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Measurement of lung tissue dynamics in artificially ventilated rats with optical coherence tomography

Abstract: Diseases of lung tissue and the airways become a major task for medical care and health care systems in modern industrial countries in the future. Suitable treatment methods and strategies for lung support and artificial ventilation are of dare need. Besides the obvious importance as life-saving intervention, the effects of usually used over-pressure ventilation onto the sensitive alveolar tissue are insufficiently understood. Therefore, it is of great interest to characterize lung tissue during artificial ventilation at the alveolar level. Those measurements can be used to link micromechanics of alveolar structures to mechanical properties of the whole lung like compliance and resistance measured at the ventilator device. This can be done only in animal experiments due to the fact that imaging techniques used in human diagnostics like CT or MRT fail to resolve alveolar tissue structures. The disadvantage of high-resolution techniques like optical coherence tomography (OCT) or intravital microscopy (IVM) is the need of a surgical access to the lung due to the limitation in penetration depth of these techniques. Furthermore, imaging dynamic processes with high-resolution imaging techniques during uninterrupted artificial ventilation is a challenging task. In this study, we present a measurement setup for combined imaging of conventional pressure-controlled ventilated rats and the visualization of volume changes of alveolar structures during one cycle of breath. A custom-made OCT system in combination with a triggered scanning algorithm was used to acquire time-resolved 3D OCT image data. Furthermore, this system was combined with a self-adapting autofocus function for intravital microscopy to track the lung surface keeping the tissue in focal plane. The combination of new dynamic measurement modes for OCT and IVM allows new insights into alveolar tissue and will promote the understanding of mechanical behavior during artificial ventilation.

Keywords: mechanical ventilation, lung imaging, optical coherence tomography.

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1 Introduction

Securing sufficient lung ventilation is a major task in modern intensive care [1]. Not only as life-saving measure but also as a long term treatment, mechanical ventilation is a critical factor for patient's survival for a broad range of diseases regarding lung tissue and the respiratory system. Beside its enormous relevance, the effects of mechanical ventilation using positive pressure onto the sensitive lung tissue isn't sufficiently understood. Furthermore, insufficient ventilation settings can cause massive damage of alveolar structures and hamper the convalescence. Therefore, the individual adjustment of ventilation parameters for each patient will help to increase patient´s outcome after mechanical ventilation treatment and will prevent lung tissue from irreversible damage. We use high-resolution imaging techniques in animal models to understand how artificial ventilation impacts lung tissue and especially the sensitive alveoli. Therefore, optical coherence tomography (OCT) in combination with intravital microscopy (IVM) allows the visualization of alveolar structures and structural changes during uninterrupted artificial ventilation [2,3]. The custom-made ventilator enables us to mimic different ventilator settings and strategies like volume- or pressure-controlled ventilation of rats. With this setup, new insights into the mechanical behaviour of lung tissue and tissue dynamics can

be achieved supporting the development of new and more protective ventilation strategies.

2 Methods

2.1 Imaging setup

To investigate lung tissue structures and tissue dynamics, high-resolution optical coherence tomography and intravital microscopy was performed using the same beam path to obtain the same region of interest. The OCT system is described in detail by Meissner et. al [4]. It consists of a superlumineszenz diode centered at 840 nm (FWHM 50 nm) resulting in a resolution of 6 µm axial and 11 µm lateral in air and features a data acquisition rate of 12 kHz. While OCT provides 3D image information, IVM only allows 2D imaging of the lung surface. Due to a four-fold magnification, the IVM depth of focus is very small. Therefore, axial movement of the lung due to the mechanical ventilation results in a loss of focal plane and hampers measurements of structural changes from these images. To overcome this disadvantage for studying lung tissue dynamics, we introduced a tuneable liquid lens, which can be controlled by a pulse width modulated signal to shift the focal plane in a range of 2 mm which is sufficient for ventilated rats. The depth-dependent image information from the OCT scanning is used to control the liquid lens and to track the lung surface. Therefore, the whole axial tissue movement can be visualized without losing sharpness partly.

2.2 Mechanical ventilation

The custom-made ventilator for small animals consists of two independent piston pumps and magnetic valves to control flow direction [3]. It allows the individual choice of ventilation settings in a broad range to investigate the impact of different parameters on lung tissue. In this study, artificial ventilation was performed in a pressure-controlled manner. Breathing rate was set to 60 breaths per minute and inspiration to expiration ratio was 1 to 1 while ventilation pressure was set between 2 mbar and 11 mbar for end-expiration and end-inspiration, respectively. The experimental setup is shown in Figure 1. The OCT system and the ventilator are connected to provide trigger information after each ventilation cycle.

