FRICTION AND MORPHOLOGY OF PLEURAL MESOTHELIA

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Abstract

To verify the hypothesis that by enmeshing lubricants, microvilli reduce the coefficient of kinetic friction (μ) of pleural mesothelium, μ was measured during reciprocating sliding of rabbit's visceral against parietal pleura before and after addition of hyaluronan, and related to the morphological features of the microvillar network. Because no relation was found between μ or μ changes after hyaluronan and microvillar characteristics, the latter are not determinants of the frictional forces which oppose sliding of normal mesothelial surfaces under physiological conditions, nor of the effects of hyaluronan. Addition of hyaluronan increased μ slightly but significantly in normal specimens, probably by altering the physiological mix of lubricants, but decreased μ of damaged mesothelia, suggesting protective, anti-abrasion properties. Indeed, while sliding of an injured against a normal pleura heavily damaged the latter and increased μ when Ringer was interposed between the surfaces, both effects were limited or prevented when hyaluronan was interposed between the injured and normal pleura before onset of sliding.

Keywords: Friction; Pleura; Hyaluronic Acid; Respiratory Mechanics

1. Introduction

Since their first unequivocal identification by transmission electron microscopy (Odor, 1954), microvilli on the free surface of mesothelial cells have been the subject of extensive morphological investigation (Andrews and Porter, 1973; Wang, 1974; Michailova, 2004), but their functional significance has remained elusive.

It has been suggested that mesothelial microvilli are involved in pleural liquid filtration and exchange, because their presence implies a larger surface area available for membrane metabolic activities (Odor, 1954). Furthermore, the higher density of microvilli on the visceral relative to the parietal pleura (Wang, 1974) can be explained in the framework of the parietal-secreting and the visceral-absorbing theory of the pleural liquid (Zocchi, 2002). This explanation, however, cannot account for the craniocaudal differences in the density of microvilli (Wang, 1974).

It is also possible that mesothelial microvilli have a mechanical role, acting as a scaffold for lubricating substances which decrease friction between pleural surfaces during reciprocating sliding. Light and electron microscopy studies have shown in fact that cationic dyes, which react with mucopolysaccharides and sialylated proteins, regularly stain the free surface of mesothelial cells (Andrews and Porter, 1973; Wang, 1974; Ohtsuka et al., 1997), and in rabbits, in which microvilli are more abundant in the caudal than in the cranial part of the pleural cavity, the coefficient of kinetic friction (μ) tends to be smaller in the former than the latter location (D'Angelo et al., 2004).

On the other hand, the suggestion that mesothelial microvilli enmesh glycoproteins and mucopolysaccharides for lubrication purposes (Andrews and Porter, 1973; Wang, 1985) has received no experimental support yet. If this suggestion is appropriate, we hypothesize that local friction between two intact mesothelial surfaces would decrease with increasing density of microvilli. The main purpose of the present research is therefore to test whether the properties of the mesothelial coating, in terms of density and characteristics of microvilli, are relevant in the determination of μ under normal conditions.

The identification of the lubricating substances has been hindered by the lack of knowledge of the precise composition of the mesothelial coating and its organization. Moreover, it is technically difficult, if not impossible, to remove selectively a substance from the coating, without damaging the coating or the cell surface itself. However, several molecules, including hyaluronic acid, sialomucins and phospholipids, have been proposed as boundary lubricants. Among these substances, hyaluronan has risen particular interest because normal mesothelial cells produce thick hyaluronan containing coats *in vitro* (Heldin and Pertoft, 1993; Blom et al., 1995), and *in vivo* they

secrete hyaluronan (Honda et al., 1986), as its concentration is larger in the pleural fluid (Wang and Lai-Fook, 1998) than in serum (Engstrom-Laurent et al., 1985). Additionally, hyaluronan is able to decrease μ of blotted mesothelial surfaces (Bodega et al., 2012). Hence, we hypothesize that if mesothelial microvilli act as scaffolds for lubricants, and if not all their binding sites are occupied, then the addition of hyaluronic acid should decrease friction.

Finally, some evidence suggests that hyaluronan may have a role in preventing abrasion between mesothelial surfaces. During remesothelialization the superficial density of microvilli (Mutsaers et al., 1996) and the production of hyaluronic acid (Horiuchi et al., 2003) increase, possibly protecting cells from mechanical injury during the regenerative process. The observation that hyaluronic acid concentration in the pleural fluid rises several folds during hyperventilation (Wang and Lai-Fook, 1998) may have an analogue meaning. An additional purpose of this investigation is therefore to test whether hyaluronan is able to prevent or reduce abrasion damage to an initially intact mesothelial surface sliding against a damaged one.

In summary, the primary aim of this study is to test whether dynamic friction between normal mesothelial layers changes with changing morphological characteristics of microvilli, the secondary aims are to test whether the effect of the addition of exogenous hyaluronan on friction is dependent on the morphological characteristics of microvilli, and whether exogenous hyaluronan is able to protect mesothelia from sliding-induced damage.

2. Methods

2.1. Measurements of kinetic friction coefficient

Fifteen rabbits (weight range 2.7-3.2 kg) were deeply anesthetized with a mixture of pentobarbital (20 $mg \cdot kg^{-1}$) and urethane (0.5 $g \cdot kg^{-1}$), heparinized (0.1 $mg \cdot kg^{-1}$) and killed by exanguination. After removal of the skin and of superficial muscles, the anterolateral part of chest wall, the diaphragm and the lungs were excised and kept at room temperature (20-25°C) in Ringer bicarbonate solution (in mM: Na^+ 139, K^+ 5, Ca^{2+} 1.25, Mg^{2+} 0.75, Cl^- 119, HCO_3^- 29, D-glucose 5.6), through which 95% O_2 and 5% CO_2 was continuously bubbled.

