Published in final edited form as:

Respir Physiol Neurobiol. 2011 July 31; 177(2): 114–119. doi:10.1016/j.resp.2011.03.030.

Influence of the softness of the parietal pleura on respiratory sliding mechanisms

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Abstract

The pleural surfaces of the lung and chest wall slide against each other with low friction. Normal load support can be effected either by a combination of quasi-static fluid pressure and solid-solid contacts of relatively stiff asperities, or by shear-induced hydrodynamic pressures in the pleural fluid layer. To distinguish between these mechanisms, we measured surface topography and spatial distribution of stiffness of rat parietal pleura using atomic force microscopy. The topography of the pleural surface has unevenness at length scales smaller than the thickness of pleural fluid, similar to mesothelial cell diameters. The estimated maximum normal contact pressure that could be borne by asperities of the soft pleura is much less than that required to support a substantial difference between pleural fluid pressure and the pleural surface pressure. These results suggest that during sliding motion, unevenness of the pleural surface is smoothed by local hydrodynamic pressure, preventing any significant contribution of solid-solid contacts.

Keywords

Parietal pleura; AFM; Elastohydrodynamic lubrication; Rat; Topography

1. Introduction

During breathing, the pleural surfaces of the lung and chest wall slide reciprocally relative to each other. The lung and chest wall surfaces experience local, variable normal loads responsible for the conformity of their shapes, and they are lubricated by pleural fluid, sliding with very low friction. A controversy exists concerning the potential existence of contacts between the pleurae under normal breathing. Following one argument (Agostoni, 1986), the measured difference between the pleural fluid pressure and the pressure on the surfaces of the lung and chest wall would require the presence of load support from

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asperities on the two surfaces coming into contact. Sliding in the presence of solid-solid contact could be facilitated by a boundary lubrication mechanism. In this picture, a substantial part of the normal loads would be balanced by contact forces at pleural asperities, where contact pressure would depend on the geometry of deformation and local elastic properties of surfaces. A test of this idea involves evaluation of the magnitude of contact pressure that the asperities could exert.

An opposing view, supported by the finding of a continuous fluid layer separating the pleural surfaces, maintains that hydrodynamic pressures in the pleural fluid maintain separation of the pleural surfaces (Lai-Fook, 2004). Sliding without solid-solid contact would be facilitated by elastohydrodynamic lubrication whereby soft uneven surfaces sliding against each other generate hydrodynamic fluid pressure that supports normal loads and redistributes fluid, promoting a more uniform fluid layer (Dowson and Jin, 1986). Computational studies based on fluid dynamic models show that the hydrodynamic pressure smoothes roughness of the pleura (Lai et al., 2002; Gouldstone et al., 2003a) and the distribution of pressure depends on the wavelength of roughness and the elastic properties of the surface (Moghani et al. 2009). During sliding, the pleurae need to be deformed to conform to each other, and the small-scale unevenness of the pleurae would not be significantly greater than the pleural fluid thickness, which averages 8–20 μm in vivo (Lai-Fook and Kaplowitz, 1985).

Both of the mechanisms above depend on the surface topography and elastic properties (stiffness) of the pleura. Previous studies have found a relatively smooth pleural surface with asperities ranging from tens to hundreds of microns (Albertine et al., 1991; Lin et al., 2008). However, there has not been any observation of the surface topography based on mechanical measurements of fresh tissue, thus avoiding uncertainties caused by fixation, freezing, or dye accumulation. The stiffness of pleural tissues, or resistance to deformation, has been measured using indentation techniques with millimeter to centimeter sized punches (Lai-Fook et al., 1976; Hajji et al., 1979; Gouldstone et al., 2003b). To examine the deformability of the pleura at the micron scale, relevant to pleural deformation in vivo, we recently measured the stiffness of the parietal pleura of rats using atomic force microscopy (AFM) to probe tissue surface in a fluid environment (Kim, Butler, Loring, unpublished). We found a stiffness lower than the values measured using larger probes, and characteristic indentation responses with both homogenous and significantly heterogeneous tissue properties depending on location on the pleura.