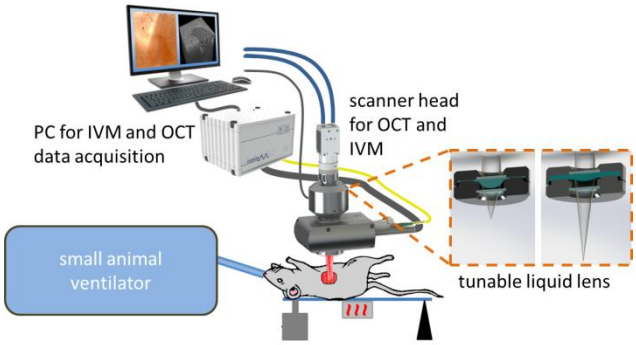

Fig. 1: Measurement setup for studying lung tissue dynamics. Mechanical ventilation is performed by a custom-made ventilator and the structural changes of subsurface alveolar structures is visualized by OCT and IVM. An additional tunable liquid lens tracks the lung surface and keeps the focal plane for IVM.

2.3 Animal experiments

All experiments were approved by the animal care and use committee of the local government authorities (Landesdirektion Sachsen; AZ 24-9168.11-1/2012-17) and were performed in accordance with the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 7th edition, 1996). Rats were anaesthetized and connected to the ventilator by a tracheal tube. To get optical access to the lung, a thoracic window is dissected by removing muscle tissue between two ribs keeping the pleura intact to prevent lung collapse. Animal temperature is maintained over the whole experimental time and fluid replacement is provided by a tail catheter.

3 Results

Exemplary measurement data during in vivo experiments are shown in Figure 2. The pressure-time-curve at the top of Figure 2 shows the used pressure-controlled ventilation mode with a rate of 60 breaths per minute between 2 and 11 mbar and an inspiration to expiration ratio of 1 to 1. Four representative time points (during inspiration (A), at max. inspiration (B), during expiration (C) and at max. expiration (D)) were chosen to better illustrate the different positions and structures of the alveolar tissue in the OCT and IVM images.

It becomes clearly visible that the use of the tunable focus lens is essential to keep the lung surface in focal plane for IVM over the whole ventilation cycle (compare to first row in Figure 2). The lateral tissue movement in small animals like rats is low and therefore, the movement of alveolar structures can be tracked during the ventilation cycle

(an exemplary alveolus is marked by the white circle in Figure 2).

The second row shows the corresponding OCT enface images. The pleura was hidden in this projection to show the alveolar structures approximately 50 µm beneath.

The lower diagram shows the area measurement of alveoli 1 (blue) and alveoli 2 (green) marked in the OCT enface images. As expected in a healthy lung, the area increases during inspiration and decreases during expiration. For a better comparability, the area at end-expiratory pressure is used for normalization.

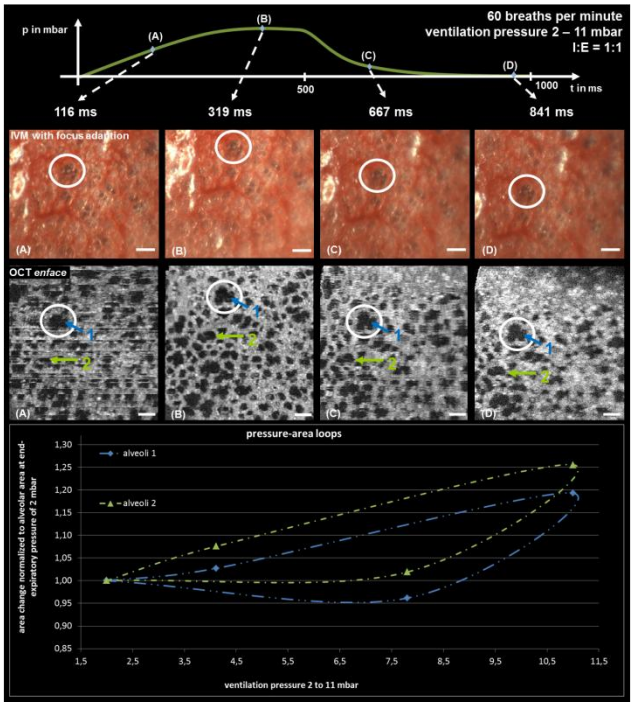

Fig. 2: OCT and IVM images at four time points during one ventilation cycle. The tunable liquid lens allows the tracking of the lung surface keeping a sharp image during axial movement. Both imaging techniques show the same region of interest and can be used for studying structural changes of alveolar tissue. The lower diagram shows a representative measurement of alveoli area changes during ventilation normalized to the area at end-expiratory pressure. Scale bar is 150 µm.

4 Conclusion

Emerging techniques like OCT provide new insights into tissue structures and dynamics as it was not possible before. The combination of time-resolved 3D OCT and intravital microscopy can promote the understanding of the mechanical behaviour of lung tissue during mechanical ventilation. Our further investigations will link the mechanical properties of microscale alveoli from OCT and IVM measurements to respiratory parameters like resistance and compliance of the whole lung. This will help to understand how mechanical ventilation affects the sensitive lung tissue and facilitates an individual adjustement of the treatment for each patient.

Author's Statement

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