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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.

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 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,

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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 (300–500 g) 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 (>200 mg/kg i.p.). The thoracic cavity was opened immediately after death, the intercostal muscles and intervening ribs were excised en bloc (~4 mm × 4 mm × 2 mm) between the 3rd and 7th rib while avoiding contact with the parietal surfaces to prevent abrasion. All measurements were completed within 5 h 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 ~4 nN. 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 h 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 μm × 80 μm 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, $0 < \delta < 0.8$ μm) 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 ($\mathrm{RMS_{whole}}$) 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 $\mathrm{RMS_{whole}}$. 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 $\mathrm{RMS_{whole}}$ 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

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

where $\sigma^2 = (1/[N-1]^2)\sum_{l=1}^{n_x}\sum_{m=1}^{n_y}(f_{l,m} - \bar{f})^2$, $N = n_x n_y$ 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

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

and

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