In the present study, we used the same technique to determine the surface unevenness and spatial distribution of stiffness of the pleural surface. In force mode AFM, spatially resolved force measurements with precise control of position (force-mapping) were performed on soft tissue surfaces (Heinz and Hoh, 1999). Stiffness and surface height at each indentation location were determined from the vertical position of AFM probes and cantilever deflection by fitting with Hertz's elastic model of homogeneous material (Hertz, 1882). The resulting stiffness map and topography of the pleura were compared to microscopic observations of surface features. The major findings from our study are the following:

1. There are significant structural inhomogeneities which are spatially clustered on the pleura and are associated with very low stiffness of the superficial layers.
2. The estimated maximal normal contact pressure exerted by asperities on the surface is much smaller than that needed to support the normal load suggested by Agostoni (1986).
3. Surface roughness of the pleura at the micron scale was less than the average pleural fluid thickness.

2. Materials and methods

2.1 Tissue measurement

We studied parietal pleural surface of the chest wall of four Sprague-Dawley rats (300 – 500g) under a protocol approved by the Institutional Animal Care and Use Committee of Beth Israel Deaconess Medical Center. To prevent fibrin formation on the mesothelial surface, heparin (5000 units i.p.) was injected ~ 5 min before an overdose of sodium pentobarbital (>200mg/kg i.p.). The thoracic cavity was opened immediately after death, the intercostal muscles and intervening ribs were excised en bloc (~4×4×2mm) between the 3rd and 7th rib while avoiding contact with the parietal surfaces to prevent abrasion. All measurements were completed within 5h after death.

A commercial AFM (MFP-3D; Asylum, Santa Barbara, CA) was used with borosilicate spherical tips, nominal diameter 5 μm, glued to triangular Au-coated silicon nitride cantilevers with a nominal spring constant $k = 0.06$ N/m (Novascan Technologies, Ames, Iowa). For all measurements, the velocity of piezoelectric displacement was 2 μm/s. Maximum forces were set to ~4nN. Two protocols were used for indentation measurement:

2.1.1. Spatial distribution of stiffness—One specimen immersed in saline from one rat was probed for the in-plane spatial distribution of stiffness. Stiffness was sampled at intervals of 1.6 μm and the accumulated stiffness data were analyzed to produce a stiffness map.

2.1.2. Surface topography—For the surface topography measurements, we soaked tissue in saline with 6 g/dl of bovine serum albumin (Sigma-Aldrich, St Louis, MO) for 24 hr and probed the tissue in the same solution (see below). Arrays of 16×16 force-displacement (F vs. z) curves with their spatial coordinates (x, y) were acquired over an 80×80μm area; the local height was assessed from the contact point z_c (point of departure from zero force). The array of z_c as a function of coordinates (x, y) was assembled into a 3-D surface profile.

2.2 Data analysis

The classical elasticity-based Hertz model for homogeneous material describes the force/depth relation for spherical punch indentations. Taking the mesothelial tissue as incompressible and a sphere of radius R , this relation reduces to

$$F = \frac{16ER^{1/2}}{9} \delta^{3/2} \quad (1)$$

where E is Young's modulus, and δ is the indentation depth (Hertz, 1882; Johnson, 1985). Given the force obtained from deflection of the cantilever (d) using Hooke's law, $F = kd$, and indentation depth obtained from piezoelectric displacement of the cantilever holder (z), $\delta = z - z_c - d$, where z_c is the contact point, Eq. 1 becomes

$$z - d - z_c = \left(\frac{9}{16ER^{1/2}} \right)^{2/3} (kd)^{2/3} \quad (2)$$

From measurements of piezoelectric displacement z and cantilever deflection d , the stiffness E and contact point z_c can be extracted from Eq. 2.

