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Morphometry of subpleural alveoli may be greatly biased by local pressure changes induced by the microscopic device

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ABSTRACT

Microscopy of subpleural alveoli has become an important technique to analyze alveolar morphology during mechanical ventilation. Mere contact of a microscope with the lung, however, may alter the local pressure at the pleural surface and thus the transmural pressure of the alveoli under view. The effect of local pleural pressure changes on alveolar morphology during microscopy has not been systematically evaluated hitherto.

We developed a new microscopic device enabling control of the pressure directly at the field of view. In 6 isolated rat lungs we systematically varied the transmural pressure of subpleural alveoli by varying both the local pleural pressure and the alveolar pressure. Results show fixation pressure, alveolar pressure and local pleural pressure significantly influenced alveolar size and the number of alveoli per field of view.

Our study demonstrates the important impact of local pleural pressure on the morphology of subpleural alveoli. We conclude that local pressures need to be determined during microscopy of subpleural alveoli to avoid misinterpretation of changes in alveolar geometry.

1. Introduction

Microscopy of subpleural alveoli has become an important technique to analyze alveolar dynamics, i.e. the changes in alveolar morphology during mechanical ventilation. During mechanical ventilation, the alveolar pressure is being varied with tidal ventilation and resulting changes in dimension and morphology of alveoli can be observed by microscopy. The method has been used to investigate alveolar stability in normal lungs (Carney et al., 1999), as well as in injured lungs (Dirocco et al., 2006; Pavone et al., 2007b; Schiller et al., 2001). Alveolar instability in combination with repeated intratidal recruitment and derecruitment (R/D) is being considered as an important promoter of ventilator induced lung injury (Marini and Gattinoni, 2004; Otto et al., 2008; Slutsky, 1999). Thus alveolar microscopy might serve as experimental on-line tool monitoring R/D on the level of subpleural alveoli and might be a valuable research tool for the development of lung protective ventilator strategies (Carney et al., 2005; Pavone et al., 2007b).

Subpleural alveoli exhibit, in general, a similar shape and expansion as the inner alveoli of the lung (D'Angelo, 1972). However, microscopy of subpleural alveoli may be vulnerable to artifacts, as local manipulations are necessary to install the microscope on the pleural surface: the chest needs to be opened, which per se alters pleural pressure. The microscopic device is being attached to the visceral pleura mostly by the application of negative pressure to the surrounding tissue (suction fixation) and in addition, a coverslip is being placed on the lung (Dirocco et al., 2007; Nieman et al., 1981; Perlman and Bhattacharya, 2007). Whether suctioning or glue is used for lung fixation, these techniques may limit or promote local lung expansion. While this is of minor importance under fixed volume conditions, unpredictable changes of the local pressure exerted on the alveoli from the pleural site may be induced by a mechanical interaction with the microscope when alveoli change their size. Such interaction might impede the interpretation of microscopic images. With current techniques, no information is available on the changes of local pleural pressure imposed by microscopy.

We developed a microscopic device that allows controlling the local pleural pressure in the field of view during microscopy. This study was performed to evaluate how variation of the local pres-

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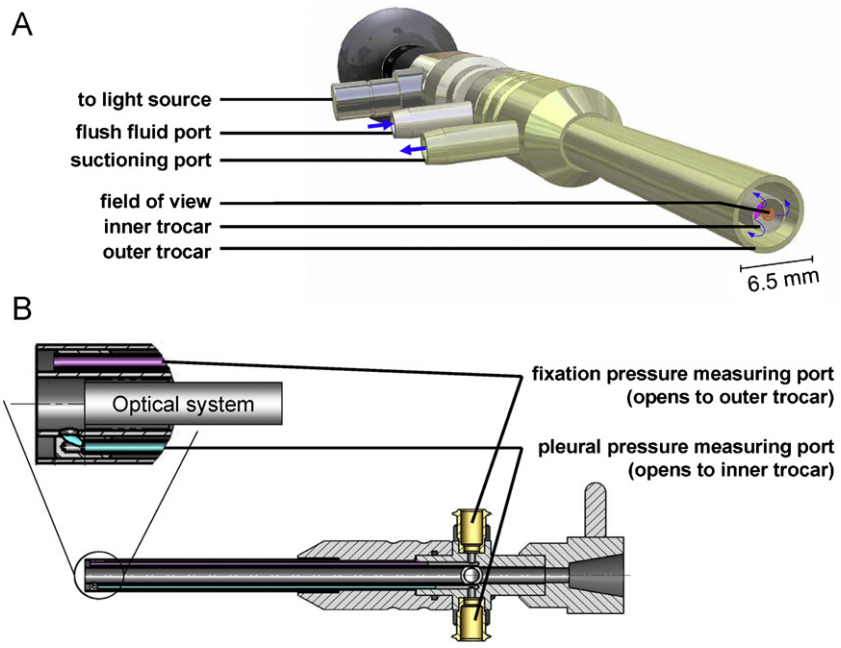