Specimens were cut from anterolateral rib cage, the diaphragm, and the corresponding zones of the lung. Specimens were discarded in case of accidental damage during handling.

The apparatus used to measure frictional force has been already described in details (D'Angelo et al., 2004). Briefly, the specimen from the rib cage or the diaphragm was mounted with the parietal pleura facing upwards on a sliding platform, connected through inextensible treads

to the core of a differential transformer (Lynearsyn Sanborn 565 DT). An electric motor drove sinusoidally the sliding platform over a distance of 1 cm with a maximum peak velocity of 3 $cm \cdot s^{-1}$. The lung specimen was tied to a plexigas piston (cross section 0.62 cm^2), with the visceral pleura facing downwards. The piston was mounted to one end of a balance arm, which was held stationary at its fulcrum by a force transducer. The balance arm could rotate to maintain contact between the specimens. The mesothelial surfaces were positioned visibly parallel to each other, the balance arm held horizontal, and frictional force in the direction of motion was measured by the force transducer. Five counterweights could be added to the other end of the balance arm to change the normal force applied to the specimens, the resulting contact pressure ranging from ~1 to ~9 cmH_2O. The signals from the transducers were acquired at 200Hz by 16 bit A/D converter (NI PCIe-6361; National Instruments, Austin, TX), stored on a desk computer, and analyzed offline. The relation between load and friction was linear in all experimental conditions, its slope, corresponding to μ (D'Angelo et al., 2004).

The study was approved by the Ministry of Health and was performed in compliance with Directive 86/609/EC.

2.2. Experimental protocol

In 43 pairs of specimens (group A) μ was measured before and 5 minutes after addition of hyaluronan (Sigma-Aldrich 53747, St. Louis, MO; m.w. $1.63 \cdot 10^6$ Da) at the concentration of 2.5 $mg \cdot ml^{-1}$ in Ringer. The reversibility of hyaluronan effect was assessed by measuring μ after washing away hyaluronan with Ringer. Thereafter all specimens were processed for light microscopy, while transmission electron microscopy (TEM) was performed on 31 parietal specimens.

To evaluate hyaluronan anti-abrasion properties, we first assessed by light microscopy in 17 pairs of specimens (group B) the degree of injury induced to a normal mesothelial surface by a short period (~ 2 min) of sliding against a mesothelial surface damaged by blotting (D'Angelo et al., 2004) and re-wetted with Ringer. This degree of injury was then compared to that observed in 7 additional pairs of specimens (group C) when before the short period of sliding, hyaluronan solution was placed between the normal and blotted surface, where it rested for 5 min. In all cases, μ was assessed before blotting with Ringer and after blotting with either Ringer (group B) or hyaluronan solution (group C) between sliding surfaces.

2.3. Histology

After completion of mechanical measurements, the specimens were removed from their support, and after clearing part of the tissues below the mesothelial surface, pinned on a cork. Care was taken to keep tissues always wet with Ringer and to avoid interfering with the area where sliding occurred (up to 1.2 cm^2).

To visualize mesothelial cells by light microscopy, the mesothelium was processed for silver staining as previously described (Gottlob and Hoff, 1968). A three grades injury score (IS) was used to classify the specimen as normal, injured, or heavily injured, according to the extent of the surface of the specimen covered by normal mesothelial cells (>95%, IS=0; 95-50%, IS=1; <50%, IS=2).

For TEM, the specimens were immersed in 3% glutaraldehyde buffered with 0.1M Sorensen's phosphate buffer (pH 7.4) for 12-24 h. The specimens were cut into small blocks and then postfixed on ice with 1% OsO_4 in 0.1M Sorensen's phosphate buffer, washed with distilled water and stained en bloc with 2% aqueous uranyl acetate, dehydrated in acetone and embedded in araldite resin. Semithin sections, toluidine blue stained, were prepared for preliminary light microscopy. Ultrathin sections were stained with lead citrate and examined under the electron microscope (Model EM10, Carl Zeiss, Oberkochen, Germany). At least 30 electron microphotographs (8000x) were randomly taken from each specimen, corresponding to 350–450 μm of free pleural surface length. Using a custom-made program (LabView and IMAQ Vision for LabView; National Instruments, Austin, TX), on each photograph a blind operator measured the length of the free pleural surface, and counted a) the number of sections of microvilli visibly connected to the pleural surface, and b) the number of the sections of microvilli not visibly connected to the pleural surface and their mean distance from the surface. The number of sections of microvilli visibly connected to the pleural surface divided by the free pleural surface length was taken as an index of the surface density of microvilli (D_{MV}). Because each microvillus necessarily stems from a mesothelial cell, isotropic distribution of microvilli in the proximity of the pleural surface cannot be assumed, and the length for unit volume of microvilli cannot be measured with the usual stereological methods on very thin sections (Howard and Reed, 1998). We reasoned that, in the absence of a stereotyped orientation of microvilli, for a given average distance between the surface and the sections not connected to the surface ($N_{Sn\text{-}c}$), the ratio between the number of these sections and those visibly connected to the surface (N_{Sc}) should increase with increasing microvillar length. Conversely, for a given $N_{Sn\text{-}c}/N_{Sc}$ ratio, the average distance between the surface and the sections not connected to the surface should increase with increasing microvillar length. Therefore the length of microvilli was tentatively indexed by the $N_{Sn\text{-}c}/N_{Sc}$ ratio multiplied by average distance of the sections not connected to the surface (L_{MV}). Finally, the distance from the pleural surface that

included 90% N_{Sn-c} was taken as an index of the overall thickness of the microvillar layer (T_{MV}). It should be stressed that these indices are not intended to represent the real values of the corresponding parameters, but are thought to be proportional to those values.

All morphologic measurements were independently performed by two observers in a blind fashion.

