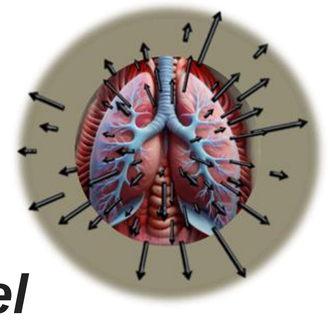
Investigating lung tissue strain in lung injury and ventilator-induced stress;

An experimental approach on macroscopic and microscopic level



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Ventilator-induced lung injury (VILI)

What if life-saving machines are actually harming the lungs?

Our HALRIC pilot project explores the mechanisms underlying mechanical ventilation-induced lung injury. Mechanical ventilation is routinely used during general anaesthesia, but also in cases of critical respiratory failure, such as in patients with acute respiratory distress syndrome (ARDS); a life-threatening condition that affects approximately 750 000 patients each year in the European Union and is associated with an annual mortality rate of around 40%. Globally, ARDS accounts for about 10% of intensive care unit admissions, representing more than 3 million patients annually.

Severe cases of ARDS require life-saving mechanical ventilation; however, this intervention may initiate or exacerbate lung injury, a complication known as ventilator-induced lung injury (VILI). Using X-ray microtomographic (µCT) 4D imaging and lung tissue histology, we aim to identify biomarkers and better understand extracellular matrix (ECM) alterations that may predict or explain the onset of VILI.

Aim of the project

- ❖ Assemble a multidisciplinary team with expertise in:
  - Preclinical imaging
  - Lung injury models
  - Mechanical ventilation and ventilator biomechanics
  - Clinical pathology of ventilator-induced injury
  - Tissue processing and ex vivo high-resolution tomography imaging
  - Precision-cut lung slices (PCLS) and ex vivo cyclic stretch models
  - Biomarker identification and signalling pathway analysis
- ❖ Optimize in vivo and ex vivo systems to study VILI at macro- and microscales
- ❖ Validate the in vivo and ex vivo systems in a pilot study
- ❖ Initiate the identification exploration of imaging and biomarkers associated with the onset of VILI

Acquired data and preliminary results

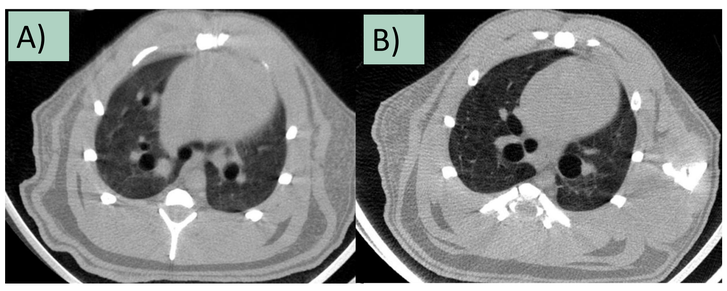


Figure 1: CT protocol optimization. Changing parameters and post-processing settings enabled image improvement, as an initial step during the pilot study. Same rat was scanned at two occasions A vs. B. before disease onset.

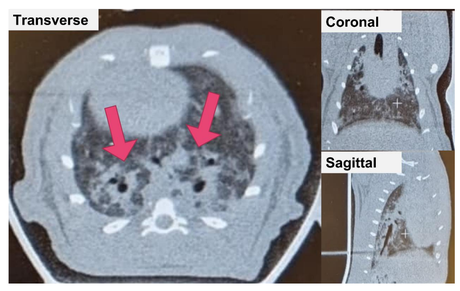


Figure 2: Image of a rat with ARDS, shown in three directions. Arrows pointing at widespread lesions in the lungs.

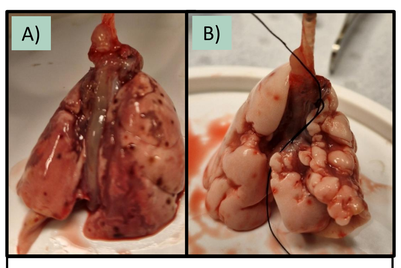


Figure 3: Rat lungs dissected and inflated manually with air to visualize injury on a macroscopic scale, after ARDS.

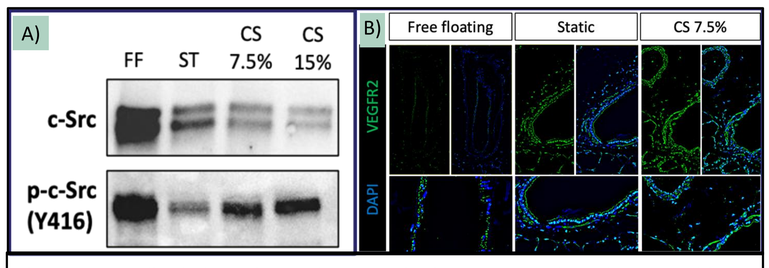


Figure 4: Antibody-based protein analysis (Western Blot) and Immunohisto-chemistry staining (B) of PCLS under free-floating, static, 7.5% and 15% strain conditions. Representative Western blot showing total c-Src protein (top) and phosphorylated c-Src levels (bottom). VEGFR2 and DAPI (cell nuclei) stain. Both c-SRC and VEGFR2 are involved in mechano-transduction signalling linked to cellular responses to stretch and mechanical strain.