Fig. 1. (A) Schematic view of the microscope and the trocar system (pressure measuring ports omitted). The volume between the lens and the focal plane is flushed with fluid. Therefore at the field of view, no part of the system has direct contact to the pleural surface. (B) Position of pressure measuring ports within the trocar system (flush fluid and suctioning port omitted).

sures at the pleural surface changes alveolar morphology during microscopy.

2. Materials and methods

2.1. Microscopic technique

We developed a new type of endo-microscope for minimal invasive alveolar microscopy in the otherwise closed thorax. The custom-built system consists of a technical endoscope (epi-objective lens with cold-light source epi-illumination, field of view 10°, viewing direction 0°) with a total outer diameter (OD) of 2.3 mm including rigid lenses and circular light guide which is placed in a concentric double-trocar system (System manufactured by Schoelly fiberoptic GmbH, Denzlingen, Germany). The trocar system (Fig. 1A) consists of an inner trocar for flush fluid (OD 3.3 mm) and an outer trocar for suction fixation (OD 6.5 mm). The distal end of the trocar system, which forms the focal plane, protrudes past the lens by 2 mm in the axial direction. During microscopy, the volume between the lens and the focal plane (the inner trocar volume) is filled with fluid via a flush fluid port. Thus, the lens has no direct mechanical contact with the pleural surface. In addition to a flush fluid port and a suctioning port (the latter opening to the outer trocar), the system has two ports that open at the tip into the lumen of the inner and outer trocar respectively. These ports allow direct pressure measurement within the lumina of inner and outer trocar during microscopy via fluid filled lines (Fig. 1B).

The focus of the lens system was set to the plane given by the distal end of the trocar system. A CCD camera (Flexiscope IQ101 with ZOOM-Adapter, Schoelly fiberoptic GmbH, Denzlingen, Germany) was connected to the microscope. The ZOOM was set to maximum (f = 50 mm). Images were recorded as bitmap files at a resolution of 725 × 756 pixels corresponding to 0.96 mm × 1.09 mm on the pleural surface (Ulead Video studio 8, ULEAD systems Inc., Taiwan).

The pressure of the flush fluid within the inner trocar between the lens and the pleural surface (local pleural pressure) was controlled by connecting the flush fluid port to a fluid filled reservoir. The fluid level was set relative to the level of the endoscope tip to obtain the desired local pleural pressure. Negative pressure for suction fixation, which was applied to the space between inner and outer trocar (fixation pressure) was controlled by an adjustable water valve connected to a vacuum pump. Application of negative fixation pressure mostly sealed the inner trocar system, so that during the experiments no relevant fluid flow was necessary from the fluid filled reservoir to the inner trocar in order to maintain the adjusted local pleural pressure.

2.2. Isolated lung preparation

We tested our hypothesis in isolated, non-perfused rat lungs. After approval by the Commission for Animal Experimentation of the University Medical Center, 6 female Wistar rats weighting 280–350 g were anesthetized with isoflurane (Forene, Abbott, Wiesbaden, Germany) and quickly exsanguinated by opening the right carotid artery. The chest was opened and the lungs were carefully removed. The trachea was cannulized and the lungs were freely suspended from the trachea into a chamber which was continuously moistened by nebulized water to prevent drying of the lung surface. After the lungs were re-expanded by raising the airway pressure to 40 cmH_2O for a period of 10 s, the microscope was attached to the pleural surface of the left lung by setting fixation pressure to −5 cmH_2O. Subsequently the inner trocar and the pressure measuring ports were filled with normal saline. Fixation pressure and pleural pressure measuring ports were connected to pressure transducers (Medex MX960) using fluid filled rigid lines. Airway pressure was measured using a piezo-resistive transducer (SI-special instruments, Noerdlingen, Germany). All signals were digitized and visualized on-line (NI PCI 6289, National instruments, Austin, TX). Prior to each experiment, all transducers of the system were calibrated.

2.3. Study protocol

Subpleural alveoli of excised rat lungs were observed in three series in which the pressures at the pleural surface and static