2.4. Statistical analysis

Results are presented as mean±SEM, except IS which is presented as median and range. Statistical significance of the difference between mean values was assessed by analysis of variance (ANOVA). Univariate, factorial within, or mixed between-within models were used as appropriate. IS data were compared using the Mann–Whitney or Wilcoxon signed-rank test. Linear regressions were computed with the least squares method. The level of significance was taken at $P \leq 0.05$.

Concerning the relation between microvillar indices and μ under normal conditions, a sample size of 23 specimens was chosen to afford a power of at least 0.80 in order to find a significant relation with $\alpha=0.05$ between D_{MV} and μ in the presence of a coefficient of determination of 0.30 between D_{MV} measured on one of the surfaces and μ. If microvillar density changes on the two opposed surfaces in the same direction, as available literature suggests (Wang, 1974), the actual power should be greater.

Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL), except sample size calculation, which was performed with G-Power (Faul et al., 2009).

3. Results

3.1. Morpho-functional relations of intact mesothelial surfaces wetted with Ringer.

In 11 pairs of specimens of group A, the value of μ was substantially higher than 0.051, i.e. the upper 99% confidence limit computed from previously published data (D'Angelo et al., 2004), and the results from this high μ group are presented at the end of the Section. In the remaining 32 pairs, μ averaged 0.023±0.002.

Light microscopy showed the presence of a continuous layer of mesothelial cells (Fig. 1, panel A). Only isolated mesothelial cells were missing, and the area of denuded pleura never exceeded 10% of the entire surface. Median and range of IS were 0 and 0-1, respectively.

TEM, performed on 23 specimens with an average μ of 0.021±0.001, confirmed the presence of a continuous and well preserved mesothelial layer with a variable microvilli distribution and density (Fig. 2). D_{MV}, L_{MV}, and T_{MV} averaged 0.86±0.06 μm^{-1}, 3.74±0.41 μm and 1.41±0.09 μm. Their between and within specimens variability, expressed as coefficient of variation, was 31, 52, and 29%, and 37, 62, and 35%, respectively.

No relation was found between μ and D_{MV}, L_{MV}, or T_{MV} (Fig. 3).

3.2. *The effects of hyaluronan addition to intact mesothelial surfaces.*

In 32 specimens, the addition of hyaluronic acid increased μ from the initial value with Ringer (0.023±0.002) to 0.028±0.002 ($\Delta\mu$=0.005±0.001, $P<0.001$). On an individual basis, μ increased significantly in 11 specimens with hyaluronan addition, and remained unchanged in 21. Moreover, the initial values of μ were significantly correlated to those after the addition of hyaluronan (Fig. 4). Finally, when hyaluronan was removed by washing with Ringer, μ returned to its initial value ($\Delta\mu$=0.000±0.001).

No relation was found between μ changes with hyaluronan and D_{MV}, L_{MV}, or T_{MV} (Fig. 5). Assessment of these relations was done on the assumption that microvillar morphology was the same during sliding with hyaluronan and after hyaluronan washout with Ringer.

3.3. Protective properties of hyaluronan.

In the 17 specimens of group B, μ before blotting was 0.025±0.003. Unilateral blotting increased μ to 0.058±0.003 ($\Delta\mu$=0.033±0.003, $P<0.001$).

Light microscopy performed on the blotted surface after sliding showed severe mesothelial damage with the disappearance of large parts of the cellular layer (IS=2, 1-2) (Fig. 1, panel B). Damage on the initially intact surface was substantial (1, 0-2) (Fig. 1, panel C), but less than that of the directly blotted surfaces (P=0.002). This effect was not due to reciprocating sliding *per se*, because non-blotted specimens of group A were practically intact (IS=0, 0-1) (Fig. 1, panel A), IS difference between the two groups being highly significant (P=0.001).

In line with light microscopy, TEM, performed on two non-blotted specimens, showed that part of the mesothelial layer was absent (Fig. 6, panel A), the remaining cells presenting signs of cellular damage, such as prominent vacuolization of the cytoplasm, and scanty microvilli (Fig. 6, panel B).

In the 7 specimens of group C, μ before blotting was 0.025±0.003, i.e. not significantly different from that of group B (P=0.740). With the addition of hyaluronan before establishing contact between the blotted and intact surface, μ increased by 0.009±0.003 (P=0.213), a change markedly smaller than that of group B (Δμ=0.023±0.003, P<0.001).

Light microscopy performed on the non-blotted surface showed that addition of hyaluronan before sliding largely prevented mesothelial damage of this surface, IS (0, 0-1) being similar to that observed for the intact surfaces of group A.

3.4. The high μ group

In 11 pairs of specimens, μ was high during the initial measurement with Ringer (0.124±0.026), fell after addition of hyaluronic acid (0.060±0.009, Δμ=-0.064±0.019, P=0.012) and returned to its initial value after washing with Ringer (0.120±0.027, Δμ=-0.004±0.024). Furthermore, the relation between μ before the addition of hyaluronan and the changes of μ with the addition of hyaluronan was closely described by a linear function (Fig. 7). Oldham's transformation (Gill et al., 1985) confirmed the dependence of the effect of hyaluronan on the pre-addition value of μ (R=0.951, P<0.001).

Light microscopy and TEM showed a normal mesothelium (IS=0) only in the specimen with μ=0.054, a value very close to the upper 99% confidence limit for normality (see above). In the remaining specimens, the former technique detected a discontinuous mesothelial layer (IS=1, 1-2), while TEM showed a nearly complete absence of mesothelium, except in two specimens where surviving cells covered ~45% of the surface. Because D_{MV}, L_{MV}, and T_{MV} of these cells (0.83 μm^{-1}, 4.57, and 1.60 μm, respectively) were similar to those found in group A, the mesothelial damage of this high μ group can be attributed to loss of cells that were loosely connected to the basement membrane.

