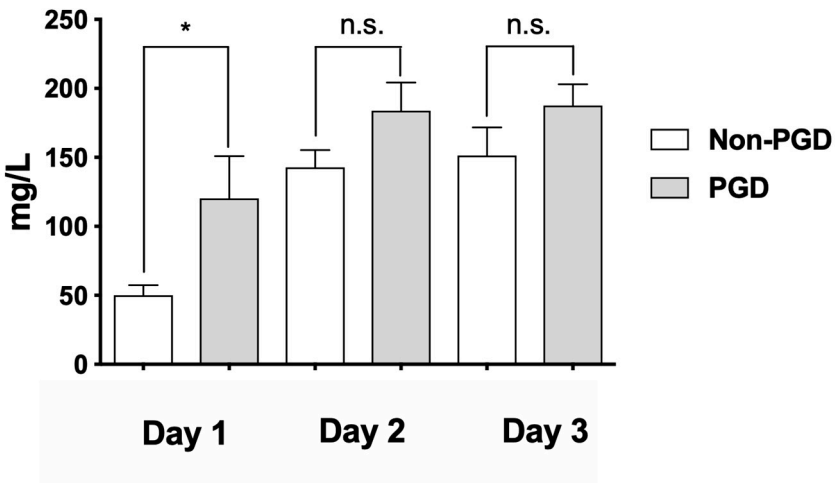

Figure 28 C-reactive protein levels in study patients
Patients in the study group who developed primary graft dysfunction (PGD) had significantly (*$p < 0.05$) higher C-reactive protein levels directly after lung transplant than patients who did not develop PGD.

Blood gases, haemodynamics, and mechanical ventilation settings

Blood gases, haemodynamics and mechanical ventilation settings were taken at the start and at the end of each mode and the patients were continuously monitored in a standard manner. During RM the same parameters were taken 3 min before RM and 3 min after RM. All patients were stable during all measurements, and no significant changes in blood gases, haemodynamics, or mechanical ventilation settings could be found.

Paper IV

Particle flow rate and particles per mass

PFR was 942 (388–4898) in the MV-NSCLC group, 1655 (968–5753) in the MV-C group and 10.520 (6933–13,462) in the NB group. Comparing the groups, a significant difference was found in PFR between MV-NSCLC and NB ($p = 0.036$) and between MV-C and NB ($p = 0.001$) but no significance was seen between MV-NSCLC and MV-C, as shown in Figure 29A. Average particle mass were 0.148×10^{-3} ng (0.128×10^{-3}–0.196×10^{-3}) in the MV-NSCLC group, 0.155×10^{-3}ng (0.095×10^{-3}–0.222×10^{-3}) in the MV-C group and 0.433×10^{-3} ng (0.387×10^{-3}–0.700×10^{-3}) in the NB group. Comparing the groups, a significant difference was found in average particle mass between MV-NSCLC and NB ($p = 0.001$) and between MV-C and NB ($p = 0.001$) but no significance was seen between MV-NSCLC and MV-C ($p = 0.920$), shown in Figure 29B.

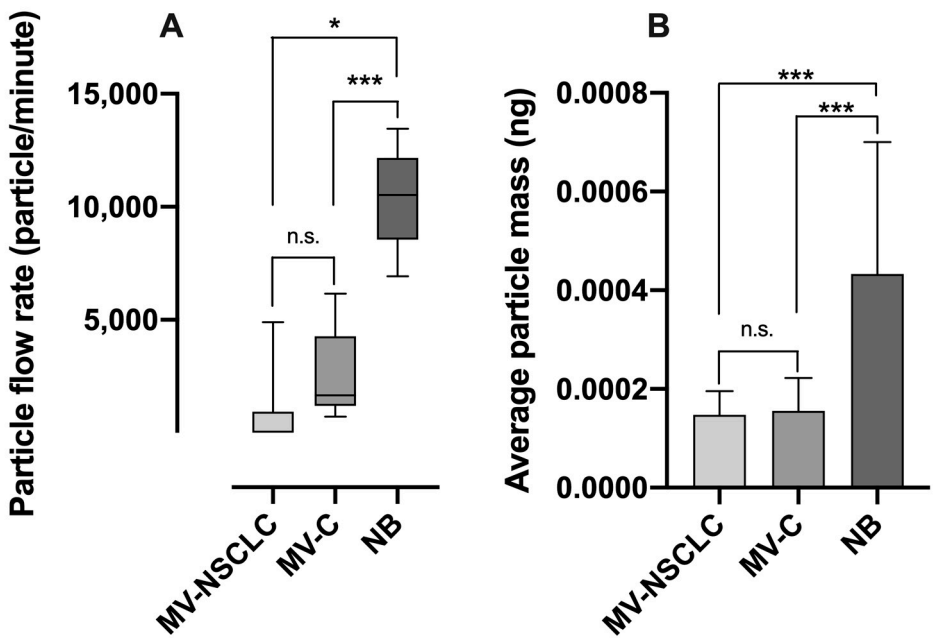

Figure 29 Particle flow rate and average particle mass in the three different groups

(A) Shows particle flow rate exhaled per minute between mechanical ventilation in non-small-cell lung cancer NSCLC (MV-NSCLC) and control (MV-C) along with normal breathing (NB). **(B)** Shows exhaled average particle mass in ng between mechanical ventilation in MV-NSCLC and control MV-C along with NB.

