Stiffness of the pleural surface of the chest wall is similar to that of the lung

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Gouldstone, Andrew, Richard E. Brown, James P. Butler, and Stephen H. Loring. Stiffness of the pleural surface of the chest wall is similar to that of the lung. J Appl Physiol 95: 2345–2349, 2003; 10.1152/japplphysiol.00588.2003.—To address the role of the parietal pleura in reduction of mesothelial shear stresses during breathing, we measured the stiffness of the parietal pleural surface of mammalian chest walls using microindentation. The pleural surface was indented over ribs and intercostal spaces with rigid flat punches (tip radii of 0.01, 0.02, and 0.1 cm) to probe stiffness at length scales comparable with those of surface asperities. We found a tissue shear modulus of 6,700 dyn/cm2 and pleural membrane tension of 4,900 dyn/cm, with a geometric standard deviation of 0.42. These values are similar to those measured for the lung by Hajji et al., using indentation (Hajji MA, Wilson TA, and Lai-Fook SJ. J Appl Physiol Respirat Environ Exerc Physiol 47: 175–181, 1979). Surprisingly, the pleural surface over ribs and intercostal spaces exhibited similar stiffness. In addition, caudal regions exhibited lower stiffness than cranial regions. In the context of elastohydrodynamic lubrication, these results suggest that shear-induced pressures during breathing deform the chest wall and lung surfaces to a similar extent, promoting spatial uniformity of pleural fluid thickness and reducing shear stresses that could lead to tissue damage. Given sufficient surface compliance, computational fluid dynamics models suggest that contact between the pleural surfaces can only occur statically and that during breathing hydrodynamic fluid pressures prevent contact (i.e., asperities are deformed in a manner that promotes gap uniformity) (3, 7). This phenomenon is referred to as elastohydrodynamic lubrication (6). However, whereas the elastic properties of the lung relevant to surface deformation have been measured (4, 5), no such measurements on the parietal surface of the chest wall have been published. An understanding of the deformability of the parietal pleural surface in vivo would provide further insight into the mechanism(s) accommodating relative sliding of lung on chest wall during breathing.

In this study, we performed displacement-controlled punch indentation experiments on the parietal surface of excised chest wall of medium-sized mammals. Cylindrical punches of radius (a) = 0.01, 0.02, and 0.1 cm were used to probe the tissue on a scale similar to observed surface unevenness (2). At this size scale, the elastic properties of the surface were found to be similar to those of the lung. The values we found for the lung were also similar to those reported by Hajji and colleagues (4, 5).

MATERIALS AND METHODS

Animals and Tissue Preparation

These experiments, approved by the institutional animal care and use committees of Harvard Medical School and Beth Israel Deaconess Medical Center (BIDMC), were completed by using chest walls and lungs of animals after their use in unrelated terminal experiments. Experiments for which these animals had been used were considered not to have a significant effect on the mechanical behavior of respiratory structures. Immediately after death, tissues were harvested from adult female and male dogs, pigs, and sheep of a range of weights (~25–45 kg) and kept moistened with physiological saline. To preserve chest wall geometry relevant to measurements of pleural stiffness (e.g., rib spacing), an intact hemithorax was harvested with attached vertebral column and sternum. Lungs were intubated and then excised without allowing them to collapse.

Histology

To characterize the anatomy of structures contributing to parietal surface stiffness, we examined microscopic cross sections of chest wall from dog and pig. Samples were immersed in an acidic formaldehyde solution (Formical-4, Decal, Tallman, NY) that was periodically changed. After preparation with standard techniques for paraffin embedding and staining, sections were viewed and photographed on a Nikon photomicroscope.

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Experimental Setup and Protocols

The brass indenter tip (Fig. 1A) had a flat cylindrical geometry and was attached to a force transducer (Grass FT0, Grass-Telefactor Instruments, West Warwick, RI). The compliance of the indenter tip and transducer was negligible compared with tissue compliance. In all cases, the cylindrical axis of the indenter tip was oriented and moved normal to the horizontal chest wall surface. Three tip sizes were used: \( a = 0.01, 0.02, \) and \( 0.1 \) cm. The transducer was mounted on a micrometer, the position of which was controlled by a stepper motor (Slo-Syn 6000i; Superior Electric; Rockford, IL) to within \( 0.0001 \) cm. Both tip displacement \( (u_z) \) and indenter load \( (L) \) were recorded (DI-220 and Windaq/Pro, Dataq Instruments, Akron, OH).

Contact between the tip and the surface was determined from the load during exploratory movements of the tip. Once located, the surface was probed with a series of eight indentations, four each to two maximal displacements \( (u_{z,max}) \) \( 0.005 \) and \( 0.01 \) cm, respectively (Fig. 1B). The rate of loading and unloading was constant at \( 0.005 \) cm/s, and there was a \( 1\)-s wait after each movement. Preliminary experiments showed little effect of indentation rate \( (0.002–0.02 \text{ cm/s}) \) on indentation response.