4. Discussion

The novel findings of this study are that neither the coefficient of kinetic friction of normal mesothelial surfaces, nor the effects of the addition of exogenous hyaluronic acid are dependent on the density and characteristics of microvilli. However, hyaluronan limits or prevents the damage of normal mesothelial surfaces sliding against an injured one, thus exerting a protective action.

The idea that microvilli acts as scaffolds for lubricating substances is rather old (Andrews and Porter, 1973; Wang, 1985); it stems from the results of electron microscopy studies, showing

that materials compatible with mucopolysaccharides or sialic proteins coat the microvilli and partially fill the space between them. Implicit in this idea is the assumption that boundary lubrication takes place between pleural surfaces *in situ*, as it has been in fact demonstrated in *in vitro* preparations using physiological loads and sliding velocities (D'Angelo et al., 2004), and confirmed in the present study. Based on this representation of the pleural surfaces, it is reasonable to hypothesize that the greater the local density of microvilli, the greater the amount of enmeshed lubricants, the lesser the coefficient of kinetic friction and its change with the addition of exogenous lubricants.

Contrary to these suggestions, no correlation was found between the coefficient of kinetic friction and the features of the microvillar network (Fig. 3), despite the wide range of μ values (0.08-0.045) and characteristics of the microvilli (0.44-1.41 μm^{-1}, 1.50-9.39 μm, and 0.64-2.04 μm for D_{MV}, L_{MV}, and T_{MV}, respectively). Similarly, the effects of hyaluronan addition was not related to density and characteristics of the microvilli (Fig. 5). A possible explanation of the former observation could be that maximal lubricant effect is already reached with the amount of lubricants corresponding to the lowest microvillar density, the excess representing a functional reserve in the presence of an increased consumption as, for example, during hyperventilation (Wang and Lai-Fook, 1998). Furthermore, it might be suggested that the increase of the coefficient of kinetic friction caused by the addition of hyaluronic acid at high concentrations (Fig. 4) is related to the modification of the mix of lubricants that covers the mesothelial surfaces.

Concerning the absence of correlation between the coefficient of kinetic friction and the features of the microvillar network, it should be considered that the appearance of the mesothelial surface after conventional fixation and dehydration, as done by us and previous investigators (Andrews and Porter, 1973; Wang, 1974; Ohtsuka et al., 1997; Michailova, 2004), may be deceptive, inducing to believe that the profile of the sliding surfaces is determined by the microvilli up to 3 μm long (Wang, 1974), while the coat, i.e. the amorphous layer stained with ruthenium red or colloidal iron, is only tens of nanometers thick. On the contrary, it is possible that this extreme thinness of the coating is artefactual. Indeed, the glycocalyx of cultured endothelial cells is ~40 nm thick when stained with ruthenium red or osmium tetroxide after glutaraldehyde fixation and alcohol dehydration, but up to ~ 6-10 μm if the same cells are fixed and dehydrated by rapid freezing and freeze substitution (Ebong et al., 2011). No corresponding information concerning mesothelial cells is presently available, but measurements performed with atomic force microscopy have shown that the stiffness of fresh rat parietal pleura (0.38±0.11 kPa; Kim et al., 2011) and that of the glycocalyx of cultured endothelial cells (~0.25 kPa; O'Callaghan et al., 2011) are not much different. Hence, if the thickness of the mesothelial coating were similar to that of the glycocalyx of

endothelial cells, the microvilli would be enmeshed inside the coating and not involved in surface contacts during reciprocating movements of the pleurae, causing the coefficient of kinetic friction to become independent of the density and morphological characteristics of the microvilli. On the other hand, the microvilli could still participate in determining the mechanical properties of this thick coating and, possibly, the frictional characteristics too, though indirectly. Furthermore, it should be noted that the absence of correlation between the coefficient of kinetic friction and the characteristics of the microvilli does not deny *per se* the relevance of the integrity of the microvillar network in determining this coefficient.

The role of hyaluronan as a lubricant of mesothelial surfaces might be open to criticism. Hyaluronic acid is certainly important in joint lubrication (Schimdt et al., 2007), but hyaluronan concentration in the synovial fluid (1-4 $mg \cdot ml^{-1}$; Castor et al., 1966) is markedly higher than that of pleural fluid (~1 $\mu g \cdot ml^{-1}$; Wang and Lai-Fook, 1998). Furthermore, treatment of pleural specimens with hyaluronidase or neuroaminidase does not modify μ (Sironi et al., 2013), suggesting that hyaluronan and sialomucins are not essential components of the lubricating system. In contrast, both pronase and phosholipase C have been shown to cause a marked increase of μ, suggesting a role for proteins and phospholipids in mesothelial lubrication. Changes of μ were, however, accompanied by a more or less extended damage of the mesothelial cells (Sironi et al., 2013; Bodega et al., 2014). Indeed, the similarities with blotting, e.g. cell injury, increase of μ with treatment and subsequent decrease of μ with hyaluronan addition, suggest that the effects of these enzymes are mainly related to cell injury. Clearly, further studies are needed to elucidate nature and role of the substances involved in mesothelial lubrication.

