



Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

<https://www.esrf.fr/misapps/SMISWebClient/protected/welcome.do>

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.



	Experiment title: LUNG INJURY AS CONSEQUENCE OF ROUTINELY ADMINISTERED MECHANICAL VENTILATION	Experiment number: MD738
Beamline: ID17	Date of experiment: from: 30 Apr 2013 to: 05 May 2013	Date of report: Feb 2015
Shifts:15	Local contact(s): Christian Nemoz	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): Professor Gaetano Perchiuzzi Professor Goran Hedenstierna Professor Anders Larsson		

Report:

Background: Due to positive pressures and tidal stretch, mechanical ventilation is thought to induce microscopic mechanical injuries in the acini causing inflammation, which can spread to the whole organism. This condition may induce multiple organ failure and ultimately death (1). Complications of mechanical ventilation can occur in normal lung, as a result of mechanical ventilation during routine anesthesia. Moreover, Ventilator-induced lung injury (VILI) is one of the major reasons for increased mortality in ventilator treated patients with acute respiratory distress syndrome (ARDS), and in patients with acute respiratory failure, in the intensive care setting. The intimate mechanisms of exaggerated mechanical stresses in the lung tissue are not known, neither in normal or injured lung. Different hypotheses such as cyclic recruitment/derecruitment (R/D) of airways and alveoli, or displacement of fluid bridges within these structures have been proposed. These phenomena can be highly dynamic and are thought to evolve progressively over time leading to gradual increases in lung elastance (2). Little is known however, about the short time-scale dynamics of R/D. The goal of this study was to quantify short-term R/D by repeatedly imaging the lung within tens of seconds, at length scales within the pulmonary acini (3) and to assess the effect of positive end-expiratory pressure (PEEP) on regional lung parenchymal microdynamics.

Methods: New-Zealand White rabbits (n=6) were anaesthetized, paralyzed and mechanically ventilated in PC mode (Vt: 8 ml/kg; RR: 40; FiO₂ 0.6; I:E 1:2, and PEEP: 3 cmH₂O). In order to enhance structural features, phase-contrast CT imaging was performed using synchrotron radiation and reconstructed with a phase-retrieval algorithm [1] with a 45.5×45.5×45.5 μm³ voxel size. Following a recruitment maneuver (20 cmH₂O, 10 s) 44 contiguous lung slices were imaged in 22 s, 3 times at approx. 100 s intervals, in 3 adjacent axial levels consecutively at PEEP 12, 9, 6, 3 and 0 cmH₂O. An example in one axial slice at PEEP0 is given in Figure 1. The imaging sequence was repeated after lung injury induced by 100 ml/kg whole lung lavage followed by injurious mechanical ventilation (Peak pressure 35 cmH₂O, Peep: 0 cmH₂O, FiO₂ 1.0) for 120 min. The volume of aerated lung was calculated on the basis of CT density. The regional distribution of R/D

was analyzed by subtracting subsequent volumetric images following image-registration, using a B-spline elastic registration algorithm (Figure 2).

Cross-sections (CSaw) of the same individual airways were measured at all PEEP levels at baseline and - in 4 rabbits - after lung injury. Strain was defined as: $(L - l)/L$; L and l: airway perimeter at 12 and 0 cmH₂O, respectively.

In order to further assess expansion and recruitment in the lung periphery, in 5 animals, the CT images were transformed into binary images, using 3 different thresholds, aiming at differentiating airspaces from tissue. We measured the numerosity of recruited airspaces and the surface they covered, as an expression of alveolar recruitment and distension at different lung volumes, using a computer algorithm which estimated the number of airspaces (NA), measured their surface area (SA) and calculated the SA/NA ratio in one Regions of Interest (size: 910x455 μ m) per image.

