Ultrasound Assessment of Ex Vivo Lung Tissue Properties Using a Fluid-Filled Negative Pressure Bath

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A relationship between tendon stress and strain and ultrasonic echo intensity has previously been defined in tendons, demonstrating a correlation between tissue stiffness and echo intensity. An analogous relationship between volume-dependent pressure changes and echo intensity changes in inflating lungs would indicate a correlation between lung compliance and echo intensity. Lung compliance is an important metric to diagnose

pathologies which affect lung tissue mechanics, such as emphysema and cystic fibrosis. The goal of this study is to demonstrate a correlation between ultrasound echo intensity and lung tissue mechanics in an ex vivo model using a fluid-filled negative pressure bath design which provides a controlled environment for ultrasonic and mechanical measurements. Lungs from 4 male Sprague-Dawley rats were removed and mechanically tested via inflation and deflation in a negative pressure chamber filled with hetastarch. Specific volumes (1, 2, 3, and 4 mL) were removed from the chamber using a syringe to create negative pressure, which resulted in lung inflation. A pressure transducer recorded the pressure around the lungs. From these data, lung compliance was calculated. Ultrasound images were captured through the chamber wall to determine echo intensity (grayscale brightness in the ultrasound image), which was then related to mechanical parameters. Ultrasound images of the lung were successfully captured through the chamber wall with sufficient resolution to deduce echo intensity changes in the lung tissue. Echo intensity (0–255 scale) increased with volumetric changes (18.4 ± 5.5 , 22.6 ± 5.1 , 26.1 ± 7.5 , and 42.9 ± 19.5 for volumetric changes of 1, 2, 3, and 4 mL) in a pattern similar to pressure (-6.8 ± 1.7 , -6.8 ± 1.4 , -9.4 ± 0.7 , and -16.9 ± 6.8 cm H₂O for 1, 2, 3, and 4 mL), reflecting changes in lung compliance. Measured rat lung tissue compliance was comparable to reported values from ex vivo lungs (0.178 ± 0.067 , 0.378 ± 0.051 , 0.427 ± 0.062 , and 0.350 ± 0.160 mL/cm H₂O for 1, 2, 3, and 4 mL), supporting proof of concept for the experimental method. Changes in echo intensity reflected changes in lung compliance in this ex vivo model, thus, supporting our hypothesis that the stiffness-related changes in echo intensity originally seen in tendon can be similarly detected in lung tissue. The presented ultrasound-based methods allowed measurement of local lung tissue compliance in a controlled environment, however, the methods could be expanded to facilitate both ex vivo and in vivo studies. [DOI: 10.1115/1.4027611]

Keywords: ultrasound, lung, compliance, negative pressure

Introduction

Pulmonary compliance is largely influenced by the mechanical properties of the lungs and surrounding tissues [1–3]. Pathological changes in lung structure can alter pulmonary compliance, which can impair the efficiency of ventilation and negatively affect the overall quality of life of the patient. For example, in emphysema the elastic tissue is destroyed and the lung becomes overly compliant. Although it is easy to inflate, the lack of elastic recoil during exhalation impairs lung emptying. Conversely, in pulmonary fibrosis the pulmonary compliance is decreased. Higher pressures are required to inflate the lung, increasing the work of breathing. The measurement of pulmonary compliance can have important diagnostic and prognostic value, although its measurement can be technically challenging [4–6]. In order to calculate compliance, volume change is measured with a spirometer and esophageal pressure is used as a surrogate for intrapleural pressure. This pressure is measured by a balloon catheter swallowed by the patient, which can be an uncomfortable procedure. A non-invasive method with which to accurately measure lung tissue mechanics in patients would therefore be highly valuable.

Ultrasound imaging has previously been used in connective tissues to not only provide visual information about a tissue but also quantitatively describe its mechanical properties (i.e., by relating tissue mechanical properties to changes in echo intensity, or the grayscale brightness in the ultrasound image) [7–10]. Ultrasound is highly portable, inexpensive, noninvasive, and requires no radiation or contrast injection. This makes it especially attractive in pediatric patients, pregnant women, patients who are sedated and mechanically ventilated, or those who have advanced kidney disease [11]. A relationship between tissue stress and strain and ultrasonic echo intensity (grayscale brightness in the ultrasound

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image) has previously been defined in tendons [7], demonstrating a correlation between tissue stiffness and echo intensity. However, the use of pulmonary ultrasound has been limited to guidance for pleural thoracentesis and the diagnosis of pulmonary hypertension, great vessel abnormalities, pneumothorax, and pleural effusion. We postulate that echo intensity could also be related to the mechanical properties of the lung, and that quantitative ultrasound of the lung could be developed as a technology to assess the mechanical properties of lung tissue.