Addition of hyaluronic acid markedly decreased μ of apparently intact mesothelial surfaces only if the initial value of this parameter was abnormally high (high μ group). Given the high hyaluronic acid concentration used, it could be that hyaluronan had formed a continuous layer, thus replacing the normal surface with an artificial one. If this were the case, no correlation should occur between μ measured before and after addition of hyaluronan, but the good correlation found between μ before and after hyaluronan addition in the normal specimens (Fig. 4) denies this supposition. Because an effect of hyaluronan addition similar to that observed in the high μ group occurred also after blotting of specimens with initially normal values of μ (group B; Bodega et al., 2012), and a substantial damage of the mesothelial layer took place under both circumstances (Fig. 1, panel B), it seems reasonable to believe that both the initial value of μ and its change with the addition of hyaluronic acid (Fig. 7) reflect the degree of mesothelial damage, rather than the amount of lubricant lacking from the surface in the high μ group, or removed by blotting and replenished with hyaluronan. It might be also suggested that damage of the mesothelial surface exposes sites

which cause μ to increase and weakly bind hyaluronan (Laurent and Fraser, 1992); we have no hint supporting this hypothesis, except the observation that washing with Ringer after hyaluronan addition returned μ to its initial value, whereas extensive washing with Ringer does not change μ of intact specimens, indicating that lubricants should be tightly bound to normal mesothelial surfaces.

Several external agents are able to damage mesothelial cells: air and isotonic saline (Ryan et al., 1973), water (Ivanova and Puzyrev, 1977), asbestos (Allison, 1973), foreign protein (Baradi and Campbell, 1974), and silica (Shade and Williamson, 1968). More recently, it has been shown that the contact between a peritoneum severely injured by electrocauterization and normal peritoneum causes the rapid loss of mesothelial cells from the latter, probably due to the mechanical stress, as it takes place before any inflammatory response (Suzuki et al., 2015). Here we show that interposition of hyaluronan between a blotted and normal mesothelium during 2 min of reciprocating sliding with velocities and under loads in the physiological range, largely limited or prevented both the damage of the non-blotted surface, as well as the increase of μ. In this model, hyaluronic acid shows therefore very good protective properties. In fact, reciprocating movements of a blotted mesothelium against an initially normal one wetted with Ringer caused substantial damage to the latter under the conditions above (Fig. 6, panel C), and a prominent increase of μ (group B).

In conclusions, the present work shows that the superficial density of microvilli and their characteristics are not determinants of the frictional forces which oppose sliding of normal mesothelial surfaces under physiological conditions, nor of the lubricant effect of hyaluronic acid, which is able to slightly increase or to markedly decrease the coefficient of friction in physiological or pathological conditions, respectively. However, hyaluronic acid reduces or prevents the injury induced by sliding of an intact mesothelial surface against a damaged one. This protective action could explain the increased secretion of hyaluronic acid into the pleural space that occurs when the reciprocating movements become potentially harmful to the pleural membrane, such as high velocities and loads during hyperventilation (Wang and Lai-Fook, 1998), or localized pleural damages during inflammatory or neoplastic processes (Pettersson et al., 1988).

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Legends

Fig. 1. Light microphotographs of parietal mesothelium stained with silver nitrate A) intact, B) after blotting, and C) non blotted, but after sliding against a blotted surface with Ringer lubrication.

Fig. 2. Transmission electron microphotographs of parietal caudal mesothelium after sliding against an intact visceral surface. The density of the microvilli is extremely irregular: microvilli can be abundant and occasionally branched (A) or scanty (B) in adjacent surface areas. Bar is 1 μm.

Fig. 3. Relationships between the coefficient of friction measured with Ringer (μ_R) in the normal μ group specimens and indexes related to density (D_{MV}, panel A) and length of the microvilli (L_{MV}, panel B), and thickness of the mesothelial layer (T_{MV}, panel C).

Fig. 4. Relations between the coefficient of friction measured before (μ_R) and after the addition of hyaluronic acid (μ_H) (*panel A and C*), and before (μ_R) and after washout of the added hyaluronic acid ($\mu_{R,Wo}$) (*panel B and D*) for specimens of the normal (*upper panels*) and high μ group (*lower panels*), respectively. Broken lines are identity lines.

Fig. 5. Relationships between the effect of hyaluronan addition, calculated as the difference between μ measured before (μ_R) and after the addition of hyaluronan (μ_H), and indexes related to the density (D_{MV}, panel A) and length of the microvilli (L_{MV}, panel B), and thickness of the mesothelial layer (T_{MV}, panel C).

Fig. 6. Transmission electron microphotographs of visceral pleural mesothelium after sliding against a blotted surface with Ringer in between. The mesothelial layer can be absent (A) or display cytoplasmic signs of damage and a not well preserved relationship with the basement membrane (B). Bar is 1 μm.

Fig. 7. Relationship between the effect of hyaluronan addition, calculated as the difference between μ measured before (μ_R) and after the addition of hyaluronan (μ_H), and the value of μ measured before the addition of hyaluronan (μ_R), for specimens from the normal (circles) and high μ group (triangles), respectively.