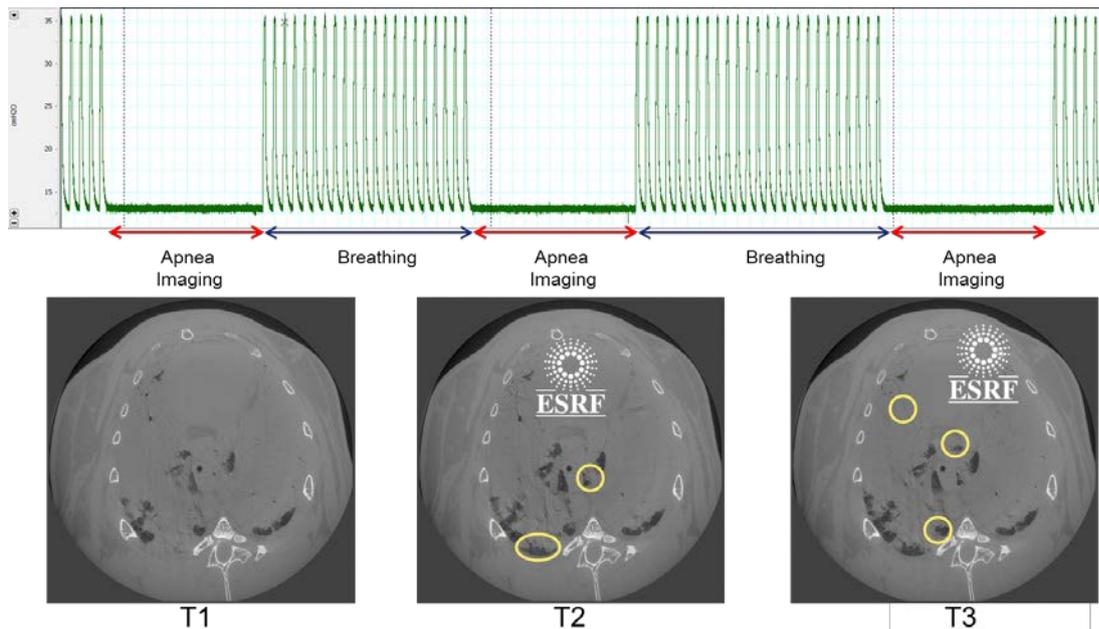


Figure 1. Top: airway opening pressure showing the sequence of image acquisition; bottom: sample images in injured lung in one axial slice level at 3 successive timepoints.

Results

1. Short-term R/D demonstrated by phase-contrast CT imaging in injured lung

Clustered areas of both recruitment and derecruitment occurred over the studied time intervals (Figure 2). Although proportionally better aeration was maintained with PEEP, R/D occurred at all PEEP levels (Figure 3). Our data demonstrate that recruitment/derecruitment in injured lung occurs over short time scales, often in clustered regions where neighboring regions showed alternating R/D behavior. These findings suggest that the critical opening pressure of peripheral lung units shows both spatial and temporal heterogeneity and that mechanical interdependence between neighboring lung regions are possibly involved in the dynamics of R/D. These results are important for better understanding of the dynamics of ventilator-induced lung injury.

