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Hydrodynamic thickening of lubricating fluid layer beneath sliding mesothelial tissues

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Abstract

The delicate mesothelial surfaces of the pleural space and other serosal cavities slide relative to each, lubricated by pleural fluid. In the absence of breathing motion, differences between lung and chest wall shape could eventually cause the lungs and chest wall to come into contact. Whether sliding motion keeps lungs and chest wall separated by a continuous liquid layer is not known. To explore the effects of hydrodynamic pressures generated by mesothelial sliding, we measured the thickness of the liquid layer beneath the peritoneal surface of a 3-cm disk of rat abdominal wall under a normal stress of 2 cm H_2O sliding against a glass plate rotating at 0–1 rev/s. Thickness of the lubricating layer was determined microscopically from the appearance of fluorescent microspheres adherent to the tissue and glass. Usually, fluid thickness near the center of the tissue disk increased with the onset of glass rotation, increasing to 50–200 μm at higher rotation rates, suggesting hydrodynamic pumping. However, thickness changes often differed substantially among tissue samples and between clockwise and counter-clockwise rotation, and sometimes thickness decreased with rotation, suggesting that topographic features of the tissue are important in determining global hydrodynamic effects. We conclude that mesothelial sliding induces local hydrodynamic pressure gradients and global hydrodynamic pumping that typically increases the thickness of the lubricating fluid layer, moving fluid against the global pressure gradient. A similar phenomenon could maintain fluid continuity in the pleural space, reducing frictional force and shear stress during breathing.

Keywords: Respiratory mechanics; Pleural space; Elastohydrodynamic lubrication; Epifluorescence microscopy; Rat

1. Introduction

With each breath, the pleural surfaces of lungs and chest wall slide past each other, lubricated by pleural fluid. The nature of the physical interaction between the pleural surfaces of lung and chest wall has been controversial. Agostoni and D'angelo (1991) have argued that the difference between estimated surface pressure over the lungs and fluid pressure is evidence for solid-to-solid contact. The necessity for contact was challenged by Lai-Fook (1987), Lai-Fook and Rodarte (1991, 2004), who suggested that fluid pressure is actually equal to surface pressure and proposed that a continuous fluid layer separates the pleural surfaces of lung and chest wall, lowering shear stress during breathing.

Several groups have explored the tribological behavior of sliding mesothelial tissues to deduce the presence and importance of contact between the sliding surfaces. Tribological behavior is commonly divided into four lubrication regimes: boundary lubrication, mixed lubrication, elastohydrodynamic lubrication, and fully developed hydrodynamic lubrication (Dowson, 1969; Adamson, 1982). In boundary lubrication, which occurs at lower sliding speeds, asperities (protuberances) on the sliding surfaces are in contact or separated by extremely thin films of lubricant, and hydrodynamic pressures are unimportant in supporting the normal load. In this regime, frictional force is relatively invariant with velocity. At the highest sliding velocities in hydrodynamic lubrication, fluid thickness is much greater than the amplitude of the surface

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roughness, hydrodynamic pressure bears the entire load, and frictional force rises with velocity. In elastohydrodynamic lubrication at intermediate sliding speeds, asperities are deformed by hydrodynamic pressures and do not come into contact because they are separated by a continuous layer of lubricant. In this regime (and in mixed lubrication, which has the features of both boundary and elastohydrodynamic lubrication), frictional force typically decreases within a range of increasing velocity. Thus, each lubrication regime has its own characteristic frictional behavior. Early tribological studies of pleural tissues measured static friction coefficients (Brandi, 1972; D'Angelo, 1975). Later, D'Angelo et al. (2004) measured a dynamic coefficient of sliding friction that did not vary with velocity during sinusoidal sliding, interpreting the findings as consistent with boundary lubrication. In subsequent experiments of mesothelial tissues sliding on a rotating glass plate (Loring et al., 2005), friction varied with velocity, consistent with elastohydrodynamic lubrication. In that study, shear force abruptly increased with the onset of constant rotation and then decreased progressively to a quasi-steady state during continued sliding (Fig. 1). The decrease in shear force was quicker at higher velocity, consistent with hydrodynamic pumping that increased the thickness of lubricant layer, reducing shear stress. In the present study, we test this interpretation by measuring fluid thickness at various locations between mesothelial tissue and a rotating glass plate under similar conditions. Our findings suggest that mesothelial tissue, sliding at speeds and under normal stresses characteristic of pleural tissues *in vivo*, exhibits hydrodynamic pumping previously invoked to explain redistribution of pleural fluid during breathing (Butler, et al., 1995). We infer that hydrodynamic pumping is also likely to be important to mesothelial lubrication *in vivo*.