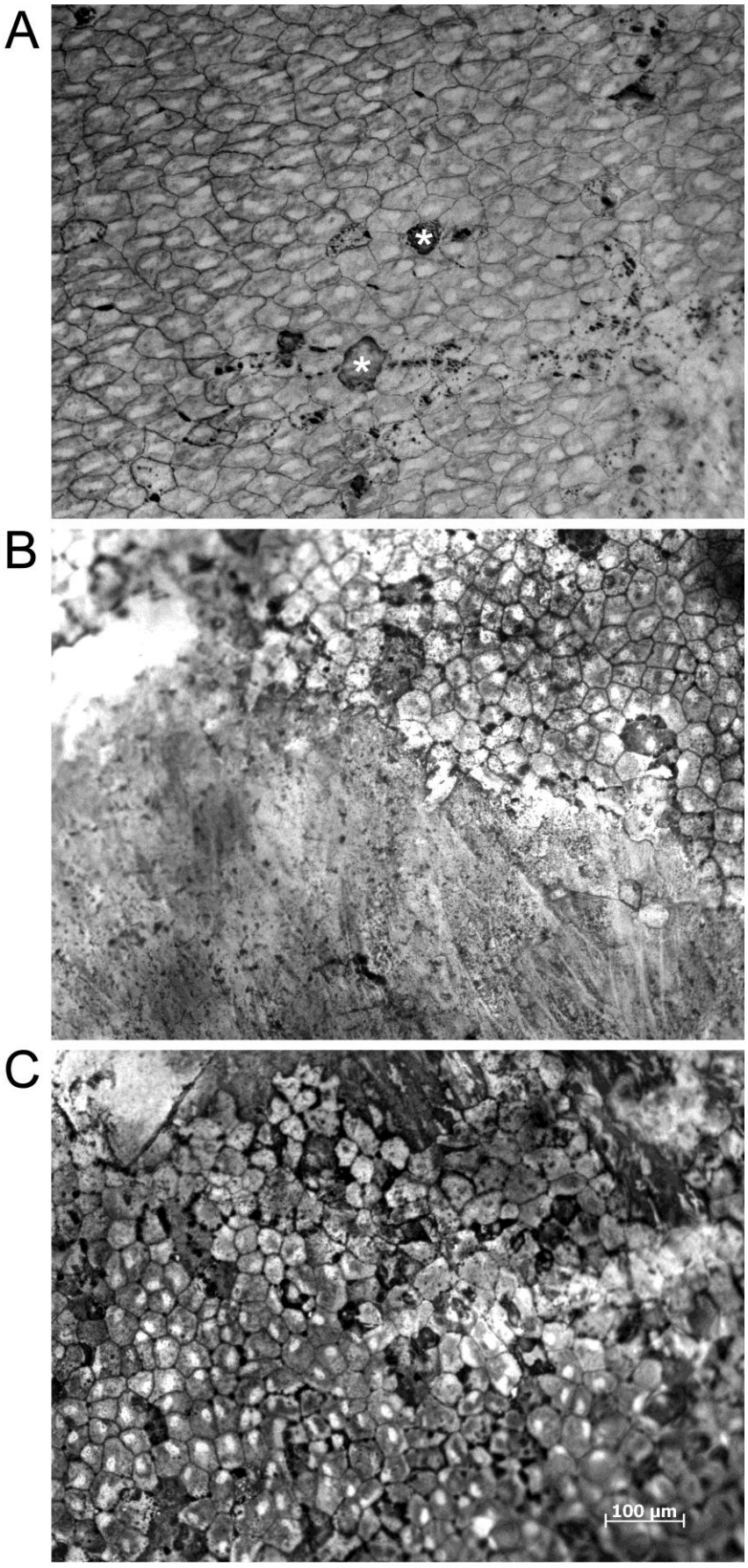

Figure 1

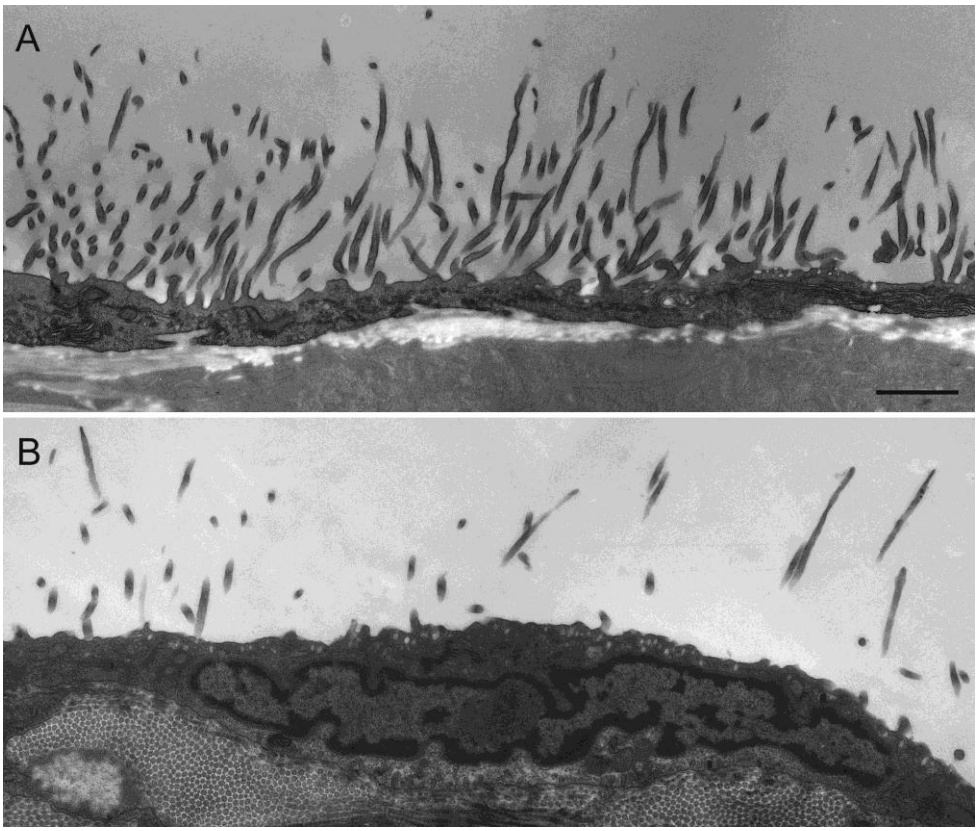

Figure 2

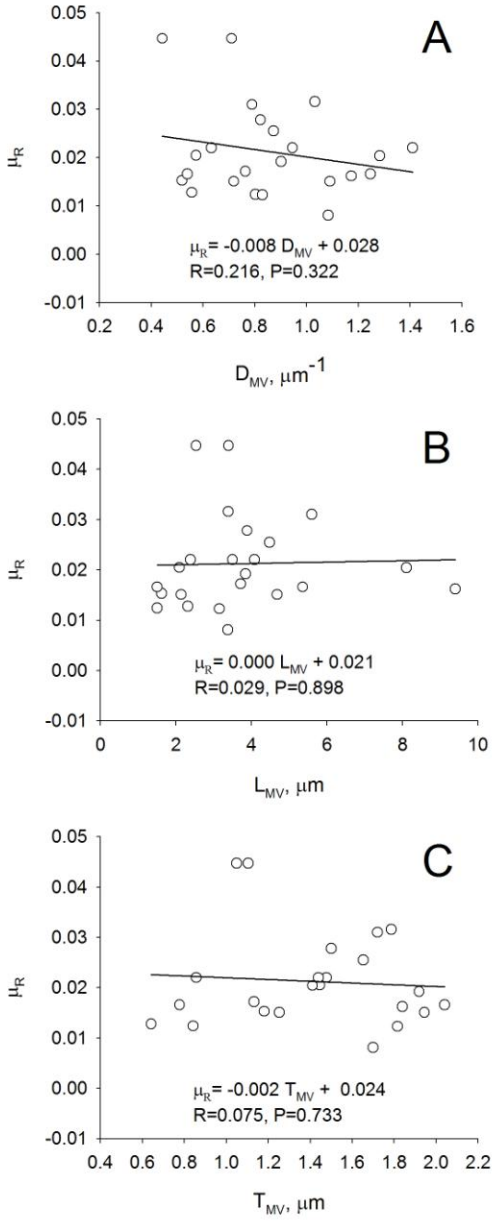
A
μ_R= -0.008 D_{MV} + 0.028
R=0.216, P=0.322
μ_R
D_{MV}, µm^{-1}
B
μ_R= 0.000 L_{MV} + 0.021
R=0.029, P=0.898
μ_R
L_{MV}, µm
C