Biochemical collection of phospholipids and proteins

DPPC was 2.50 (1.80–5.10) wt.% in the MV-NSCLC group, 2.10 (1.30–2.70) wt.% in the MV-C group and 8.35 (4.80–9.90) wt.% in the NB group, with significant differences between MV-NSCLC and NB (p = 0.001), MV-C and NB (0.004) but not for MV-NSCLC and MV-C (p = 0.102), as shown in Figure 30A. POPC was 0.65 (0.40–1.30) wt.% in the MV-NSCLC group, 0.60 (0.30–0.70) wt.% in the MV-C group and 2.05 (1.10–2.40) wt.% in the NB group, with significant difference between MV-NSCLC and NB (p = 0.001), MV-C and NB (p = 0.004) but not for MV-NSCLC and MV-C (p = 0.493), as shown in Figure 30B. DPPC and POPC ratio was 3.965 (3.03–4.70) in the MV-NSCLC group, 3.76 (2.70–4.04) in the MV-C group and 4.22 (3.88–4.59) in the NB group. Significance could be seen between MV-C and NB (p = 0.030) but not between MV-NSCLC and MV-C (p = 0.222) or between MV-NSCLC and NB (p = 0.491), as seen in Figure 30C.

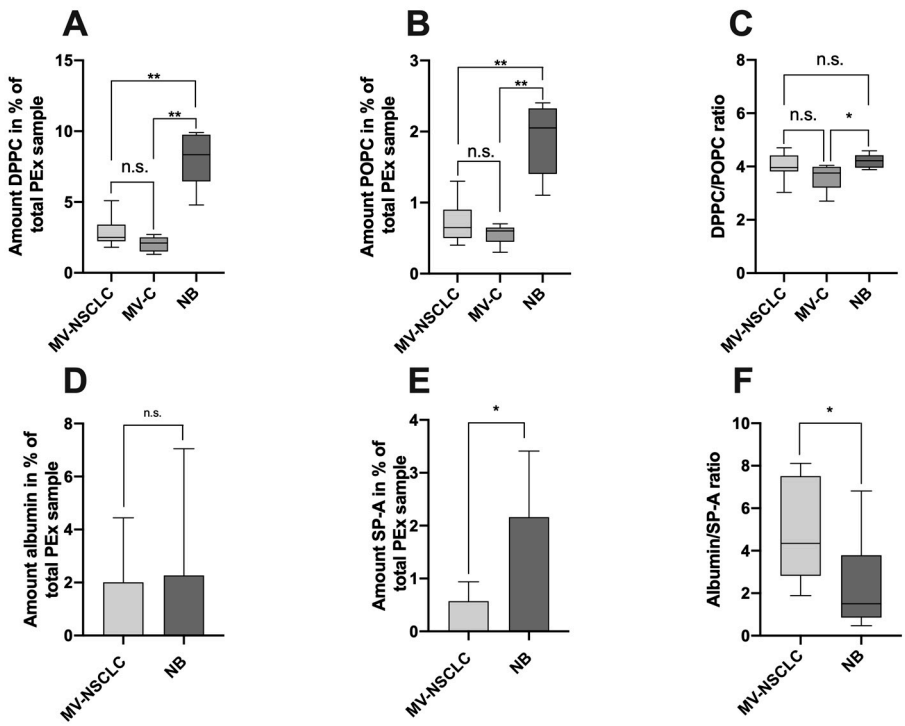

Figure 30 Analysed particles in the different groups

(A) Shows weight percent protein (wt.%) of total PEx sample for phospholipids di-palmitoyl-phosphatidyl-choline (DPPC) comparing non-small-cell lung cancer patients on mechanical ventilation (MV-NSCLC), control on mechanical ventilation (MV-C) and non-intubated normal breathing (NB) patients. **(B)** Shows wt.% of total PEx sample for palmitoyl-oleoyl-phosphocholine (POPC) comparing MV-NSCLC, MV-C and NB. **(C)** Shows the ratio DPPC/POPC between MV-NSCLC, MV-C and NB. **(D)** Shows wt.% of total PEx sample for surfactant A (SP-A) comparing MV-NSCLC and NB patients. **(E)** Shows wt.% of total PEx sample for albumin comparing MV-NSCLC and NB. **(F)** Shows the albumin/SP-A ratio between MV-NSCLC and NB.

For albumin and SP-A unfortunately two samples from the MV-C group did not reach detection levels and therefore we only present MV-NSCLC and NB for albumin and SP-A. Albumin was 2.01 (0.77–4.44) wt.% in the MV-NSCLC group and 2.27 (1.59–7.05) wt.% in the NB group (p = 0.485) and SP-A in the MV-NSCLC group was 0.57 (0.11–0.94) wt.% and 2.16 (0.30–3.41) wt.% in the NB group (p = 0.024) as shown in Figure 30D and 30E. Albumin and SP-A ratio was 4.35 (1.89-8.11) in the MV-NSCLC group and 1.51 (0.47-6.81) in the NB group (p = 0.041), as shown in Figure 30F.

One-lung ventilation and double-lung ventilation during lung surgery

During mechanical ventilation in lung surgery, measurements were possible in a total of nine out of 26 patients when going from OLV to DLV. During OLV, PFR was 119 (29-1930) compared to DLV 171 (30–2239) (p = 0.002) as shown in Figure 31A. PFR was 157 (18–3403) during OLV and 171 (24–4348) during DLV in the MV-NSCLC group (p = 0.7) and 83 (29–274) during OLV and 316 (30–1955) during DLV in the MV-C group (p = 0.3). During OLV average particle mass were 0.399 x 10^{-3} (0.268 x 10^{-3}–0.834 x 10^{-3}) compared to DLV 0.604 x 10^{-3} (0.368 x 10^{-3}–0.678 x 10^{-3}) (p = 0.5) as shown in Figure 31B.