To determine the contact point and the stiffness, we performed least squares fitting for each indentation curve. These procedures are described in greater detail in the previous manuscript (Kim et al., unpublished). Briefly, F vs. z data were fitted to the Hertz model over a range of depths (fitting window, $0 < \delta < 0.8 \mu\text{m}$) and the modulus E , here referred to as overall stiffness E_o , and the contact point z_c were recovered from Eq. 2. After contact point analysis, the mean squared depth-wise error over the whole range of F vs. z data ($\text{RMS}_{\text{whole}}$) was computed for each curve, as an index of deviation from the homogeneous elastic model at large depths beyond the fitting window. An average of overall stiffness (E_o) for each rat was determined after excluding the 10% of indentation curves with the highest $\text{RMS}_{\text{whole}}$. Compared to overall stiffness (E_o) over fitting window, the stiffness was computed for each pair of force/depth. Using z_c from the analysis above, Eq 2. gives an apparent pointwise stiffness E_p at each indentation depth (Costa and Yin, 1999). Finally, the local slope of each force-displacement curve was analyzed by fitting over depth intervals of 400 nm, recovering piecewise stiffness E_{pw} at four mean depths (Domke and Radmacher, 1998).

By collecting E_o and $\text{RMS}_{\text{whole}}$ values for different grid locations, the spatial distribution over sampling area was obtained. The measure of randomness in the spatial distribution was analyzed by computing the spatial autocorrelation function (Cox and Lewis, 1966). The autocorrelation function A of a 2-D $n_x \times n_y$ array f at lag (k_x, k_y) is given by

$$A(k_x, k_y) = \frac{1}{\sigma^2(n_x - k_x)(n_y - k_y)} \sum_{l=1}^{n_x - k_x} \sum_{m=1}^{n_y - k_y} (f_{l,m} - \bar{f})(f_{l+k_x, m+k_y} - \bar{f}) \quad (3)$$

where $\sigma^2 = \frac{1}{(N-1)^2} \sum_{l=1}^{n_x} \sum_{m=1}^{n_y} (f_{l,m} - \bar{f})^2$, $N = n_x n_y$ and (k_x, k_y) represents a shift of f . The means for the two terms in the fluctuation product and that used in the variance are computed separately, as they comprise slightly different sets due to the lag;

$$\bar{f} = \frac{1}{N} \sum_{l=1}^{n_x} \sum_{m=1}^{n_y} f_{l,m}$$

and

$$\bar{f}' = \frac{1}{(n_x - k_x)(n_y - k_y)} \sum_{l=k_x}^{n_x} \sum_{m=k_y}^{n_y} f_{l,m}$$

and

$$\bar{f}'' = \frac{1}{(n_x - k_x)(n_y - k_y)} \sum_{l=1}^{n_x - k_x} \sum_{m=1}^{n_y - k_y} f_{l+k_x, m+k_y}$$

For later use, we note that in a spatially uniform distribution of independent fluctuations, the random quantity $A \sqrt{(n_x - k_x)(n_y - k_y)}$ has mean zero, and its standard deviation is

asymptotically equal to unity. Data were processed using Matlab (Mathworks, Inc., Natick, MA).

3. Results and discussion

3.1 Stiffness of the pleura

The overall stiffness (E_o) of parietal pleura of rat chest wall are summarized in Table 1. Stiffness values were not significantly different between saline and albumin environment, and similar to our previous findings of 0.38–0.95 kPa measured using spherical AFM probes (Kim et al., unpublished). The pleural surfaces exhibited two distinct indentation responses, Hertzian and non-Hertzian, reflecting homogeneity in tissue properties and significant departures from structural homogeneity, respectively. Fig. 1 shows representative F vs. δ curves and the dependence of stiffness on indentation depth for each curve. Most curves fitted the Hertz model with good agreement and showed a plateau of pointwise stiffness (E_p) to a fairly large depth, suggesting homogeneous tissue properties (left panel). By contrast, about 10% of curves over wide ranges of depth showed large deviations from the homogeneous elastic model (right panel). Many non-Hertzian F vs. δ curves showed a common feature that pointwise stiffness is very low at small depths and rises above the plateau at larger depths (inset in right panel). Considering that indentation strains are relatively small, departures from Hertzian behavior are most likely due to structural inhomogeneities normal to the surface. Note E_p in Fig. 1 is an indicator of deviation from the linear model, but its change depends more on the total depth than local slopes of the force-displacement curve. To quantify departures from homogeneity, accumulated error from linear homogeneous model (RMS_{whole}) was computed in the expanded range. F vs. δ curves were divided between the 10% of curves with the highest RMS_{whole} (the non-Hertzian curves) and the remaining 90% of curves (Hertzian). Piecewise stiffness (E_{pw}) was computed at 4 depth intervals of 400 nm. Fig. 2 shows the depth dependence of E_{pw} for the F vs. δ curves plotted in Fig. 1 and data of rat R1 (Table 1). Clearly, E_{pw} of the Hertzian group is higher than that of non-Hertzian group ($p < 0.001$, unpaired two-tailed Student's t test). E_{pw} is independent of depth in the Hertzian group ($p > 0.15$, ANOVA) but increases significantly with depth in the non-Hertzian group ($p < 0.0001$). Hertzian and non-Hertzian responses were correlated with the spatial distribution of stiffness (see Section 3.2).