μ_R= -0.002 T_{MV} + 0.024
R=0.075, P=0.733
μ_R
T_{MV}, µm

Figure 3

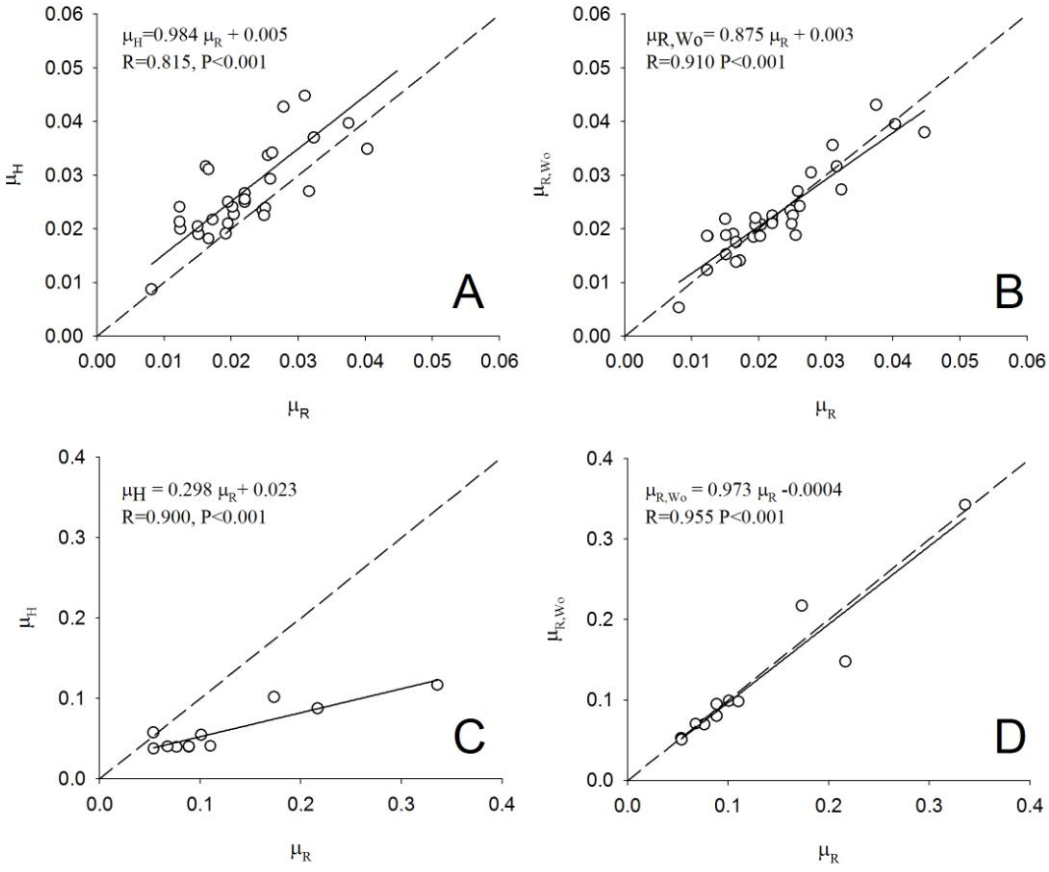
μH=0.984 μR + 0.005
R=0.815, P<0.001
A
μR,Wo= 0.875 μR + 0.003
R=0.910 P<0.001
B
μH = 0.298 μR+ 0.023
R=0.900, P<0.001
C
μR,Wo = 0.973 μR -0.0004
R=0.955 P<0.001
D
μH
μR,Wo
μR