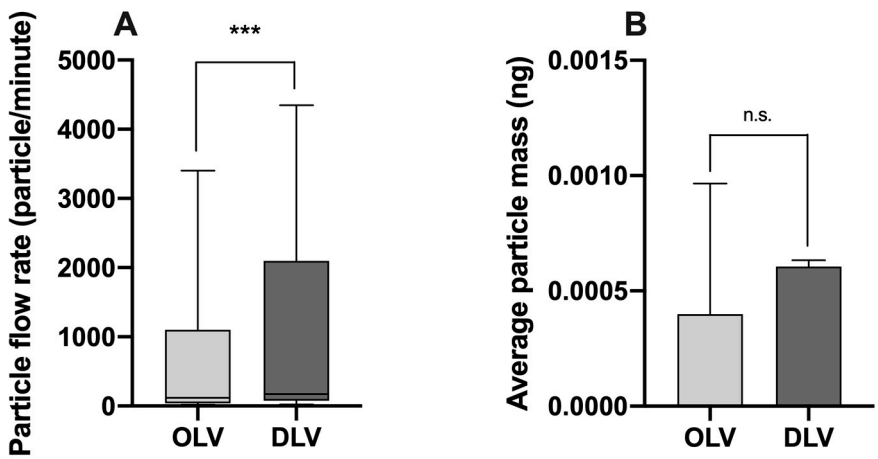

Figure 31 Particle flow rate during one-lung and double-lung ventilation
(A) Shows particle flow rate exhaled per minute measured for 5 min of one-lung ventilation (OLV) before opening up the other lung and followed by measurements for 5 min of double-lung ventilation (DLV). **(B)** Shows exhaled average particle mass in ng measured for 5 min of OLV before followed by measurements for 5 min of DLV.

Haemodynamics

Haemodynamics and mechanical ventilation parameters in the different groups are shown in Table 2. Peak pressure was statistically different during MV-NSCLC but not for any other parameters, as seen in Table 8.

Table 8

Haemodynamics during mechanical ventilation in non-small-cell lung cancer (MV-NSCLC) and mechanical ventilation in the control group (MV-C). Blood pressure (BP). Mean arterial pressure (MAP). End tidal carbon dioxide ($EtCO_2$). Tidal volume (TV).

Parameters	Start MV-NSCLC	Stop MV-NSCLC	P-value	Start MV-C	Stop MV-C	P-value
BP systole (mmHg)	106 ± 3	104 ± 2	0.65	102 ± 5	104 ± 2	0.87
BP diastole (mmHg)	59 ± 2	57 ± 1	0.24	59 ± 3	59 ± 2	0.98
MAP (mmHg)	76 ± 2	73 ± 1	0.23	75 ± 3	76 ± 3	0.78
Pulse (beats/minue)	76 ± 3	71 ± 4	0.41	78 ± 4	74 ± 4	0.41
$EtCO_2$ (kPa)	5.06 ± 0.12	4.72 ± 0.11	0.11	5.30 ± 0.20	4.84 ± 0.18	0.14
Saturation (%)	99 ± 1	99 ± 1	0.15	99 ± 1	99 ± 1	0.1
Respiration Frequency	15 ± 1	16 ± 1	0.61	16 ± 1	17 ± 1	0.84
TV inspiration (ml)	453 ± 15	470 ± 13	0.43	437 ± 27	418 ± 27	0.65
TV expiration (ml)	440 ± 14	453 ± 15	0.56	420 ± 26	404 ± 32	0.72
Peak Pressure (cmH_2O)	15 ± 1	19 ± 1	0.01	15 ± 1	17 ± 1	0.32
Air leakage (ml)	13 ± 2	21 ± 1	0.31	16 ± 4	14 ± 7	0.79

Paper V

Cumulative incidence of BOS grade ≥ 2 and death

Type of transplant

Incidence of BOS (grade ≥ 2) is presented (percentage of probability ± SE) by type of transplant (DLTx and SLTx) as seen in Figure 32. The incidence of BOS among DLTx-recipients was 16 ± 3% at 5 years, 30 ± 4% at 10 years, 35 ± 5% at 15 years, and 37 ± 5% at 20 years, compared to SLTx recipients whose incidence of BOS was 11 ± 3% at 5 years, 20 ± 4% at 10 years, 24 ± 5% at 15 years, and 24 ± 5% at 20 years ($p > 0.05$). The mortality rate for DLTx recipients was 19 ± 3% at 5 years, 23 ± 4% at 10 years, 28 ± 4% at 15 years, and 43 ± 7% at 20-years compared to the mortality rate of SLTx-recipients, which was 34 ± 5% at 5 years, 55 ± 6% at 10 years, 56 ± 6% at 15 years, and 71 ± 8% at 20 years ($p < 0.05$). Kaplan-Meier survival is displayed after development of BOS (grade ≥ 2) until follow-up/death seen in Figure 33. Survival curves are divided into patients that have underwent DLTx versus SLTx ($p > 0.05$).

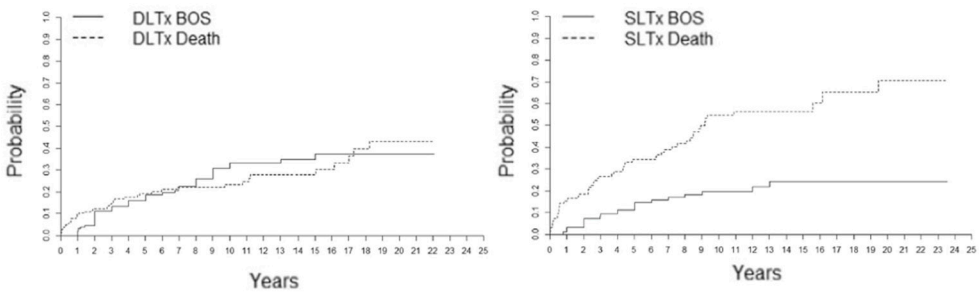

Figure 32 Cumulative incidence of bronchiolitis obliterians after lung transplantation
Cumulative incidence of bronchiolitis obliterians (BOS) grade ≥ 2 and mortality after lung transplantation in double-lung transplantation (DLTx) and single-lung transplantation (SLTx) recipients. Note that DLTx and SLTx recipients have the same risk of developing BOS, but DLTx has a significantly better chance of survival despite the presence of BOS.