3.2 Spatial distribution

Fig. 3 shows the spatial distributions of stiffness (E_o) and RMS_{whole} in a $40 \times 10 \mu\text{m}$ area of the parietal surface of one rat. Stiffness and RMS_{whole} values for the example locations shown in Fig. 1, which have typical Hertzian and non-Hertzian responses, are displayed with special symbols. The RMS_{whole} value of the F vs. δ curve in Fig. 1b, marked by square symbols in Figs. 3b and 3c, is much larger than RMS_{whole} of the Hertzian response in Fig. 1a, indicated by star symbols in Figs. 3b and 3c. Fig. 3b shows that the locations of non-Hertzian response are not randomly distributed but spatially clustered, whereas, in most of locations, RMS_{whole} is negligibly small. Indeed, most of locations near the right bottom edge of the stiffness map show high RMS_{whole} values and low stiffness. In Fig. 3c, RMS_{whole} is plotted against stiffness for all points. This plot shows a coupling between abnormally low stiffness and non-Hertzian response. To characterize the randomness in the spatial variation of stiffness and to exclude the possibility that artifacts, such as a false engagement of tip or optical interference, produced the non-Hertzian response, we performed a 2-D autocorrelation analysis of stiffness and RMS_{whole} distributions. Table 2 summarizes estimated autocorrelations $A(k_x, k_y)$ for two specimens, R1 (Fig. 3) and S1 (data from Kim et al., unpublished) at several partial lags (k_x, k_y) . Recalling that the statistic

$A \sqrt{(n_x - k_x)(n_y - k_y)}$ is asymptotically normal (mean 0 and SD 1) for independent

distributions, we found that both stiffness and $\text{RMS}_{\text{whole}}$ distributions of R1 and S1 have this statistic nearly or greater than 2 for Euclidean lags of 1 and $\sqrt{2}$, approximating rejection of independence at $p < 0.05$. The results of stiffness distribution in Fig. 3a and its spatial autocorrelation suggest that there are multiple components within the pleural tissue varying on length scales at least as large as microns. Clustered locations of high $\text{RMS}_{\text{whole}}$ and very low stiffness shown in Figs. 3a and 3b suggests that heterogeneous responses are not shared over many surface components, but restricted in specific regions or few components. Fig. 4 shows electron microscope image of the diaphragmatic surface with microvilli on some of the flat mesothelial cells. We note that in Fig. 3a the stiffness varies on a length scale approximating the size of cells in Fig. 4. These observations suggest that spatial distributions of stiffness and $\text{RMS}_{\text{whole}}$ in Figs. 3a, b are due to surface features on the pleura. When probed at the micron scale, indentation stiffness would be sensitive to structural inhomogeneity such as microvilli on the pleura at superficial depth (Sokolov et al., 2007). The parietal pleura, covered mostly with thin flat mesothelial cells, some of them covered with microvilli as in Fig. 4, would exhibit mostly homogenous stiffness at superficial depth, but locally heterogeneous responses where microvilli exist. This distinct difference of indentation responses between locations would be less significant as the depth increases, because stiffness at deeper indentation would be dominated by the cells or other components of the submesothelial region. Consistently, our previous studies confirmed less variation in elastic properties with deeper probing (Kim et al., unpublished). The indentation stiffness measured in the current study represents a resistance to deformation, or load carrying capacity of the pleural tissue. The existence of surface components with inhomogeneity such as microvilli or glycocalyx layer on the pleura would contribute to lower friction (Andrews and Porter, 1973; D'Angelo et al., 2004), but would not support a substantial normal load due to its very low stiffness (see Section 3.4).

