SB³C2023
Summer Biomechanics, Bioengineering and Biotransport Conference
June 4-8, Vail, CO, USA

VISCERAL PLEURA MECHANICS: A COMPARISON BETWEEN PORCINE AND RAT LUNG TISSUE

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INTRODUCTION

The visceral pleura is a thin layer of stiff tissue on the surface of the lung, encapsulating the spongy parenchyma. Among its many roles, the visceral pleura helps seal the lung tissue which conducts air exchange. Visceral pleura injuries cause air leaks and are amongst the most common complications in thoracic surgery [1]. Patients undergoing a lobectomy have an 8% to 26% chance of experiencing a prolonged air leak (PAL), defined as air seepage into the pleural cavity persisting for more than 5 days after the procedure [2,3]; Patients experiencing PAL after a lung resection experience higher morbidity rates and lengthier hospital stays [4]. Today there are many lung sealants both on the market and in development to help reduce PAL in thoracic surgery, however, the ability of the sealants to reduce PAL are often inconclusive [5]. Sealants must adhere to and synchronously stretch with the visceral pleura at the site of injury, and therefore characterizing the mechanical properties of this biological tissue is central to understanding how to improve sealants and patient outcomes [6].

Currently, only a few studies characterize the mechanics of the visceral pleura and such studies are limited to uniaxial tests [7]. In-vivo rat and porcine lung studies focus on the rupture performance of lung sealants, not the mechanical behavior of the visceral pleura [8,9]. To date, there has been no mechanical characterization of rat visceral pleura while porcine pleura has been tested only uniaxially. Given the multi-axial loading experienced by the visceral pleura with each dynamic breath, unidirectional testing is an inadequate representation of tissue physiology. This study addresses this knowledge void in the literature by investigating the material properties of both rat and porcine visceral pleura using equibiaxial tensile tests, and considering regional and anisotropic dependencies.

METHODS

Fresh porcine (N=3) and rat (N=3) lungs were obtained from local vendors (Sierra for Medical Science, IACUC exempt). Visceral pleura specimens were collected from both ventral and dorsal sides of the lung for both porcine and rat lungs (Figure 1). The sheets of visceral pleura tissue were carefully separated from underlying parenchyma while tracking of sample orientation to be aligned with the cranial-caudal (CC) and medial-lateral direction (ML). A total of six rat visceral pleura specimens (4x4mm test area) were collected (Figure 1A). Porcine lobes (Figure 1B) were substantially larger than the rat, allowing for additional consideration of upper (cranial) versus lower (caudal) lobe pleural mechanics. A total of 16 porcine samples (1x1cm test area) were collected.

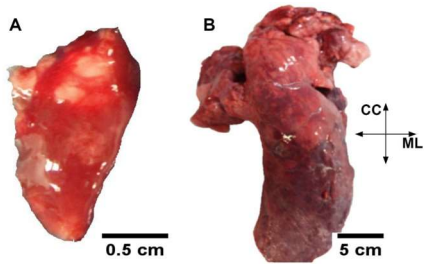

Figure 1: (A) Rat lung and (B) pig lung single lobes where the reflective outmost layer is the intact visceral pleura. Cranial-caudal (CC) and medial-lateral (ML) orientations are shown.

Tissue thickness was measured using a digital thickness gauge for porcine specimens (Mitutoyo, Kawasaki, Japan) and a confocal microscope for rat specimens (Zeiss, Oberkochen, Germany) [10]. Each specimen was loaded onto rakes and immersed in a 1X phosphate buffered saline solution bath maintained at 37°C [11]. Samples underwent equibiaxial tension tests using a commercial planar biaxial machine (1.5N load cell, BioTester, CellScale Biomaterials Testing, Waterloo, ON, Canada). Extensive preliminary tests informed the testing parameters utilized to avoid tissue damage: 60% strain was applied simultaneously in both the CC and ML directions at a strain rate

of 0.2%/s. Two preconditioning cycles were followed by a third cycle used for data analysis [11]. All tissues were refrigerated at 4°C upon receipt and experiments were conducted within 72 hours postmortem [12].

The First Piola-Kirchhoff engineering stress was calculated and the resulting bilinear stress-strain loading curves (Figure 2) were analyzed by fitting lines through both the initial and final slopes of the curve (R^2>0.95) to determine the tissue initial and final moduli [11,13]. The maximum stress at 60% strain was noted and the strain energy was calculated as the area under the loading portion of the stress-strain curve. Each metric was explored for the CC and ML orientations, and the ventral and dorsal sides of the lung. Additionally, thickness variations in the porcine visceral pleura suggested upper (79 ± 12 μm) versus lower (104 ± 25 μm) regions should also be examined; the rat visceral pleura thickness was consistent (11 ± 1 μm). No region dependent mechanics were found, and thus ventral-dorsal and upper-lower sample groups were pooled together. Data comparisons were performed using a student's t-test. Statistical significance was defined at *p<0.05, where **, ***, and **** are p<0.01, p<0.001 and p<0.0001 respectively.