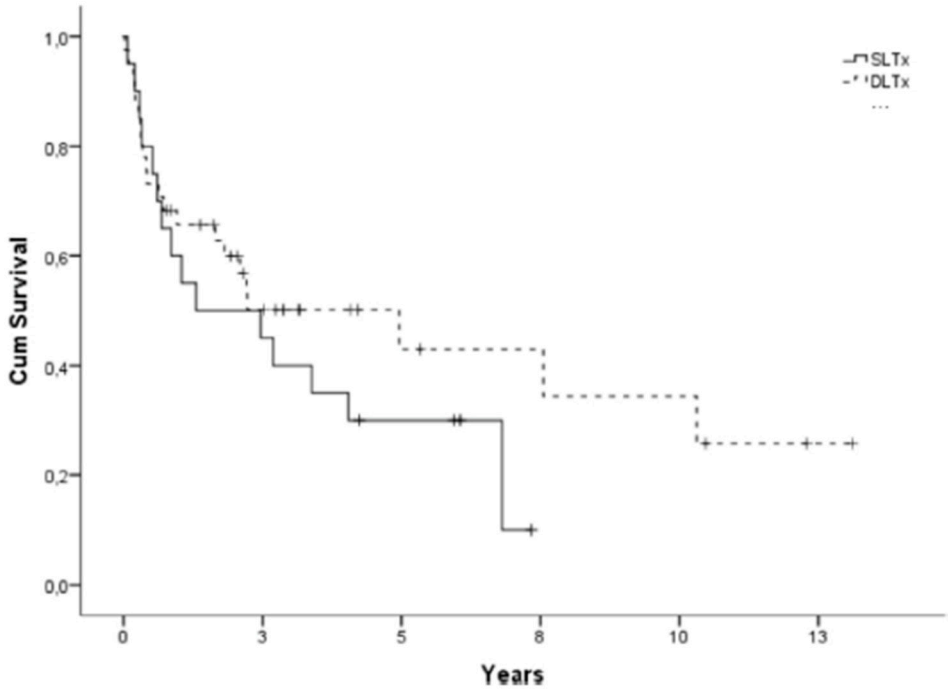

Figure 33
Kaplan-Meier figure displaying survival between single-lung transplantation (SLTx) and double-lung transplantation (DLTx) after development of BOS grade ≥ 2 until death/follow-up (p > 0. 05).

Major indications

The incidence of BOS (grade ≥ 2) is presented by different diagnostic groups in Figure 34. The incidence of BOS among CF-patients was 20 ± 6% at 5 years, 37 ± 8% at 10 years, 37 ± 8% at 15 years, and 44 ± 10% at 20 years. For AAT1-patients it was 4 ± 3% at 5 years, 14 ± 6% at 10 years, 26 ± 8% at 15 years, and 26 ± 8% at 20 years. For COPD-patients it was 13 ± 4% at 5 years, 19 ± 5% at 10 years and 24 ± 7% at 15 years. For PF-patients, the incidence of BOS was 25 ± 8% at 5 years, 34 ± 9% at 10 years, 34 ± 9% at 15 years and 34 ± 9% at 20 years while for PH-patients it was 6 ± 4% at 5 years, 19 ± 7% at 10 years, 19 ± 9% at 15 years, and 19 ± 7% at 20 years ($p < 0.05$). The mortality rate for CF-patients was 12 ± 5% at 5 years, 15 ± 5% at 10 years, 19 ± 7% at 15 years, and 37 ± 19% at 20 years. For AAT1-patients it was 26 ± 6% at 5 years, 41 ± 8% at 10 years, 44 ± 8% at 15 years and 68 ± 10% at 20 years. For COPD-patients it was 32 ± 6% at 5 years, 55 ± 8% at 10 years, and 58 ± 8% at 15 years. For PF-patients it was 26 ± 8% at 5 years, 38 ± 9% at 10 years, 38 ± 9% at 15 years, and 52 ± 16% at 20 years, and for PH it was 30 ± 8% at 5 years, 34 ± 8% at 10 years, 39 ± 9% at 15 years, and 54 ± 12 at 20 years ($p < 0.05$).

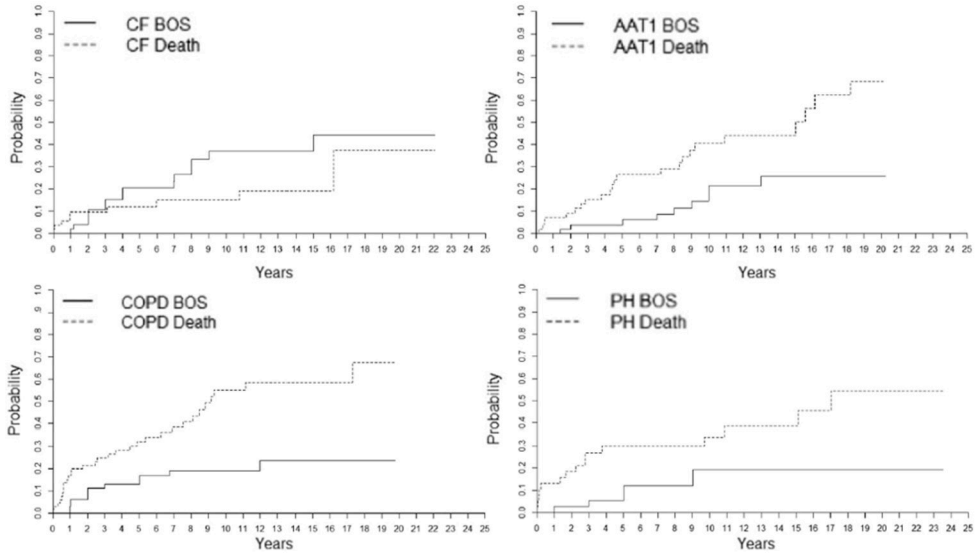

Figure 34 Cumulative incidence of bronchiolitis obliterans syndrome (BOS) and mortality after lung transplantation (LTx) in different groups