Therefore, the goal of this first proof-of-principal experiment was to develop a test setup in which lung tissue mechanics could be related to echo intensity. To that end, we evaluated ex vivo lung compliance in normal rats by a testing method which uses negative pressures to inflate the lungs in a fluid-filled bath specially designed to allow ultrasound imaging to compute lung tissue compliance.

Materials and Methods

Animal Preparation and Sample Collection. All methods were approved by the University of Wisconsin IACUC committee. Four male Sprague-Dawley rats (250–270 g, ~2 months old) were euthanized via carbon dioxide exposure in accordance with AVMA Guidelines on Euthanasia [12] and lungs were isolated according to previously described techniques [13,14]. Briefly, the rat was secured supine and the trachea was isolated and cannulated with polyethylene tubing. A midline sternotomy was performed, the chest wall removed, and the lung carefully dissected from the surrounding tissue. During isolation, lungs were inflated with 3 ml of air using a syringe connected to the polyethylene tubing to minimize atelectasis during removal; the syringe remained connected until the lungs were mounted in the hetastarch-filled chamber when they were allowed to passively deflate to residual volume.

Mechanical Testing. Lungs were mounted in a polymethyl methacrylate testing chamber of known volume (115 mL). A schematic of the chamber is given in Fig. 1. This chamber has three

ports—the first port connects the lung to the outside environment and a second is connected to a pressure transducer, allowing for the measurement of the internal chamber pressure. The third is connected to a syringe which is used to create negative pressure within the chamber, inflating the lungs. After removal, the lungs were placed inside the chamber and the trachea was connected to the first port. Lungs were oriented with the trachea pointing downwards to prevent contact between the lungs and the chamber wall. If positioned horizontally, the lungs float upwards in the chamber, causing interaction between the chamber wall and the lungs and also movement out of the ultrasound frame. The chamber was then filled with hetastarch (6% hetastarch in 0.9% sodium chloride; Novaplus, Lake Forest, IL) in order to maintain tissue moisture and facilitate ultrasound wave transmission. Hetastarch is a complex mixture of derivatized amylopectin molecules of various molecular sizes and is clinically used as an iso-oncotic plasma volume expansion agent [15]. The chamber was then sealed on all sides.

A pressure transducer (Hospira Inc., Lake Forrest, IL) was attached to the second port in order to measure the pressure within the chamber relative to the outside environment. The catheter was calibrated before each use (Veri-Cal; Utah Medical Products, Midvale, UT) and zeroed before each measurement by temporarily opening it to the environment. The transducer was interfaced with an amplifier (Grass Technologies, West Warwick, RI). Data were acquired with a PowerLab data acquisition device and analyzed using the LabChart software package (AD Instruments, Colorado Springs, CO).

A 10 mL syringe was connected to the chamber's third port. Lungs were inflated by withdrawing a known volume of hetastarch from the chamber, creating a negative pressure. Because the chamber is sealed, the amount of hetastarch removed is equal to the amount of air inflating the lungs. Lungs were deflated by returning the volume to the chamber. Because this deflation was not completely passive, only inflation maneuvers were analyzed. The negative change in pressure within the chamber during inflation was used in conjunction with the known fluid volume change to plot the pressure-volume curves and calculate static compliance using the equation:

$$C = \frac{\Delta V}{\Delta P} \quad (1)$$

where ΔV represents the change in lung volume and ΔP represents the difference between the pressure in the chamber before the maneuver and the plateau pressure at the end of inflation. Static compliance was used because, unlike dynamic compliance, it is not influenced by airway resistance.

Each lung underwent a test sequence where it was inflated and deflated a total of three times at each of the four volumetric changes (1, 2, 3, or 4 mL, for a total of twelve total inflation/deflation maneuvers). Repeatability was evaluated by comparing the three repetitions at each volumetric change. This entire test sequence was repeated two more times, providing additional data to test reproducibility of the protocol.

Ultrasound Imaging. Dynamic ultrasound images were acquired in B-mode (GE LOGIQe ultrasound machine) with a GE 12L-RS Linear Array Transducer (General Electric, Fairfield, CT). The ultrasound transducer (operating at 12 MHz) was held in fixed position at the side of the bath (see Fig. 1) using a custom-built clamp. Lungs were imaged at 20 frames per second throughout mechanical testing. Ultrasound settings (including gain, time gain compensation, frequency, and focus position) were held consistent between tests and across test specimens.