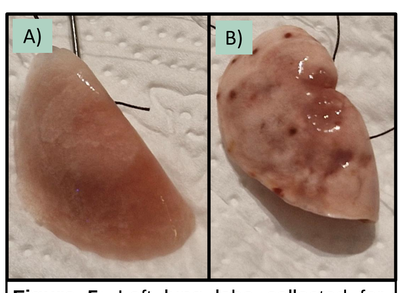


Figure 5: Left lung lobe collected for histological assessment, showing lung collected from a healthy rat (A) vs. rat with lung injury (ARDS) (B).

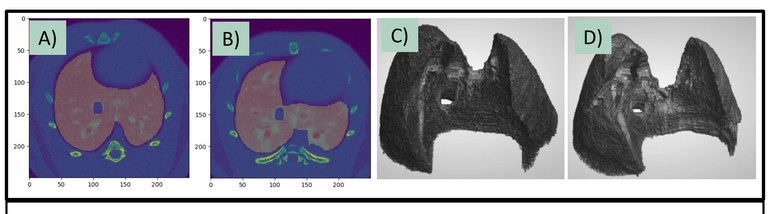


Figure 6: Image segmentation by InfraVis showing transverse mid-section of (A) healthy lungs and (B) lungs from a rat with VILI. Corresponding (C) healthy and (D) VILI lung as whole Region of Interest, showed as 3D view.

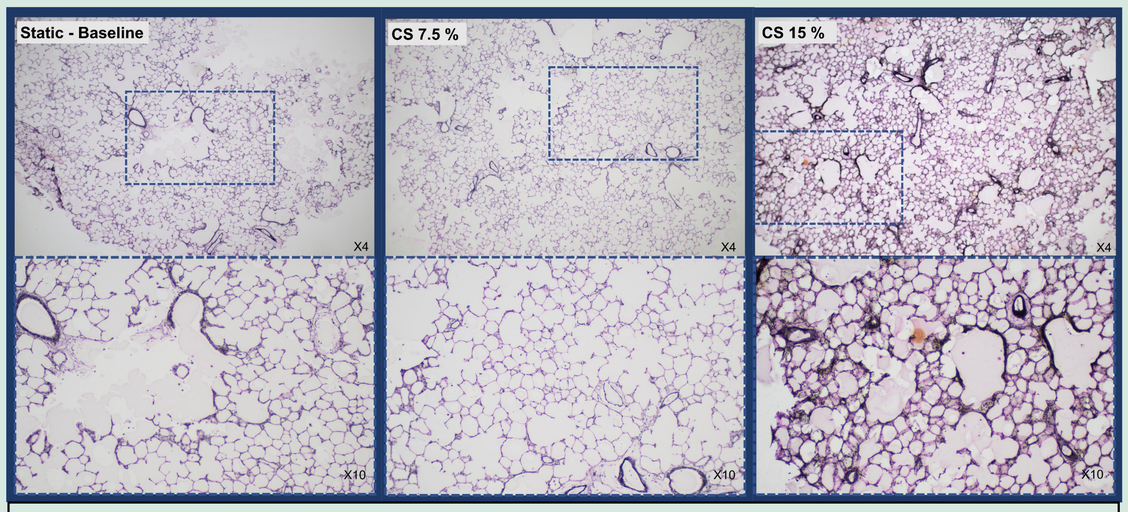
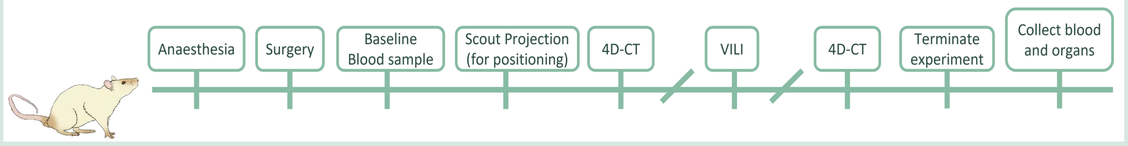


Figure 7: PCLS exposed to Static stretch (baseline with only attached lung tissue slices), compared to 7% and 15% cyclic stretch (CS), showing increased positive elastin staining. Top panel showing 4x magnification and bottom panel is 10x magnification.

Methods

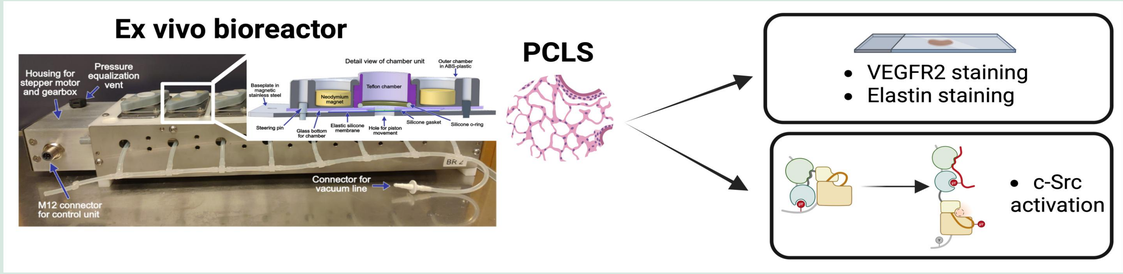
In vivo model of VILI combined with:

- 4D-CT lung imaging
- Histology and tissue staining



Ex vivo bioreactor system combined with:

- Histology and tissue staining
- Western blot analysis of candidate signaling pathways



The team and affiliations

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