The figure display comparison of cystic fibrosis (CF), alpha1-antitrypsine deficiency (AAT1) recipients, chronic obstructive pulmonary disease (COPD) recipients and pulmonary hypertension (PH) recipients. CF recipients had a significantly higher risk of developing BOS grade ≥ 2 compared to AAT1 recipients ($p < 0.05$), but AAT1 had a significantly higher mortality ($p < 0.05$), indicating that CF recipients might withstand BOS better than AAT1 recipients. Recipients with CF and COPD had the same incidence of BOS grade ≥ 2 ($p > 0.05$), but COPD recipients had a significantly higher mortality ($p < 0.05$), indicating that CF recipients might withstand BOS better than COPD recipients. CF recipients had a significantly higher risk of developing BOS grade ≥ 2 compared to PH recipients. However, CF and PH recipients showed the same mortality, indicating that CF and PH recipients with BOS have the same chance of survival.

Major indications compared group wise

The patient groups of major indications were compared. Recipients with CF had higher risk of developing BOS compared to AAT1 recipients ($p = 0.048$), but AAT1 recipients had a higher mortality ($p = 0.020$). Recipients with CF and COPD had the same incidence of developing BOS ($p = 0.164$), but COPD recipients had a higher mortality ($p = 0.001$). Recipients with CF had higher risk of developing BOS compared to PH recipients ($p = 0.055$), but CF and PH recipients had the same mortality ($p = 0.057$), as seen in Figure 34. The group 'other' describes a heterogenous group of patients who underwent LTx due to bronchiectasis, sarcoidosis, bronchioalveolar cancer, silicosis, BOS and graft-vs-host disease (GVHD). The group had a higher incidence of BOS compared to COPD ($p = 0.007$), AAT1 ($p = 0.001$) and PH ($p = 0.002$) patients. The group also showed significant lower risk of death compared to COPD ($p = 0.037$), AAT1 ($p = 0.281$), and PH ($p = 0.300$) patients.

Age and BOS

The incidence of BOS (grade ≥ 2) for patients ≤ 50 years of age was 15 ± 3% at 5 years, 30 ± 5% at 10 years, 35 ± 5% at 15 years, and 38 ± 6% at 20 years. For patients > 50 years of age it was 14 ± 3% at 5 years, 22 ± 4% at 10 years, and 26 ± 5% at 15 years (p = 0.238). The mortality rate for patients ≤ 50 years of age was 20 ± 4% at 5 years, 28 ± 4% at 10 years, 34 ± 5% at 15 years and 41 ± 7% at 20 years. For patients > 50 years of age the mortality rate was 29 ± 4% at 5 years, 44 ± 5% at 10 years, and 45 ± 5% at 15 years (p = 0.019), as seen in Figure 35.

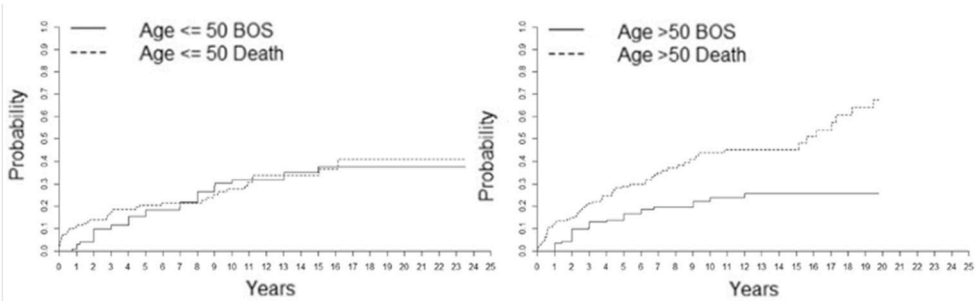

Figure 35 Competing risk analysing the impact of age on the development of bronchiolitis obliterans syndrome (BOS) and the risk of death after lung transplantation (LTx).
Age had no impact on the development of BOS grade ≥ 2, but recipients 50 years or older had a 9% higher mortality 5 years post-transplant and a 16% increased risk 10 years post-transplant compared to recipients younger than 50 years (p < 0. 05)

Different time periods and BOS

For the period 1990–2002, the incidence of BOS (grade ≥ 2) in all recipients was 9 ± 3% at 5 years, 23 ± 4% at 10 years, 27 ± 4% at 15 years, and 29 ± 4 at 20 years. The overall mortality rate for the same time period was 24 ± 4% at 5 years, 36 ± 4% at 10 years, 40 ± 5% at 15 years, and 57 ± 6% at 20 years. Between 2003 and 2014, the incidence of BOS was 8 ± 2% at 2 years, 17 ± 3% at 4 years, 21 ± 4% at 6 years, 24 ± 4% at 8 years, and 29 ± 5% at 10 years. The overall mortality rate for the same time period was 14 ± 3% at 2 years, 22 ± 4% at 4 years, 27 ± 4% at 6 years, 32 ± at 8 years, and 36 ± 5% at 10 years.

Mortality

Postoperative cause of death before and after 12 months is shown in Table 9. The group called 'other causes' is defined as mortality caused by myocardial and cerebral ischaemia, and multiple organ failure as well as other causes related to the patient's age and health status.