3.3 Surface topography

We found that the tissue swells during AFM measurement when immersed in saline, probably due to osmosis induced by the oncotic pressure of proteins in the tissue. This swelling caused a gradual outward movement of the surface during contact point z_c measurements, limiting our ability to estimate the height of the surface. The outward drift of the surface was minimized in three rats (R2–R4) by soaking the tissue in an albumin solution (6 g/dl) for 24 hr before measurements (Lai-Fook, personal communication). The effect of soaking on stiffness was tested using two rats, and the average stiffnesses was not significantly different from that measured in saline (Table 1). Fig. 5 shows that zero force points of repeated indentation curves sampled in albumin solution are relatively consistent. Fig. 6 shows the 3-D profile of the parietal pleura over an $80 \times 80 \mu\text{m}$ area. We believe this is the first measurement of the surface topography of the parietal pleura using physical contact, i.e. force detection. Previously, the morphology of the unfixed pleura has been acquired mostly using 3-D fluorescent microscopy in liquid or by microscopy of the cut surface of frozen lung and chest wall (Agostoni et al., 1969; Lin et al., 2008). In the fluorescent microscopic studies, the contrasting dye may accumulate in materials or structural components that extend above the surface, such as the glycocalyx layer or microvilli, making the surface appear rougher than it would be mechanically. On the other hand, the quick-freezing procedure captures the in-situ shape of the parietal pleura, which could have been smoothed to conform to the opposing visceral pleura. With the force-displacement measurement based on contact point analysis, the horizontal resolution is limited by the distance between indentations, but 3-D maps can be obtained without uncertainties in the dye location or freezing artifacts.

The surface profile displayed in Fig. 6 depicts two morphological features of the parietal pleura. First, the vertical roughness of the pleural surface without respiratory motion is on

the order of 10 μm , which is smaller but comparable to the fluid thickness of 8–20 μm previously measured using in-situ microscopy (Lai-Fook and Kaplowitz, 1985). Second, a characteristic length scale of the horizontal surface unevenness seems to be a few tens of μm , comparable to the range of the characteristic length of the surface components shown in Fig 4. The origin of this length could be that of a mesothelial or muscle cell, or a collagen bundle, but is unknown. This roughness of the pleura, comparable to the range of the characteristic length of the surface components, supports the hypothesis of hydrodynamic flattening of the pleural surfaces during respiratory motion (Gouldstone et al., 2003a). The pleurae have to deform in the course of sliding without inducing significant damage of the surfaces. Considering their softness, pleural surfaces with asperities depicted in Fig. 6 could easily conform to each other through the agency of local hydrodynamic pressure without requiring solid-solid contact (Lai-Fook, 2004).

3.4 Tissue-tissue contact

In addition to elastohydrodynamic lubrication, competing mechanisms such as boundary lubrication have been proposed to explain the extremely low friction coefficient between the pleural surfaces (Agostoni, 1986). The importance of boundary lubrication, which requires tissue-tissue contact in pleural space, depends on the pressure distribution across the pleural space and the normal pressure on asperities of the surface. The existence of contact between pleurae in vivo has been inferred from the measured difference between the pleural surface pressure and the pleural fluid pressure. In early measurements by Miserocchi et al. (1981), the pressure difference is ~10 cmH_2O near the top of a dog's chest in the lateral posture at functional residual capacity (FRC). For small animals, the difference is smaller due to a smaller lung height. We used the pressure gradients and pressures reported by Agostoni et al. (1969) and Agostoni and D'Angelo (1970) to estimate the pressure difference in small animals; ~1.5 cmH_2O in rats and ~2 cmH_2O in rabbits in the same posture at FRC. The normal pressure on an asperity can be estimated from Hertz theory; force in Eq. 1, normalized by contact area gives contact pressure. The pressure between two identically soft surfaces in contact is given by