Figure 4

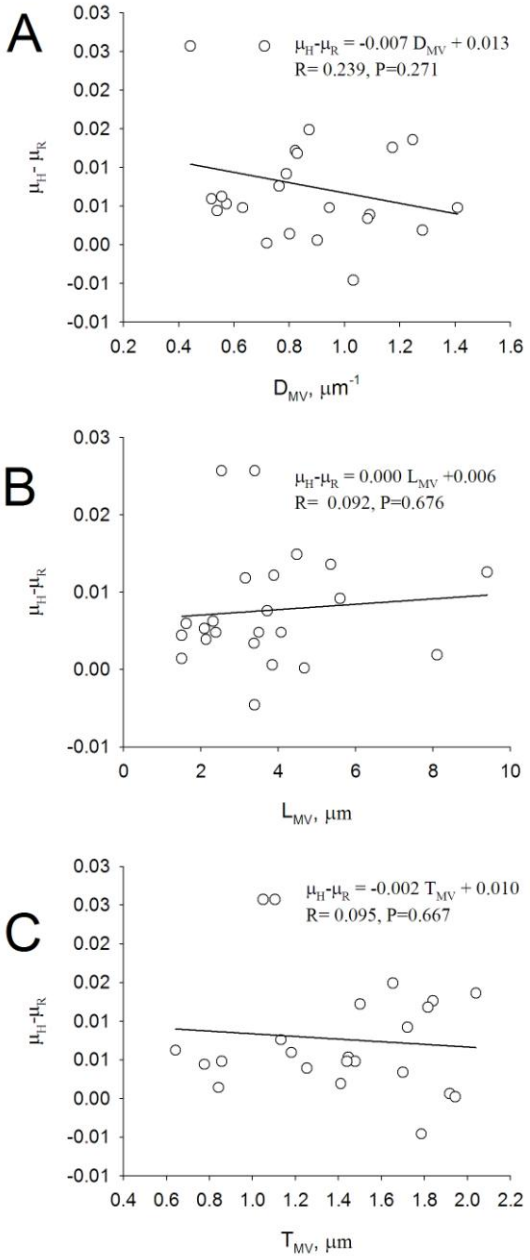
A
μ_H-μ_R = -0.007 D_{MV} + 0.013
R= 0.239, P=0.271
μ_H-μ_R
D_{MV}, µm^{-1}
B
μ_H-μ_R = 0.000 L_{MV} +0.006
R= 0.092, P=0.676
μ_H-μ_R
L_{MV}, µm
C
μ_H-μ_R = -0.002 T_{MV} + 0.010
R= 0.095, P=0.667
μ_H-μ_R
T_{MV}, µm

Figure 5

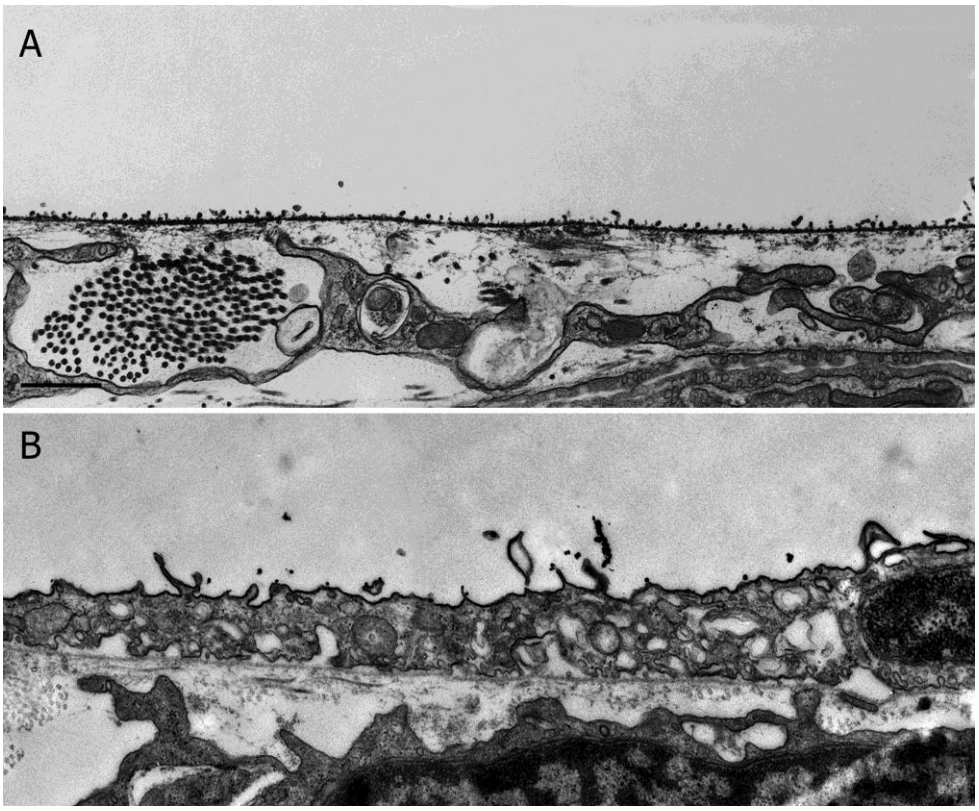

Figure 6

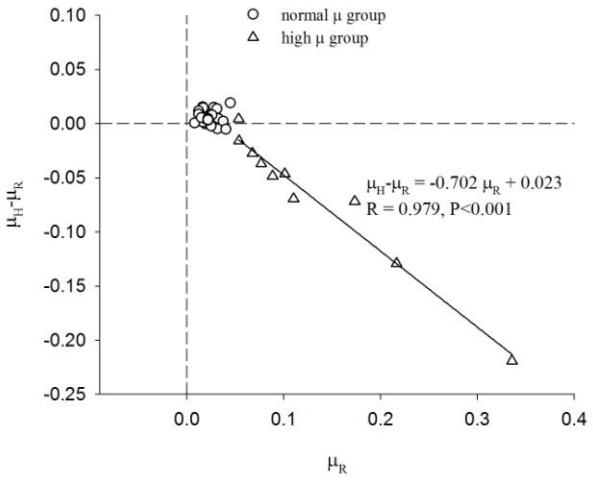
normal μ group
high μ group
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
0.0
0.1
0.2
0.3
0.4
μ_H-μ_R
μ_R
μ_H-μ_R = -0.702 μ_R + 0.023
R = 0.979, P<0.001

Figure 7