Table 9
Cause of death according to recipient transplantation type and time after transplantation

Tx-type; Cause of Death	< 12 months	> 12 months	*p*-value
Total: 278			
SLTx ($n = 97$)			
Total number of deaths	17	51	0.158
Death from Organ Rejection	2 (12%)	10 (20%)	
Death from Infection	4 (23%)	16 (31%)	
Death from Malignancy	1 (6%)	10 (20%)	
Death from Other Causes	10 (59%)	15 (29%)	
DLTx ($n = 172$)			
Total number of deaths	16	47	0.388
Death from Organ Rejection	4 (25%)	20 (42%)	
Death from Infection	4 (25%)	6 (13%)	
Death from Malignancy	1 (6%)	6 (13%)	
Death from Other Causes	7 (44%)	15 (32%)	
HLTx (n = 9)			
Total number of deaths	1	4	0.576
Death from Organ Rejection	0 (0%)	1 (25%)	
Death from Infection	0 (0%)	0 (0%)	
Death from Malignancy	0 (0%)	0 (0%)	
Death from Other Causes	1 (100%)	3 (75%)	

Discussion

Pre-clinical and clinical implementation

This exploring work was started out to customise the PExA device to be used in conjunction with mechanical ventilation. The device has never before been used in intubated subjects on mechanical ventilation. To be able to use the PExA device in conjunction with mechanical ventilation a closed respiratory circuit has to be constructed. The PExA device is connected to the outflow track and rebreathing is eliminated by the use of non-rebreathing valve and the respiratory circuit has no additional air leakage.

In Paper I measurements were performed during *in vivo* studies, at post-mortem and during EVLP. In Paper II the feasibility was studied *in vivo* over a period of consecutive days with the animals anaesthetised continuously. No adverse event (mild, moderate or severe) was observed. There were no signs of airway leakage, signs of rebreathing, altered pressure levels or impact on haemodynamic parameters.

After this initial pre-clinical work, an application was submitted to the local ethical board and ethical clearance to perform the first pilot study in humans was granted.

Before clinical implementation, intensive care and operating theatre personnel were informed and educated about the PExA device. During all clinical measurements a trained anaesthesiologist was always in the room along with an attending nurse.

In Paper III the PExA technique was used in conjunction with mechanical ventilation postoperatively in LTx patients and measurements were performed daily until extubation. In Paper IV the PExA technique was used on patients having lung surgery and also during OLV. In these two different clinical settings no adverse event (mild, moderate or severe) was observed. There were no signs of airway leakage, signs of rebreathing, altered pressure levels or impact on haemodynamic parameters.

In conclusion, we did not detect any harmful effects in any of the intubated subjects using the PExA device, and we believe that this technique can be used safely for intubated patients on mechanical ventilation either in intensive care or in the operating theatre.

Particle flow between different ventilation modes

In Papers I–III mechanical ventilation was performed in the same subjects in two different ventilation modes: VCV and PCV. We studied differences in particle flow pattern in the different modes and during RM in both VCV and PCV.

VCV and PCV have been compared and studied extensively both in intensive care and during surgery [72, 102, 103, 203]. In Papers I and II all animals had healthy lungs at the start of mechanical ventilation. In Paper I during *in vivo* studies, we found that VCV resulted in a significantly lower particle count than PCV; a similar pattern was seen in Paper II during the first day of measurement, but the difference in the patterns was not significant. The PExA device detects differences in these two ventilation modes that can be seen in two different cohorts. What is also interesting is that in Paper II during the second day, VCV had a higher particle flow than PCV and on day 3 the particle flow was similar in the two modes. In Paper II we also studied the eight different sizes according to mean diameter measured by the OPC, where size 1 was the smallest and size 8 the largest. This study demonstrated that the composition between the eight different particle sizes had the same pattern in VCV and PCV, so the relationship between different particles is very similar, while it is their total count that differs, as seen in Figure 21. The same pattern is seen in Paper I in Figure 16, 17 and 18. The total flow patterns might indicate a change in the impact that mechanical ventilation has on the lung, which can be detected by measuring particles in exhaled air.

In Paper II on the last day of measurement one animal developed acute clinical signs of ARDS although no signs were seen during the first 2 days. This animal showed markedly increased total particle flow compared to the other animals. When studying the particle pattern within the eight different sizes divided by the OPC, a higher total particle count in both VCV and PCV was seen, as seen in Figure 21. It is a very interesting finding that signs of a severe lung injury, such as ARDS, could be detected before clinical signs such as increased levels of FiO_2 and difficulties in ventilation occurred. The particle size that stood out was size 6 and it increased on days 1 and 2 but on day 3 it was severely reduced and also the pattern among the eight different sizes appeared similar to that of the other five animals. If this change in the relationship among the particle sizes is reproducible it is a very tempting hypothesis that particles in exhaled air could be used to detect signs of early lung injury and thereby be addressed.

The use of RM is an area of ongoing research and to date a full understanding of the impact of RM is not known fully. Interestingly in Papers II and III after 2 days there was no difference in particle flow before and after RM as seen before, which might indicate that RM has a time limit. There is no real consensus on when to do an RM and for how many days during mechanical ventilation it should be done

[204-208]. This thesis gives further evidence on the importance of cautiously used RM after days on mechanical ventilation.

In this thesis a gentle and relatively short RM was used. In both Papers II and III in both modes an increase in the total particle count occurred after the RM compared to before the RM, as seen in Figure 22 and Figure 27. Even though it was not done as an RM in Paper I, it has similarities to an RM when increasing PEEP from 2 to 10. When releasing PEEP 10 back down to PEEP 2 there was an increase in particle flow in both VCV and PCV, just like in Papers II and III. Most likely the RM reopens alveoli, and this renders a higher particle flow, but the effect was over within a few minutes. RM also gave a different particle flow, depending on mode and time within the RM. VCV had an increased particle flow while PCV had an decreased particle flow during RM. It is possible that increasing PEEP from 5 to 10, changing the I:E ratio and reducing the number of breaths does not have the same impact on particle flow in VCV as in PCV. The results from these findings should most likely best be interpreted in that the therapeutic option for patients should be adjusted to their specific lung function and needs and should be addressed individually.