RESULTS

The stress-strain response shown in Figure 2 illustrates rat and porcine CC and ML directional responses. Overall, rat visceral pleura experienced higher stresses throughout the strain range compared to porcine tissue.

Maximum stress, strain energy, initial, and ultimate moduli were compared between species. Porcine visceral pleura had an initial modulus of 1.21 ± 0.11 kPa and 0.97 ± 0.09 kPa in the CC and ML directions respectively, while rat was nearly double that of the porcine moduli (CC: 2.82 ± 0.41 kPa; ML: 2.80 ± 0.35 kPa). The ultimate modulus between species differed only in the CC direction (pig: 14.3 ± 1.26 kPa; rat: 8.64 ± 0.87 kPa). Despite the greater ultimate modulus for porcine tissue, the maximum stress was greater in rat specimens along both CC and ML directions compared to porcine counterparts. The strain energy was also significantly greater along both CC and ML orientations for the rat compared to porcine visceral pleura.

Intraspecies comparisons were also made. Regional dependencies were not found when comparing ventral and dorsal samples within each species. Despite the sizeable variation in upper versus lower lobe thicknesses of porcine samples, no regionally dependent differences were observed in the mechanical responses of porcine tissue. Anisotropic CC and ML analysis of rat visceral pleura did not exhibit any significant differences. However, porcine specimens demonstrated statistically significant greater CC values for the ultimate modulus, maximum stress, and strain energy compared to ML (Figure 3).

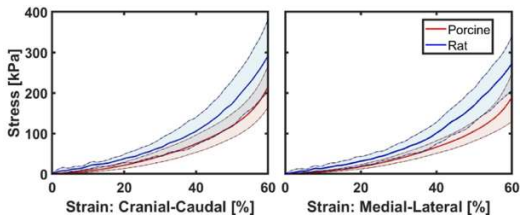

Figure 2: Average ± standard deviation stress-strain behavior for rat and porcine visceral pleura tissue in both the cranial-caudal and medial-lateral orientations.

DISCUSSION

This study compares porcine and rat visceral pleura mechanical properties via equibiaxial planar testing for the first time. Intraspecies' mechanical properties demonstrate that porcine and rat visceral pleura are homogenous with no regional variation across the lung. Rat visceral pleura was observed to behave isotropically, however, porcine specimens were anisotropic. Previous literature notes potential anisotropy for canine visceral pleura but concluded isotropic behavior due to experimental errors while determining the tissue reference state [14]. This current study is the first to report biaxial species variability and directional dependency of the visceral pleura. The anisotropic response documented in this biaxially analyzed study will be further substantiated histologically in the future, to investigate the role of the underlying fiber orientation on the directional dependency observed in porcine but not rat visceral pleura.

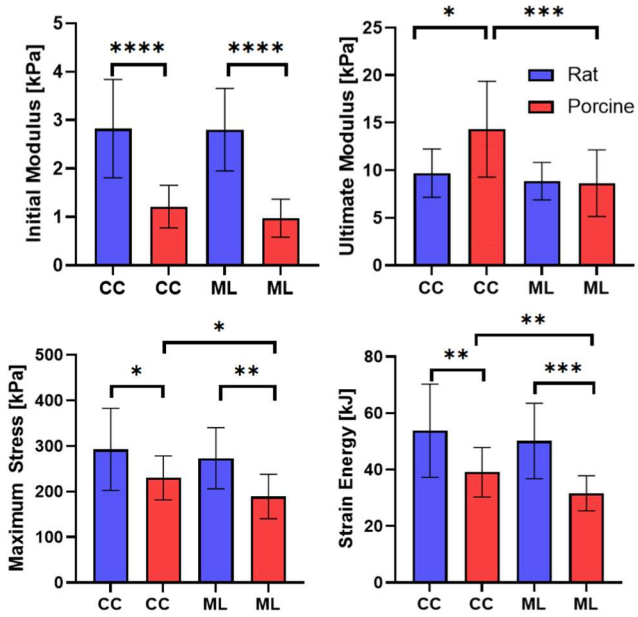

Figure 3: (A) Initial and (B) ultimate moduli, in addition to the (C) maximum stress and (D) strain energy values in both the cranial-caudal (CC) and medial-lateral (ML) directions.

Rat and porcine visceral pleura material properties are notably disparate and indicate variability from species to species. As such, the effects of differing visceral pleura mechanics among animal models should be considered when constructing and testing pleural sealants. The mechanical properties of modern tissue sealants are often tunable, and thus, comprehensively characterizing the mechanics of the visceral pleura can aid the optimization and effectiveness of pleural sealants [6]. Our future studies will also investigate human cadaveric visceral pleura lung tissue to assess the applicability of animal models for clinical translation.

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