$$\frac{F}{\pi a^2} = \frac{16E}{9\pi R^*} a \quad (4)$$

where a is the radius of contact and R^* is the harmonic mean radius of curvature ($1/R^* = [1/R_1 + 1/R_2]/2$) of two surfaces with principal radii of curvatures R_1 and R_2 (Johnson, 1985). If we assume that an asperity with radius R_1 and a flat surface ($R_2 = \infty$), both having $E = 1\text{kPa}$, deform and conform to each other, Eq. 4 gives the contact pressure. Quantitatively, this is ~2.9 cmH_2O at maximum strain ($a \approx R_1$), which is an upper limit of the contact pressure exerted by an asperity.

Agostoni's hypothesis requires that the aggregate of asperities in contact areas support the unbalanced pressure between pleural fluid and pleural surface. The maximum normal pressure at each asperity estimated above is close to the magnitude of the pressure difference suggested by Agostoni, but the normal pressure needs to be multiplied by the fraction of contact area to give the effective normal pressure. It was observed previously that the pleural fluid layer is continuous with narrow variation in thickness (Agostoni et al., 1969; Lai-Fook and Kaplowitz, 1985). Therefore, if the fraction of contact observed in cross sectional images was less than 10%, the area fraction in contact is likely to be at most order 1%, and so the effective normal pressure would be far less than the reported pressure difference. If we assume that contact between the pleurae balances the pressure difference, either the asperities on the pleural surfaces would be severely strained or the contact area would be

much larger than that suggested by microscopic observations. The results from this first semi-quantitative analysis of the normal contact pressure of pleural surfaces, based on the stiffness at length scales approximating those in vivo, thus support the conclusion of Lai-Fook and others that surface pressure and liquid pressures in the pleural space are equal, and is inconsistent with any significant role played by surface-surface asperity contact between the pleural surfaces.

4. Conclusion

We studied the surface topography and spatial distribution of mechanical properties of the parietal pleura using micron-sized probes. The force-based topography provides evidence that the vertical unevenness of the surface is generally less than the average thickness of pleural fluid in vivo. Spatial distribution of stiffness and autocorrelation confirmed the association between significant superficial inhomogeneities and very low stiffness. The characteristic lengths in the spatial distributions of both mechanical properties and topological profile are comparable to the size of mesothelial cells. Importantly, the maximum contact pressure estimated based on the pleural stiffness excludes the possibility of any significant solid-solid contact mechanism in load support during respiratory sliding.

The flatness and softness of the pleura observed in the current study suggest that during respiratory motion, the uneven pleural surfaces promotes elastohydrodynamic lubrication and smoothness by shear-induced hydrodynamic pressure, thus maintaining a relatively uniform fluid thickness and preventing tissue-tissue contact.

Acknowledgments

The authors thank Dr. Stephen J. Lai-Fook for helpful comments and suggestions, and Dr. Richard E. Brown for the electron microscopic study presented in this report. Fig. 4 was prepared by Dr. Brown at Harvard School of Public Health. AFM measurements were performed at Harvard Center for Nanoscale Systems (CNS), a member of the National Nanotechnology Infrastructure Network (NNIN), which is supported by the National Science Foundation under NSF award no. ECS-0335765. This work was supported by a Grant HL-63737 from the National Institutes of Health.