In Paper IV we studied exhaled particles in air both during OLV and DLV. When opening up a previously closed lung, as occurs when going from OLV to DLV, a significant increase in particle flow occurred. During both OLV and DLV there was no change in average particle mass, only in particle flow, which would indicate that more airways are opened up which gives higher particle flow but no change in composition. In Paper IV when comparing with regard to particle flow and particle mass in both mechanically ventilated (MV-NSCLC and MV-C) and NB, a significant difference was seen between mechanically ventilated and NB but not between MV-NSCLC and MV-C. This most likely indicates that closing and opening of the distal airway will increase particle flow and induce PEx, similar to the situation seen in RMs.

Blood flow and its relation to particle flow

Blood flow through the lungs is displayed schematically in Figure 2. In Paper I by using the EVLP and by keeping a stable ventilation the pulmonary flow was adjusted to different percentages of total flow, which was 4 L/min. At 25% of pulmonary flow no particle flow occurred and at every increased step by 25% a significant increase in particle flow was detected, as seen in Figure 15B. This strongly suggests that blood flow has an impact on particle flow. By increasing PEEP to 10, and in most cases in Papers I–III, the particle flow was decreased and this may, to some extent, be related to reduced blood flow. By altering the capillary wall permeability by using drugs with an impact on dilatation and

constriction of the capillary bed, blood flow differences in particle flow were seen. For vasodilation potassium was used followed by NA for vasoconstriction and finally Niprid for vasodilation. These alterations rendered a significant increase in the number of particles going from baseline to potassium, i.e. vasodilatation and from NA, i.e. vasoconstriction to Niprid, i.e. vasodilatation. This implies that blood flow and altered capillary wall permeability have an impact on the particle flow and alteration in particle flow may indicate the importance of pulmonary blood flow for particle homeostasis in the lung alveoli.

In Paper I a significantly lower particle flow was seen between early and late phase EVLP. A lung in EVLP circulation cannot continuously reproduce the components of RTLF and if particle flow is reduced this might reflect a depletion of surfactants in the lung. The lung is more prone towards harm when surfactant is depleted, and a tempting alternative would be to add surfactant to the EVLP if the lung is exposed to longer evaluation, in order to maintain the surfactant's great importance for surface tension and decrease the risk of lung collapse or for possible *ex vivo* regeneration.

Particle flow rate related to breathing pattern

In this thesis we compared mechanically ventilated patients to NB patients.

In Paper IV mechanically ventilated patients had considerably lower particle flow and lower average particle mass compared to NB patients. In the mechanical ventilation group, they were muscle relaxed and each patient had a PEEP of 5 to facilitate the exchange of pO_2 and pCO_2 by keeping the airway open, while in the NB group they used a breathing manoeuvre with large tidal volumes to promote opening and closing of the distal airway. These two different forms of breathing may just be the key to the difference in particle flow and particle mass, because one promotes an open distal airway and the other promotes opening and closing of the distal airway. It has been described previously that opening and closing of the distal airways generates PEx [18, 200, 209].

Analysed particles

In Papers I and IV we analysed particles biochemically and studied proteins and phospholipids in the form of: albumin, SP-A, DPPC and POPC.

In Papers I and IV we analysed DPPC and POPC. In Paper I DPPC was significantly increased *in vivo* compared with the EVLP late phase, as seen in Figure 19. EVLP late phase reflects EVLP after up to 6 hours with significantly

increased PVR as seen in Table 7 which indicates a decreased pulmonary function along with altered and demanding ventilation, using at intervals large tidal volumes and higher PEEP. It is a plausible conclusion that the decrease in DPPC in late phase EVLP indicates a potential lung injury.

In Paper IV DPPC and POPC were analysed and showed significantly lower levels for mechanically ventilated patients compared to NB, as seen in Figure 30A and 30B. Once again this suggests that breathing promotes more closing and opening of the distal airways, and NB will generate higher amounts of particles.

In Paper IV we analysed albumin and SP-A; patients on mechanical ventilation had lower wt. % of total PEx levels of SP-A and albumin/SP-A ratio compared to NB, as seen in Figure 30E and 30F. SP-A comes more from the alveoli, while albumin exists in the entire RTLF and most likely different ways of breathing mirror the part of RTLF that has been exposed to the flow of air that carries PEx out of the airways. In the SP-A and albumin analysis, both mechanically ventilated patients and NB patients had NSCLC, so the difference is more likely due to different ways of breathing then to the underlying diagnosis. Several other factors alter particle flow such as blood flow described above.

Development of primary graft dysfunction and chronic lung allograft dysfunction after lungtransplantation

In this thesis, development of both PGD and BOS after LTx have been addressed. LTx recipients have only a median survival of 5.8 years and the causes of death are strongly related to rejection [151].

In clinical practice the mildest form of PGD, i.e. stage 1, is often not seen as a condition leading to increased duration of mechanical ventilation or higher risk of complications. PGD was seen in 50% of the study cohort in Paper III and, of those, the majority (67%) developed stage 1. Those who developed PGD had a stepwise increase in particle flow from the airways over the 3 days and stayed significantly longer in mechanical ventilation than those who did not develop PGD. CRP levels in first blood samples after LTx surgery were significantly higher in the PGD group compared to those who did not develop PGD. This study has shown that there is a difference in particle flow from the airways in patients who develop PGD compared to those who do not. The increased particle flow from the airways could suggest an increased inflammatory response in the RTLF and the significantly elevated CRP levels indicate an increase in the inflammatory process.

In Paper V, a large cohort of LTx recipients was studied and the development of the most common type of CLAD, BOS, was analysed. In the study long-term

survival was significantly increased for recipients of DLTx compared to SLTx recipients. The two groups had a similar risk of BOS but the results showed that DLT is associated with a better survival. These results might reflect that in later years treatment for both bacterial and viral infections and rejection treatment have been addressed more aggressively in combination with more frequent DLTx instead of SLTx the last 10-12 years.