Ultrasound videos were analyzed using EchoSoft™ (Echometric, Madison, WI). Briefly, this software tracks pixel movement using digital image correlation (DIC) to record pixel location and pixel intensity values for each frame. The echo intensity, defined

Fig. 1 Mechanical testing apparatus. The fluid-filled, plastic-walled chamber is conducive for ultrasound. The sealed bath had three outlets to connect the lung, the pressure transducer and a syringe. Specific volumes (1, 2, 3, and 4 mL) were removed from the box using a 10 mL syringe to create a negative pressure and inflate the lung. The same volume was returned to the box in order to deflate the lung. The pressure was measured using a pressure transducer. The lung was connected to the bath using a catheter which was open to the atmosphere. The ultrasound transducer (top right) was positioned longitudinally along the bath to record ultrasound videos simultaneously with mechanical data. The dotted box outlines the region of ultrasound analysis.

Fig. 2 Ultrasound image collected simultaneously with pressure measurements. The base of the lungs is shown in this image. A sample ROI is outlined on the left lung.

as the average gray scale brightness of all the pixels in the selected region in the B-mode image (0–255 ordinal scale), was averaged over the pixels in the region near the base of the left lung (bottom 0.5 cm).

Results

Ultrasound images were successfully collected (Fig. 2) and analyzed for each specimen.

Pressure change was successfully recorded during inflation at each volume change (Fig. 3(a)). Similarly, echo intensity in the lung increased with time during inflation and decreased during deflation (Fig. 3(b)).

Changes in pressure and echo intensity increased with increased volume change. The average pressure change across all four samples were -6.8 ± 1.7 , -6.8 ± 1.4 , -9.4 ± 0.7 , and -16.9 ± 6.8 cm H₂O (mean $\pm$ standard deviation) for volume changes of 1, 2, 3, and 4 mL, respectively (Fig. 4). Echo intensity changes of 18.4 ± 5.5 , 22.6 ± 5.1 , 26.1 ± 7.5 , and 42.9 ± 19.5 (on 0–255 ordinal scale) were observed for volume changes of 1, 2, 3, and 4 mL (Fig. 4).

Data were evaluated for reproducibility throughout the three volume change protocol repetitions. The second and third pressure measurements varied less from the mean than the first reading root mean squared error (RMSE) for first, second, and third

Fig. 4 Average changes in pressure and echo intensity from all samples at all volume changes. Both parameters demonstrate increased changes with increased volume demonstrating similar trends. Error bars represent standard error ($n = 4$).

readings were 4.56, 1.31, and 1.35, respectively); thus, the second and third measurements for each volume change in each sample were averaged for compliance calculations.

Overall compliance for each volume change was found to be 0.178 ± 0.067 , 0.378 ± 0.051 , 0.427 ± 0.062 mL/cm H₂O, and 0.350 ± 0.160 for 1, 2, 3, and 4 mL, respectively. The relationship between compliance and changes in echo intensity is shown in Fig. 5.

Discussion

The purpose of this study was to evaluate the ability of ultrasound echo intensity to assess lung mechanics. We were able to validate that ultrasound echo intensity changes increase with greater volume changes in a manner similar to pressure changes (Fig. 4); this information can then be related to lung compliance, a key marker for lung mechanics. We measured lung tissue compliance in an ex vivo model using a fluid-filled negative pressure chamber so that we could report actual compliance (by changing a

Fig. 3 Representative (a) pressure change and (b) ultrasound echo intensity data during inflation and deflation, collected for three trials on one lung. Echo intensity changes were taken near the base of the left lung. The pressure waveform and echo intensity waveforms are essentially the same shape in time.

Fig. 5 Relationship between overall compliance and echo intensity

known volume and measuring pressure) and also incorporate ultrasound imaging on that same model to introduce a new dimension of data acquisition.

Compliance values were numerically similar to previously reported compliance values for in vivo [16] and ex vivo [13] rat lung preparations. The compliance measurement in rat lungs was found to increase as the volume change was increased up to 3 mL and then a slight decrease was seen at 4 mL. Classic studies in pulmonary physiology demonstrate a nonlinear relationship between pressure and volume, the determinants of compliance [1]. At low lung volumes compliance is low, with the greatest compliance observed at moderate inflation volumes. Our results are consistent with this general behavior. As anticipated, ultrasound echo intensity measurements likewise demonstrated volume-dependent changes. Volume-dependent echo intensity changes that are related to pressure changes in inflating lung tissue are analogous to strain-dependent echo intensity changes that are related to stress changes in the stretching tendon [7], and the stiffness-related changes in echo intensity first seen in tendon [9] are here confirmed as compliance-related changes in lung echo intensity.