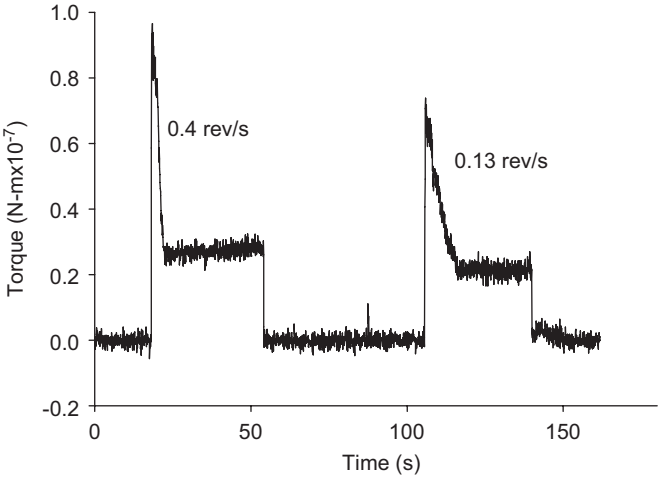

Fig. 1. Data replotted from Fig. 3 of Loring, et al. (2005). Torque applied to a disk of mesothelial tissue sliding on a glass plate rotating at rates indicated. Torque increased abruptly with the onset of motion and then progressively decreased to a steady state with continued sliding. Note that the time required to reach steady state decreased with increasing rotation rate.

2. Materials and methods

For mesothelial tissue, we used the peritoneum and underlying muscle of 30 male Sprague–Dawley rats (400–500 g) under a protocol approved by our Institutional Animal Research Committee. We used physiologic saline to simulate pleural liquid, which is a Newtonian fluid with a viscosity only 1.5 times that of water. To prevent fibrin formation on the tissue surface, heparin (5000 units i.p.) was administered 5 min before lethal anesthetic overdose (sodium pentobarbital, 200 mg/kg). Immediately after death, skin and subcutaneous tissue were reflected and the ventral abdominal wall excised, avoiding abrasion of the mesothelial surface and keeping it wet with saline.

The experimental apparatus consisted of a rotating glass plate sealed to the inner race of a ball bearing (Fig. 2). The glass plate covered with saline was rotated against a disk of mesothelial tissue glued with cyanoacrylate to the rim of an inverted metal cup (~3.7 cm diameter) that was vertically positioned with 0.1 mm resolution using a calibrated rack and pinion. The space within the metal cup over the tissue was pressurized to 2 cm H_2O to apply a uniform normal stress to the tissue surface. This created a pressure gradient driving fluid from beneath the tissue to the surrounding reservoir to simulate the non-gravitational pressure gradients within the pleural space caused by elastic deformation of the lung and chest wall (Loring et al., 2005). An inverted epifluorescence microscope measured the thickness of the layer of saline between the rotating glass and tissue (see below).

The 2 to 5-mm thick tissue sheet with the mesothelial surface facing outward was fixed under minimal tension so that it became slightly convex when pressurized. The tissue and glass plate were bathed for approximately 5 min in saline solution containing fluorescent latex microspheres (1.9 μm diameter) to allow the microspheres to adhere to glass and tissue surfaces. Unattached microspheres and solution were rinsed off before data collection. To avoid post-mortem changes in tissue properties, experiments were concluded within 2 h after death.

Images of microspheres at the focal plane appeared as small bright spots, whereas those at greater distance from the lens appeared as rings (Fig. 3). We used the linear relationship between ring radius and distance from the focal plane to measure the vertical distance between microspheres on the tissue and glass plate (one pixel diameter = 4 μm); the difference in ring size indicated the thickness of the fluid between glass and tissue. Microspheres on the tissue, glass, and in the fluid were distinguishable from images taken during rotation (Fig. 3). Microspheres on the tissue were stationary, those on glass moved in concentric circular arcs, and those that had become dislodged and were free in the fluid moved in non-circular trajectories.

In initial experiments, it became apparent that the height of the tissue-cup had a large effect on the response to rotation. (See Results.) For most studies, the height of the tissue-cup was set to a low position, ~1 mm above that at which the tissue with no pressure applied would have rested

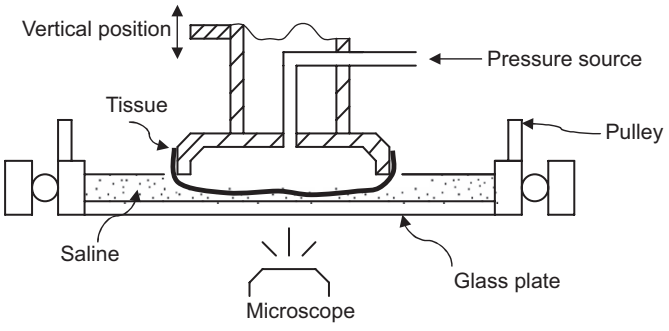

Fig. 2. Apparatus for fluid thickness measurement. The ball bearing with its glass bottom was rotated by a stepper motor and belt (not shown) over an inverted epifluorescence microscope. Tissue was fastened over the opening of an inverted metal cup (~3.7 cm diameter) and positioned close to the glass plate. Air pressure applied a spatially uniform normal stress to the top of the tissue.