References

- Agostoni, E. Handbook of Physiology. The Respiratory System. Mechanics of Breathing. Vol. III. American Physiology Society; Bethesda, MD: 1986. Mechanics of the pleural space; p. 531-559.
- Agostoni E, D'Angelo E. Comparative features of the transpulmonary pressure. *Respir Physiol.* 1970; 11:76–83. [PubMed: 5491116]
- Agostoni E, Miserocchi G, Bonanni MV. Thickness and pressure of the pleural liquid in some mammals. *Respir Physiol.* 1969; 6:245–256. [PubMed: 5773392]
- Albertine KH, Wiener-Kronish JP, Bastacky J, Staub NC. No evidence for mesothelial cell contact across the costal pleural space of sheep. *J Appl Physiol.* 1991; 70:123–134. [PubMed: 2010367]
- Andrews PM, Porter KR. The ultrastructural morphology and possible functional significance of mesothelial microvilli. *Anat Rec.* 1973; 177:409–426. [PubMed: 4127780]
- Costa KD, Yin FDP. Analysis of indentation: Implications for measuring mechanical properties with atomic force microscopy. *J Biomech Eng.* 1999; 121:462–471. [PubMed: 10529912]
- Cox, DR.; Lewis, PAW. The Statistical Analysis of Series of Events. Chapman and Hall; London: 1966. p. 87-96.
- D'Angelo E, Loring SH, Gioia ME, Pecchiari M, Moscheni C. Friction and lubrication of pleural tissues. *Respir Physiol Neurobiol.* 2004; 142:55–68. [PubMed: 15351304]
- Dowson D, Jin ZM. Micro-elastohydrodynamic lubrication of synovial joints. *Eng Med.* 1986; 15:63–65. [PubMed: 3709914]

- Gouldstone A, Brown RE, Butler JP, Loring SH. Elastohydrodynamic separation of pleural surfaces during breathing. *Respir Physiol Neurobiol.* 2003a; 137:97–106. [PubMed: 12871681]
- Gouldstone A, Brown RE, Butler JP, Loring SH. Stiffness of the pleural surface of the chest wall is similar to that of the lung. *J Appl Physiol.* 2003b; 95:2345–2349. [PubMed: 14600162]
- Hajji MA, Wilson TA, Lai-Fook SJ. Improved measurements of shear modulus and pleural membrane tension of the lung. *J Appl Physiol.* 1979; 47:175–181. [PubMed: 468657]
- Heinz WF, Hoh JH. Spatially resolved force spectroscopy of biological surfaces using the atomic force microscope. *Trends Biotechnol.* 1999; 17:143–150. [PubMed: 10203772]
- Hertz H. Über die Berührung fester elastischer Körper (On the contact of elastic solids). *Journal für die Reine und Angewandte.* 1882; 92:156–171.
- Johnson, KL. *Contact Mechanics.* Cambridge University Press; Cambridge: 1985. p. 90-104.
- Lai J, Gouldstone A, Butler JP, Federspiel WJ, Loring SH. Relative motion of lung and chest wall promotes uniform pleural space thickness. *Respir Physiol Neurobiol.* 2002; 131:233–243. [PubMed: 12126924]
- Lai-Fook SJ. Pleural mechanics and fluid exchange. *Physiol Rev.* 2004; 84:385–410. [PubMed: 15044678]
- Lai-Fook SJ, Kaplowitz MR. Pleural space thickness in situ by light microscopy in five mammalian species. *J Appl Physiol.* 1985; 59:603–610. [PubMed: 4030613]
- Lai-Fook SJ, Wilson TA, Hyatt RE, Rodarte JR. Elastic constants of inflated lobes of dog lungs. *J Appl Physiol.* 1976; 40:508–513. [PubMed: 931871]
- Lin JL, Moghani T, Fabry B, Butler JP, Loring SH. Hydrodynamic thickening of lubricating fluid layer beneath sliding mesothelial tissues. *J Biomech.* 2008; 41:1197–1205. [PubMed: 18367193]
- Miserocchi G, Nakamura T, Agostoni E. Change pattern of pleural deformation pressure on varying lung height and volume. *Respir Physiol.* 1981; 43:197–208. [PubMed: 7280377]
- Moghani T, Butler JP, Loring SH. Determinants of friction in soft elastohydrodynamic lubrication. *J Biomech.* 2009; 42:1069–1074. [PubMed: 19358993]
- Domke J, Radmacher M. Measuring the elastic properties of thin polymer films with the atomic force microscope. *Langmuir.* 1998; 14:3320–3325.
- Sokolov I, Iyer S, Subba-Rao V, Gaikwad RM, Woodworth CD. Detection of surface brush on biological cells in vitro with atomic force microscopy. *Appl Phys Lett.* 2007; 91:023902.1–3.