An interesting observation was that depending on initial diagnosis for LTx, there was an impact on the clinical outcome for BOS whereby CF and PF patients had a more favourable outcome compared to other diagnoses despite also being diagnosed with BOS. CF patients also had lower cumulative mortality even though this group has a higher probability of developing BOS compared to, for example, COPD and AAT1 recipients. This is an important aspect for clinical evaluation of CF patients for possible LTx, since patients with CF are more prone to develop CF-related arthropathy and severe and lethal chronic infections such as *Aspergillus*, *P. Aeruginosa* and *B. Cepacia* [210]. The study also demonstrated that CF patients had a significantly higher incidence of diagnosed BOS compared to PH but they have the same overall survival rate, indicating that patients developing BOS with CF or PH have a similar chance of survival despite having BOS.

Ethical aspects

The studies were performed according to the principle of the Helsinki Declaration of human rights and approved by the Regional Ethical Review Board in Lund, Sweden.

The animal studies was approved by the Ethics Committee for Animal Research, Lund University, Lund, Sweden, Dnr for Paper I; M 154-13 and for Paper II; 8401/2017. All animals received care according to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, as well as to the USA Principles of Laboratory Animal Care of the National Society for Medical Research, and the Guide for the Care and Use of Laboratory Animals, published by the National Academies Press (1996).

Papers III, IV and V were approved by the local Ethics Committee for Research with Dnr 2017/396, 2017/519 and 2016/638 respectively. For Papers III and IV all patients also signed a written informed consent.

Conclusions

Paper I

For the first time in the world particle flow from the airways has been measured during mechanical ventilation. The study demonstrated that VCV compared to PCV resulted in a lower particle flow from the airways *in vivo*. In both VCV and PCV large tidal volumes resulted in an increase of particle flow compared to small tidal volumes. Particle flow was also shown to be affected by pulmonary blood flow. Biomarkers in exhaled air were collected and subsequent analysis was performed. The PExA device has been proven to be safe to use in conjunction with mechanical ventilation in preclinical settings in animals.

Paper II

The results from this study showed that different ventilation modes, such as VCV and PCV, generated different particle counts during different consecutive days. The study revealed that VCV and PCV had different particle flow patterns during recruitment manoeuvres. During the 3 consecutive days, all animals showed a stepwise decrease in particle count. This study has been fundamental in proving that the PExA technique is safe to use in conjunction with mechanical ventilation both as single use but also repeatedly over days in preclinical settings in animals.

Paper III

This study has shown that the PExA device is safe to use in conjunction with mechanical ventilation in an intensive care settings in patients. Lung transplant recipients who developed PGD showed different particle flow profiles from the airways before showing clinical signs of PGD. The results showed that changes in particle flow could be seen in between two different ventilation modes, during RM and also depending on duration of mechanical ventilation.

Paper IV

We have established the safety and feasibility of the PExA device during surgery in mechanically ventilated patients. This study showed a different and detectable particle flow rate in patients on mechanical ventilation compared to patients breathing normally. The study showed that different particle composition by biochemical analysis can be detected between mechanically ventilated patients and normal breathing patients.

Paper V

The study showed that DLTx recipients showed a better chance of survival despite developing BOS compared to SLTx recipients. The highest incidence of BOS was seen among CF, PF, COPD, PH, and AAT1 recipients in descending order; however, CF and PF recipients showed a better chance of survival despite developing BOS compared to the other recipients.

Future perspectives

This thesis has predominantly addressed exhaled particles in air, a relatively new technique that has been studied in mechanically ventilated subject for the first time and this has resulted in several interesting prospects for future studies.

By using EVLP and controlling blood flow rigorously we could see that particle flow showed a very strong relationship to pulmonary blood flow. In the same study by altering the capillary wall permeability by using different vasoconstrictors and vasodilators particle flow was also altered. This is an interesting finding that is worth studying further, not only to increase the knowledge of basic physiology, but also as a possible clinical tool to evaluate the interaction in the environment between lung tissue and blood.

During EVLP reduced particle flow was seen in a later stage of EVLP and since a lung cannot reproduce RTLF components it would be interesting to study the impact of applying surfactant in lung evaluation when EVLP is for longer durations, similar to the time in this study or for a longer period.

Further studies are warranted to attempt to implement the findings that particle flow can indicate lung injury even though blood gases, ventilator settings and haemodynamic parameters are within normal limits. It would also be invaluable to study if individualising ventilation settings by reducing tidal volume and keeping blood gases above acceptable would reduce particle flow and possibly affect the risk of lung injury.

In this thesis we have studied LTx patients and found that PGD, which is an inflammatory condition in the lung, can be detected with this technique before clinical signs occur. We suggest studying other inflammatory conditions such as pneumonia but also in healthy individuals who are intubated and on mechanical ventilation for other reasons and to study their particle pattern over several days and evaluate whether or not any difference can be seen and be related to whether they develop signs of VILI or not.

Clinical signs of ARDS were seen in one animal in Paper II on the third day of measurements and the particle pattern from this animal differed markedly compared to all the other animals and at the same time no clinical signs of ARDS were seen on days 1 and 2. An intriguing thought is to address the issue with ARDS and study animals under controlled circumstances with induced ARDS and

find if the particle flow is similar to the findings in this study. Furthermore, to study signs of inflammatory release such as CRP or cytokine in PEx over time.

During RM we have seen increased particle flow compared to before RM. One can speculate about the reason for this change in particle flow and maybe it is related to opening and closing of part of the distal airways. It would be of value to investigate this further in future studies as to how this change in particle flow can be applied in a clinical setting. In two studies we have seen that the particle flow is not increased after 2 days of mechanical ventilation and it would be very interesting to study further what changes have happened to the lung for this decrease in particle flow pattern to occur after 2 days.

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