Chest Wall

The pleural surface of ribs and intercostal spaces was probed primarily along the most lateral aspect of the hemithorax. To determine whether the dorsoventral position on the chest wall affected indentation results, in some trials the pleural surface overlying both ribs and intercostal spaces was indented at each of three places: 1) the most lateral curvature of the ribs; 2) dorsally, just lateral to the vertebral bodies; and 3) ventrally, near the costo-chondral junctions of the ribs. All animals were examined with the tip of \( a = 0.01 \) cm. To determine the mechanical contribution of pleural membrane tension \( (T) \) to indentation response, three animals were also examined with the tips of \( a = 0.02 \) and \( 0.1 \) cm. To explore the effect of increases in rib-rib spacing on indentation response, in two animals, the pleural surface overlying two ribs \( (\text{ribs} 8\) and 9) and their intervening intercostal space was probed before and after expanding the rib-rib spacing to known strains. Experiments on each chest wall lasted \( \sim 30–60 \) min. To mitigate any influence of postmortem change, experiments were alternately conducted from the first to last rib and from last to first rib.

Lungs

For comparison with chest wall results, we indented lungs using the same apparatus and protocol. Lungs were examined at constant transpulmonary pressure \( (P_{tp}; 16 \text{ cmH}_2\text{O on deflation limb}) \) in areas of the pleural surface that were broad and relatively flat. Each indentation protocol was preceded by a standard lung volume history consisting of three inflations of \( P_{tp} \) from 2 to 30 cmH\(_2\)O. Preliminary experiments with lungs at a \( P_{tp} \) of 4 cmH\(_2\)O showed prohibitively noisy results and were thus not pursued.

Indentation Analysis

Data generation and manipulation. The indenter load signal was digitized at 240 Hz and filtered post hoc by using a moving boxcar average (Windaq Calc Software, DATAQ Instruments, Akron, OH). The displacement was calculated from time and programmed tip velocity. The filtered force output and indenter \( u_z \) were used to calculate the stiffness \( (L/\mu) \) by linear regression. In preliminary experiments, there was no difference between loading and unloading behavior (i.e., fully elastic response), so only the loading behavior was analyzed. There was no order effect among the four values at each maximum indentation depth, so those values were averaged for further analysis. Results were analyzed with JMP Statistical Software (SAS Institute, Cary, NC), with \( P \leq 0.05 \) considered significant.

Indentation mechanics. In the absence of a membrane component, the predicted load-displacement \( (L/\mu) \) response during indentation of a homogeneous, isotropic elastic half-space by a flat rigid punch of radius \( a \) is given by the following equation

\[
L = 4\mu(1 - v)u_z
\]

where \( \mu \) is shear modulus and \( v \) is Poisson’s ratio. However, in preliminary experiments with tips of different size, data did not fit this simple relation and suggested the presence of an elastic half-space covered with a tensed membrane. The existence of a tensed membrane on the surface of an elastic half-space creates a different indentation size effect, i.e., the load is no longer linear in punch radius \( a \).

To analyze indentations of the lung, Hajji (4) quantitatively determined the contribution of a surface membrane,
Parietal Pleural Stiffness

Alternate Measurement of Membrane T

To support the applicability of the above analysis (i.e., the assumption of a tensed membrane), an independent measurement of pleural membrane T was taken. A region of the intercostal muscles between ribs was removed (with no effect on rib-rib spacing), leaving only the parietal pleural membrane, and a rigid plastic tube (inside diameter = 1 cm) was attached to the exposed membrane with methyl methacrylate glue. The pleural membrane outside the circumference of the tube was cut away, and the tube with attached tissue was mounted vertically. Normal saline was added to or removed from the tube via a catheter, and the pressure distending the tube was recorded as the height of the fluid column. Changes in the distension of the bulging membrane were measured with a calibrated scale and dissecting microscope as vertical changes in position of the tensed membrane’s center. Radius of curvature was computed by the following relation:

\[ R = \frac{(D^2/4) + \Delta^2}{2\Delta} \]

where \( P \) is pressure, \( R \) is the radius of curvature, and \( \Delta \) is the vertical changes in position. Membrane T was calculated by using \( P = 2\pi R \) (Fig. 1C).

Histology

The parietal pleura, which functionally includes all the tissue components lining the internal chest wall surface over intercostal spaces and ribs, varied in gross appearance across the chest wall. Over intercostal spaces, intercostal muscles were variably obscured by whitish tissue. Over ribs, variations in appearance were due to 1) differences in the gross thickness of soft tissue and 2) the presence or absence of vascular elements. In the soft tissue over the ribs, we observed numerous vessels, whose blood-filled luminal diameters not uncommonly approached 0.1 cm. Thus we assert that these layers were at least 0.1 cm thick.

Microscopically, there were no obvious differences in the construction of the pleura over intercostal spaces or ribs. The mesothelium overlaid a layer of loosely organized connective tissues that was many times thicker than the mesothelium per se (Fig. 2). Intermixed within those loose connective tissue structures were islands of adipose tissue. Morphological differences between sites on the parietal surface were primarily due to thickness of the loose connective tissues layer.