Fig. 1.

Representative F vs. δ curves and dependence of stiffness on depth obtained from R1. Left panel: a typical Hertzian response, showing good agreement with the Hertz model. Right panel: a non-Hertzian response showing large deviation from the elastic model. Insets: Pointwise stiffness E_p (circle) and overall stiffness E_o (dashed line) for each curve. Depth windows for fitting are marked as vertical dotted lines. Indentations were performed with 5 μm spheres in saline. Note that stiffness is much lower during initial indentation in the non-Hertzian response.

Fig. 2.

Depth dependence of piecewise stiffness, E_{pw} , calculated over depth intervals of 400 nm. Open symbols indicate E_{pw} for F vs. δ curves plotted in Fig. 1. Filled symbols indicate averages ($\pm$ SD) of E_{pw} in the 10% of curves with the highest RMS_{whole} (non-Hertzian) and the remaining 90% of curves (Hertzian) from R1 (Table 1). Hertzian curves were analyzed only to the third interval due to missing data at the larger depth. E_{pw} is independent of depth in the Hertzian group ($p > 0.15$), but increases significantly with depth in the non-Hertzian group ($p < 0.0001$).

Fig. 3.

Distribution of stiffness (E_0) and $\text{RMS}_{\text{whole}}$ from R1. a) A stiffness map over $40 \times 10 \mu\text{m}$ area with horizontal resolution of $1.6 \mu\text{m}$. Dots display locations of indentation. b) $\text{RMS}_{\text{whole}}$ plotted along lines at each y distance in the stiffness map. Average stiffness calculation excludes 10% of data with the highest $\text{RMS}_{\text{whole}}$ (horizontal line). c) $\text{RMS}_{\text{whole}}$ versus stiffness. Star and rectangle indicate the locations of F vs. δ curves in Figs. 1a and b, respectively. All the indentations were performed with a $5 \mu\text{m}$ sphere in saline solution.

Fig. 4. Scanning electron micrograph of the parietal pleural surface (diaphragmatic) of a Sprague-Dawley rat. Scale bar = 10 μ m.

Fig. 5. Indentation curves at a fixed location from R4. Indentations repeated 8 times with a 5 μm sphere in albumin solution show adequate consistency for topographical measurements.

Fig. 6. Surface topography of parietal pleura from R4. Sampling was performed with a 5 μm sphere in albumin solution after confirming that the surface was geometrically stable (Fig. 5).

Table 1 Stiffness (E_0) of parietal pleural surface measured using spherical probes.

Environment	Rat	N*	Average stiffness $\pm$ SD (kPa)	Mean of stiffness (kPa)
Saline	R1	201	1.12 $\pm$ 0.46	1.12
Albumin solution	R2	34	0.97 $\pm$ 0.38	1.19
	R3	55	1.41 $\pm$ 0.87	
	R4 [†]	(256)	-	

* Total number of indentation curves obtained from each rat.

[†] R4 was used for profile measurement. Stiffness values from the contact point analysis as describe in method section were not accepted because the cantilever sensitivity differed between before and after the profile measurement. We speculate that the surface of a cantilever is easily contaminated in albumin solution.

Table 22-D autocorrelations of stiffness and RMS_{whole}

	Lag (k_x, k_y) *	$A \sqrt{(n_x - k_x)(n_y - k_y)}$	
		R1[†] (Fig. 2)	S1^{††}
Stiffness	(-1,0)	11.94	6.69
	(0,1)	8.05	4.49
	(-1,1)	7.40	3.55
RMS _{whole}	(-1,0)	7.53	4.38
	(0,1)	1.81	0.97
	(-1,1)	3.98	3.55

* Unit length = 1.6 μm .[†] Stiffness and RMS_{whole} from R1 were analyzed in 26×7 arrays. Distribution of stiffness and RMS_{whole} from R1 are displayed in Fig. 3.^{††} Data from S1 in Kim et al. (unpublished) was reanalyzed for autocorrelation. S1 was sampled in 22×7 arrays with the same method as R1 over a 34×10 μm area.