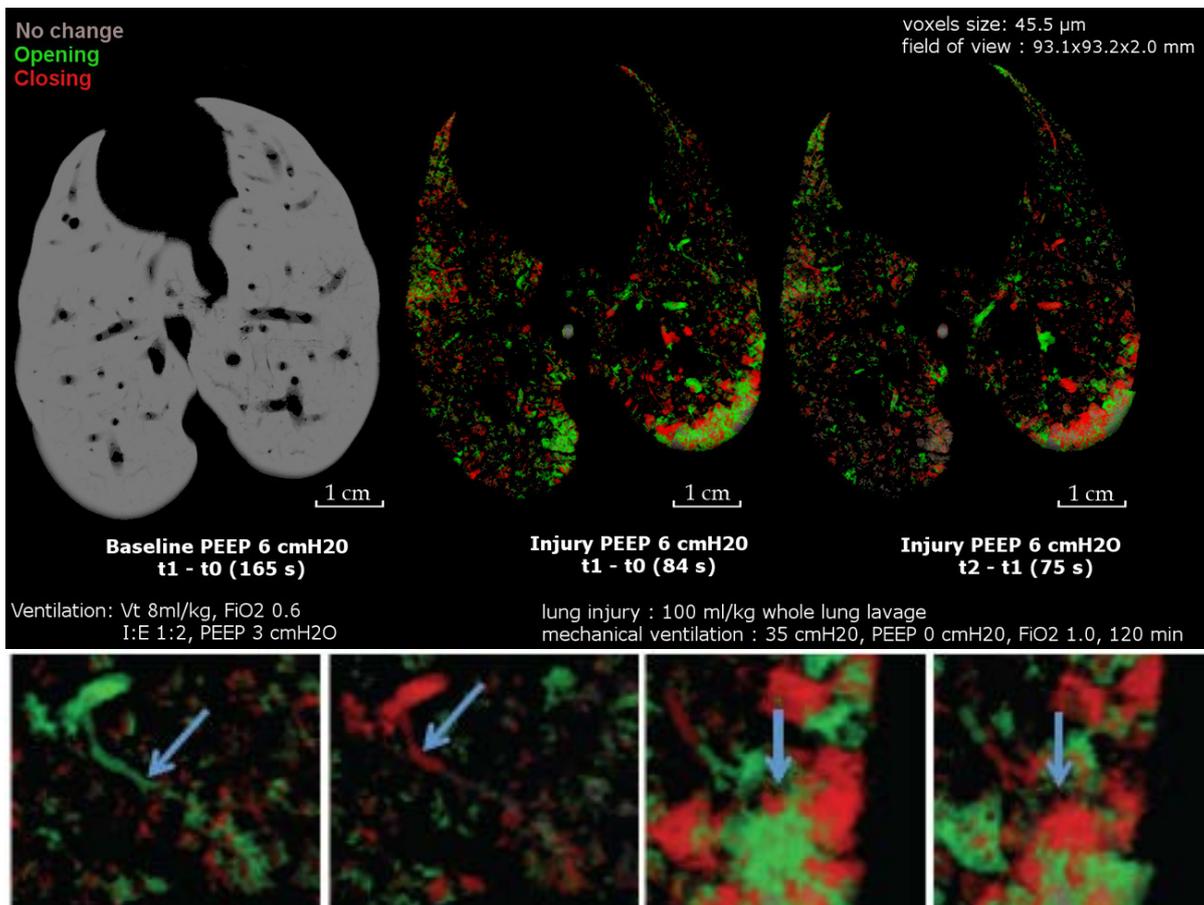


Figure 2. Rabbit Injured lungs under mechanical ventilation in vivo. Top: R/D volumetric analysis at PEEP 6 cmH₂O, showing closed airspaces that remain closed (black), opening airspaces (green), closing airspaces and open airspaces that remain open (grey).; bottom: the left two panels show a terminal bronchiole, which is initially opening (green) and few breaths later closing (red). The right two panels are magnifications showing that within an acinus, the central alveoli are initially opening (green), while those in the periphery are closing (red); a couple of breaths later the central alveoli close and then empty themselves in the peripheral ones, thereby suggesting an alternating opening/closing behaviour.

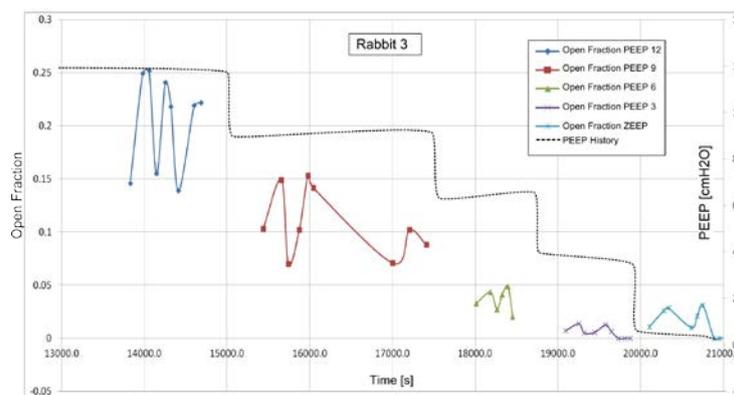


Figure 3. Short-term changes in fractional lung aeration in injured lung, at different PEEP levels.

2. Individual airway narrowing and in normal and injured lung

The same 8 airways were measured per animal and per PEEP level at baseline (n=48, radius (r): 1.7 to 0.21 mm), and after injury (n=32). Airway narrowing was significantly increased at 3 and 0 cmH₂O, after injury (Figure 3). Closure was observed at 0 cmH₂O in 2/48 (4.2 %; r: 0.35±0.08 mm at PEEP12) airways at baseline and 5/32 (15.6 %; r: 0.28±0.09 mm) airways after injury. The mean slope of CSaw vs. PEEP was significantly increased after injury (0.072±0.012 vs. 0.062±0.012, p<0001). We found a significant relation between narrowing and airway caliber at PEEP12 in injured, but not in normal lung (R²=0.67, p<0.001). A ΔP of 12 cmH₂O produced significantly larger strain after injury: 0.65±0.19 vs. 0.52±0.16 (p=0.0018). Phase-contrast CT imaging allowed in vivo assessment of individual airway behavior in normal and injured lung. Furthermore, the closing pressure and compliance of the studied airways could be estimated *in vivo* (Figure 4), and are valuable in producing a computational model of the airway tree under mechanical

ventilation, which is currently underway. These results demonstrate that the propensity for airway closure is increased in lung injury. Furthermore, similar static airway pressure changes produce significantly larger strain in the airways of injured lung.