Overall Response

Figure 3 shows a typical set of results over an intercostal space for a tip of \( a = 0.01 \) cm. There was no significant difference in stiffness among species or individual animals examined, so results from all experiments were grouped for analysis. Analysis of \( >500 \) indentations of parietal pleura with a tip of \( a = 0.01 \) cm revealed no significant difference in stiffness between examinations to maximal \( u_z \) of 0.005 and 0.01 cm, nor between examinations over intercostal regions and over ribs. In addition, there was no significant effect of dorsoventral location on indentation stiffness, with the following exception: the costochondral junctions not covered by the transversus thoracis muscle occasionally exhibited stiffness beyond the measurable range of the indenter.

Figure 4 shows the results of indentation experiments from seven individuals of three species for the
indenter tip of \( a = 0.01 \text{ cm} \). Values of stiffness approximated a log normal distribution and are thus displayed on a log scale here. The mean value of stiffness shown for each \( n \)th region represents contributions from both rib and adjacent intercostal space. ANOVA and pairwise Tukey-Kramer analysis revealed that indentation stiffness decreases by 75% cranial to caudal, specifically between the ribs 1–6 and the ribs 7–11. Also included on the figure are the results from the present lung indentation experiments; note the similarity to chest wall results.

**Calculation of Elastic Properties**

Analysis of a smaller set of 100 indentations with tips of \( a = 0.01, 0.02, \) and 0.1 cm revealed no significant difference in stiffness between the two smaller indenters and a twofold difference in stiffness between the smaller indenters and the largest indenter. This dependence of stiffness on indenter size is significantly weaker than predicted for indentation of an elastic half-space, and therefore the effect of a surface membrane was quite strong (see MATERIALS AND METHODS). From these indentation measurements, and assuming that \( v = 0.5 \) for hydrated tissue, the parietal surface was found to have a \( \mu = 6,700 \text{ dyn/cm}^2 \) and \( T = 4,900 \text{ dyn/cm} \), geometric standard deviation = 0.42. Membrane \( T \) calculated from the water column experiment was ~3,000 dyn/cm (range 2,500–3,500 dyn/cm, \( n = 3 \)), which agrees well with the values obtained from indentation experiments. The values of \( \mu \) and membrane \( T \) are also similar to those obtained for the lung by Hajji et al. (4, 5). As illustration, Fig. 4 shows predicted indentation responses for lungs at \( P_{\text{tp}} = 4 \) and 16 cmH_2O, calculated from mean elastic properties (\( T, \mu \), and Poisson’s ratio) measured by Hajji et al. (4, 5), which clearly provide close upper and lower bounds to the bulk of the chest wall data.

**Effect of Rib Spacing**

Figure 5 shows indentation stiffness as a function of rib-rib lateral strain (\( \Delta R_{so}/R_{so} \)), where \( R_{so} \) denotes rib spacing unstrained by retractors and \( \Delta R_{so} \) denotes change in rib spacing with retraction. Black and white circles denote response over intercostal spaces and ribs, respectively. For \( \Delta R_{so}/R_{so} < 0.05 \), there was little change in stiffness compared with stiffness at \( R_{so} \); for higher strains, stiffness markedly increased.

**DISCUSSION**

There were few exceptions to the otherwise mechanically homogeneous behavior of the chest wall’s pleural surface. For the most part, indentation over intercostal spaces and ribs exhibited similar results. Stiff outliers in the data are apparent on Fig. 4, especially in regions 5–7, where the difference between maximum and minimum stiffness spans two orders of magnitude. However, the predicted indentation stiffness of bone obtained with the use of our indenter tip geometry (as calculated by using values reported in Ref. 9) would be three to four orders of magnitude higher than the stiffest data points for rib on the figure. Thus the relatively similar indentation response in these regions suggests the existence of a uniform protective pleural “blanket” spanning the pleural surface of the chest wall. Even more importantly, we were surprised to find that on the size scale of the microindentations (0.01–0.1 cm) the parietal surface of the pleural space is no stiffer than its corresponding visceral surface (i.e., indentation of chest wall and lung gives similar re-
sults; see Overall response). The comparable stiffness of the two surfaces, in the context of elastohydrodynamic lubrication (3, 7), suggests that they play equally important roles in the smoothing of asperities during sliding. In addition, membrane T was found to have a large influence on indentation stiffness at this size scale, which is in agreement with the dependence of stiffness on rib-rib spacing. (However, it must be noted that rib-rib strains at which stiffness markedly increased were considered nonphysiological, because the pleura began to tear under such stretching.) A qualitative discussion on the effect of a surface membrane on the elastohydrodynamic smoothing of asperities is discussed in Ref. 3.

The significant craniocaudal dependence of surface stiffness (the tissue is least stiff in caudal regions) is relevant in that highest sliding velocities in the pleural space typically occur in caudal regions. Thus, in caudal regions where a mechanism of protection would be most necessary, the most compliant tissue exists and most easily deforms to lower potentially damaging mesothelial stresses. In addition, in regions exhibiting exceptionally high stiffness, e.g., the surface overlying the costochondral junctions, elastohydrodynamic lubrication may be achieved solely due to deformation of the visceral pleura.

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DISCLOSURES

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