Our testing method was found to be repeatable within the tested range of volume changes in normal rat lungs, particularly in the second and third measurements which had RMSE values of 1.31 and 1.35, respectively. This repeatability indicates the potential application of this method to evaluate local changes associated with pathologic lung tissue, such as changes in lung compliance due to bleomycin-induced lung fibrosis [17] and elastase-induced emphysema models [18], tracking the onset and development of disease in different regions of the lung.

The custom-built negative pressure chamber was utilized to analyze lung tissue mechanics in a more physiologic manner, but introduced methodological limitations. First and foremost, this testing method required that delicate lung tissue was dissected, removed from its native environment, and handled. Though test procedures were designed to minimize contact with the lungs, all handling and tissue preparation introduces tissue damage and may result in lung mechanical behavior deviating from native tissue behavior. Also, lungs remain viable for a short time ex vivo, again contributing to varied tissue behavior. The positioning of the lung, with the trachea downwards instead of horizontal, may also alter mechanical results. Even with a uniformly applied pressure, the distribution of ventilation in the lung has a gravitationally dependent component, which may be altered when the lungs are in the revised position. However, this positioning prevented contact

of the lung with the wall of the chamber due to floatation, which may interfere with normal lung inflation. Additionally, the syringe system utilized with the chamber relied on manual volume controlled inflation and deflation. This manually controlled volume change is not as precise as machine controlled pumps. However, our method allows comparative compliance readings at any selected point on the curve, and a precisely controlled end point is not always necessary to obtain compliance information.

Further limitations of this study include the ex vivo nature of the testing (repeated inflations and deflations in an ex vivo setting may result in loss of viability which may influence compliance values, thus, limiting the number of repetitions that can be performed) and the fact that there is no chest wall present in the test setup. True in vivo compliance includes both the chest wall and the tissue compliance. However, the purpose of the study was to show that changes in echo intensity can be related to measured changes in compliance. Thus, it was less critical that the compliance of these lungs be identical to in vivo lungs than to be able to exactly measure the compliance in the tested tissue. Additionally, echo intensity was only sampled in one region, which did not allow for assessment of normal variation within a single tissue. Fibrosis and elasticity loss is likely to be locally variable and would require sampling in multiple areas of the lung, requiring knowledge of normal variation to compare to the patchy changes seen in the pathologic cases. Furthermore, the sensitivity of this measurement is yet to be elucidated and compared to traditional pulmonary function measures.

Transition to clinical application will require overcoming several challenges. Ultrasound-based techniques have shown promising ability to noninvasively estimate mechanical properties. For example, changes in 1D ultrasound echo intensity have been used to determine changes in tissue stiffness [19], and 2D ultrasound echo intensity has been used to accurately predict stress and strain in porcine digital flexor tendons [7]. Clinical application will require development of ultrasound imaging-based methods capable of detecting such changes in lung compliance in vivo. Additionally, the ultrasound measurements performed in this study examine a small area (dependent on transducer area)—in rat lung, a more substantial area is visible in a single image, but in human lungs the area would represent a much smaller percentage of the total lung surface. Thus, regional variation in lung stiffness would have to be investigated in order to provide additional insight compared to overall, compliance versus pressure measurements. Also, in a clinical setting, high resolution images will be necessary to obtain adequate information from the lung tissue, as the tissue wall is relatively thin; this issue is further complicated by the fact that ultrasound waves do not travel through air, and the air-tissue interface can result in artifact which may preclude reliable ultrasound imaging at tissue boundaries.

Negative pressure experiments more closely mimic physiologic inflation, but controlled experiments still require removal of lung tissue to measure mechanical properties. A noninvasive method with which to measure lung tissue mechanics in patients would therefore be highly valuable. This study demonstrates consistent echo intensity changes during controlled negative pressure inflations in an ex vivo setting, revealing an acoustoelastic-like effect not previously reported. These ultrasound-based methods have the potential to translate to in vivo experiments and ultimately could allow for direct in vivo measurement of tissue compliance focused on specific regions of normal and pathologic tissues.

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