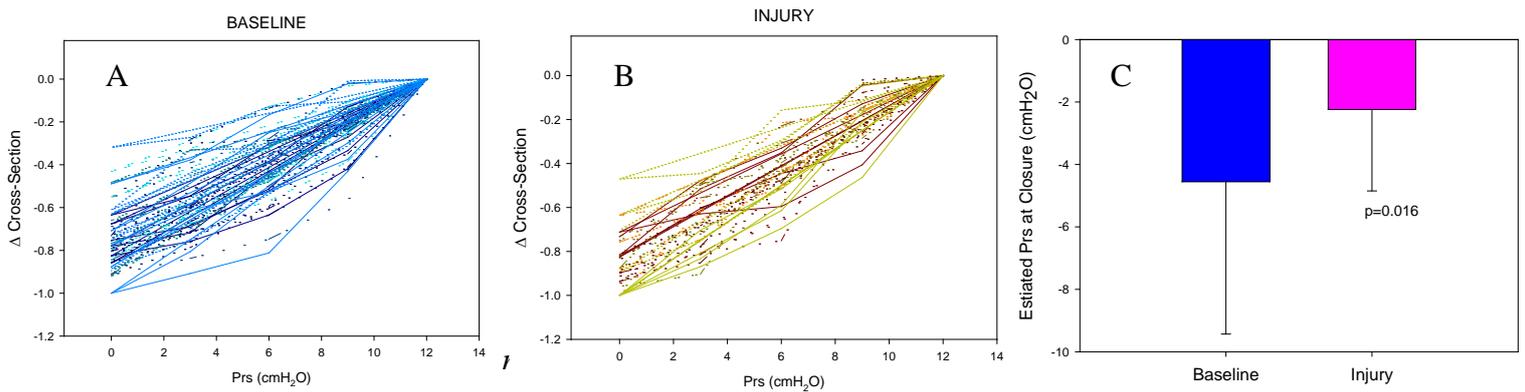


Figure 4. Relative changes in airway caliber vs. airway pressure in normal (A) and injured lung (B); mean respiratory system pressure at airway closure in normal and injured lung (C).

3. Alveolar recruitment and distension during mechanical ventilation

With increasing lung volume, SA increased in both healthy and injured lung. While NA was curvilinear with a peak at 6 cmH₂O (PeakNA), it steadily increased in injured lung (Figure 5). The SA/NA ratio in healthy lung was lower at PEEP < PeakNA than at PEEP > PeakNA. The SA/NA ratio in injured lung had a non-uniform behavior among the different animals. This analysis shows that in healthy conditions at low lung volume the predominant effect of increasing lung volume (such as during inspiration) was recruitment of airspaces. After reaching a peak, the surface area of already open airspaces increased. In injured lung, a marked mechanical inhomogeneity was found where recruitment and distension followed different patterns in individual animals, reflecting various degrees of mechanical instability, likely due to the different severity of injury.

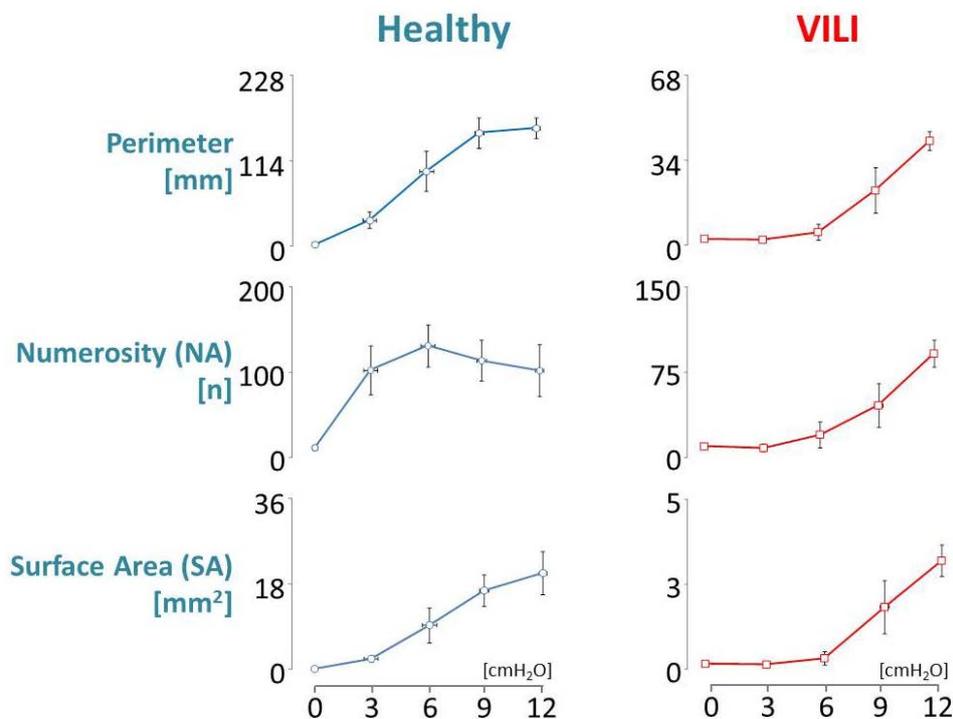


Figure 5. Perimeter, Numerosity and Surface area of lung airspaces, computed at different PEEP levels, during healthy conditions and after Ventilator Induced Lung Injury (VILI).

Conclusions: Our data demonstrate that : 1) a substantial cyclic opening and closing of terminal bronchioles and of alveoli, in contrast to the healthy lung where this phenomenon was absent; 2) a positive end-

expiratory pressure up to 12 cmH₂O, commonly used clinically in ARDS, reduced but did not entirely prevent this phenomenon. These findings are significant and further current understanding of the mechanisms leading to VILI in the mechanically ventilated lung. Further study will focus on imaging pulmonary acini at higher spatial resolutions *in vivo*.

Published abstracts

1. G. Perchiazzi, S. Bayat, J.B. Borges, L. Porra, L. Broche, A. Sindaco, M. Pellegrini, A.P. Tannoia, S. Derosa, A. Larsson, G. Hedenstierna: Alveolar Recruitment And Distension During Mechanical Ventilation: Synchrotron Radiation Computed Tomography In An In-Vivo Model of Acute Lung Injury. Proceedings of the Congress of American Thoracic Society, 2014, A2393
2. S. Bayat, P. Pisa, L. Broche, A. Tannoia, M. Pellegrini, A. Sindaco, S. Derosa, L. Porra, J.B. Borges, A. Larsson, G. Perchiazzi, G. Hedenstierna: Individual Airway Narrowing And Closure Studied By Phase-Contrast CT Imaging In Injured Rabbit Lung. Proceedings of the Congress of American Thoracic Society, 2014, A5373
3. J.B. Borges, G. Perchiazzi, L. Broche, A. Sindaco, M. Pellegrini, A. Tannoia, S. Derosa, A. Larsson, S. Bayat, L. Porra, G. Hedenstierna: Low End-Expiratory Pressure Promotes Heterogeneity Of Regional Specific Ventilation And Lung Densities During Mechanical Ventilation. Proceedings of the Congress of American Thoracic Society, 2014, A1192
4. L. Broche, A. Tannoia, M. Pellegrini, S. Derosa, A. Sindaco, J.B. Borges, L. Porra, A. Larsson, G. Hedenstierna, G. Perchiazzi, S. Bayat: Short-Term Recruitment/Derecruitment Demonstrated By Phase-Contrast CT Imaging In Injured Rabbit Lung. Proceedings of the Congress of American Thoracic Society, 2014, A5024
5. G. Perchiazzi, J.B. Borges, G. Hedenstierna, L. Porra, L. Broche, M. Pellegrini, A. Sindaco, A.P. Tannoia, S. Derosa, F.F. Todisco, T. Fiore, A. Larsson, S. Bayat: Mechanisms of pulmonary inflation during lung injury assessed by synchrotron radiation computed tomography. Intensive Care Medicine Experimental 2014, 2(Suppl 1):O23
6. G. Perchiazzi, S. Bayat, J.B. Borges, L. Porra, L. Broche, A. Sindaco, M. Pellegrini, A.P. Tannoia, S. Derosa, S. Liccese, A. Larsson, G. Hedenstierna: Artificial Ventilation of Healthy and Acutely Injured Lungs: Sequence of Recruitment and Distension of Airspaces Assessed by Synchrotron Radiation Computed Tomography. Proceedings of ESRF users' meeting 2014, session: X-ray Imaging.

References

1. Slutsky AS, and Ranieri VM. *The New England journal of medicine* 369: 2126-2136, 2013.
2. Suki B, and Bates JH. *J Appl Physiol* (1985) 110: 1109-1110, 2011.
3. Rodriguez M, et al. *The American journal of anatomy* 180: 143-155, 1987.