Surface Morphology of the Human Pleura*
A Scanning Electron Microscopic Study

Eugenio Gaudio, M.D.; Erino A. Rendina, M.D.;† Luigi Pannarale, M.D.; Costante Ricci, M.D., F.C.C.P.;† and Giulio Marinozzi, M.D.

The superficial morphology of the pleura has been observed in humans by scanning electron microscopy (SEM). Pleural samples from the visceral, mediastinal, diaphragmatic and costal normal human pleura were observed, and a thorough morphometric study was performed. The most evident feature was the ubiquitous presence of microvilli and micro pores. The secretion and absorption activities therefore should not be regarded as accomplished by different topographic zones of the pleura. Discontinuities and clefts were observed at the level of cellular junctions; absorption of high weight molecules and cells from the pleural space may be facilitated at this level, but the structures which were observed in humans may not be considered "stomata," according to the definition of such morphologic units as we give in the present study. Cilia and blebs, described in the experimental animal or in other mesothelia, were not seen in the human pleura.

Scanning electron microscopy (SEM) has proved to be an excellent tool for the study of superficial morphologic features of cells and tissues, and have thus been successfully employed to elucidate the ultrastructural characteristics of several epithelia, both in experimental animals and in man.

Mesothelial lining of serous cavities has also been studied by SEM in the experimental animal, and controversies exist among authors concerning several aspects of the mesothelium; the presence and distribution of areas with different superficial features within the lining of the same serous cavity are debated, as well as the distribution of microvilli; the morphology of cell junctions and the existence of structures such as blebs, micropores, stomata and cilia also needs further evaluation.

A number of experimental studies of the pleura by SEM have appeared in the literature, but not all doubts have been resolved concerning its fine structure. Pleural function such as secretion and absorption of fluid is also dubious, especially if a correlation with specific sites is attempted.

In addition, no exhaustive SEM observations have been carried out in man so far, owing probably to technical difficulties.

We believe it is of great importance to have precise knowledge of the anatomy of the pleural surface in man, in order to draw physiologic as well as pathologic conclusions regarding the mechanisms regulating secretion and absorption of fluid in the pleural space.

For these reasons we have undertaken the present study of the normal human pleura using scanning electron microscopy. Observations were initially undertaken in experimental animals following techniques described elsewhere† and subsequently an original method was developed to obtain good human specimens, suitable for SEM observation. This material forms the subject of our report.

MATERIALS AND METHODS

This study was carried out on 30 patients undergoing thoracic surgery procedures. Tissue specimens 1 cm to 2 cm square were taken at thoracotomy just after the chest was entered and the presence of any pleural abnormality was ruled out. Costal, mediastinal and diaphragmatic pleura were taken in patients undergoing surgery for benign esophageal diseases or T1N0 lung cancer; visceral pleura was taken in T1N0 lung cancer patients from the lobe to be resected.

Eight specimens from the four pleural leaflets were taken in lung cancer patients (20 pts); in those remaining, only six to seven specimens were taken from the parietal, mediastinal and diaphragmatic pleura.

Parietal pleura was taken after incision of the intercostal muscles before opening the pleural cavity; the pleural leaflet was incised by a scalpel circumferentially and grasped on one side by surgical forceps. The visceral pleura was taken before resection of the lobe, by applying a Crile forceps at the base of a zone of superficial lung parenchyma held by a Duval, and excising by a scalpel only an area of untouched pleura. Diaphragmatic pleura was similarly obtained, preserving a layer of phrenic muscular fibers beneath the specimen. In the operating room, specimens were immediately washed in physiologic heparinized solution to prevent fibrin deposition, fixed in 2.5 percent glutaraldehyde in sodium cacodilate buffer 0.13 M (pH 7.36) for 48 hours at 4°C.

Each fixed specimen was divided into two halves: as a control, one half was processed according to current techniques of dehydration and embedded in epoxy resins; semithin sections stained with toluidine blue were observed by light microscopy; ultrathin sections contrasted with lead citrate and uranyl acetate were observed by a Zeiss TEM9A lens.

The second half of each specimen, on the contrary, was washed in distilled water for two hours; dehydration was performed in a graded...
Visceral 69.16
Costal 56.01
Diaphragmatic 7.60

When strictly followed, this method allowed us to obtain, in humans, specimens comparable to those collected in experimental animals injecting fixative solution directly into the pleural space, with no fibrin or blood clots and no artifacts such as retraction of the surface, although a percentage of shrinking is common and not avoidable in glutaraldehyde-fixed specimens.

On the SEM micrographs, morphometric data were obtained by an image analysis computer system (Kontrol MOP-Videoplan). The results are presented as mean ± SD.

**Observations**

Despite all precautions, a number of our specimens, especially those coming from the visceral pleura, were coated by a layer of fibrin which covered all superficial features; this artifact, due to the method of fixation which necessarily could not be performed in man by intracavitary injection of glutaraldehyde, rendered several specimens of no use.

In observable specimens, the arrangement of cells, though constantly polygonal (Fig 1), was variable (Table 1); the surface area of cells of the visceral and mediastinal pleura is higher than that of cells in the costal and diaphragmatic pleura. This might be related to the underlying musculature.

Overlapping of cell borders was evident on the parietal and visceral pleura; a bulging corresponding to the nucleus and most of the other cytoplasmatic organs was evident in the center of the mesothelial cells.

The cell surface was characterized by numerous microvilli of different lengths ranging from 0.5μ to 1.9μ (Table 2). It was possible to see cells having fewer and shorter microvilli; they were seen randomly dispersed in areas showing normal density of microvilli or gathered so as to form a totally distinct zone. The overall density of microvilli is increased on the visceral and mediastinal pleura, lower on the parietal and diaphragmatic pleura.

Micropores are ubiquitous over the mesothelial surface. They were mainly evident, however, in zones with decreased microvilli (Fig 2).

The length of microvilli, ranging between 0.5μ and 1.9μ, is higher where the density is increased, reaching its peak on the visceral pleura (Fig 3).

It was also possible to see intercellular clefts surrounded by cells, the borders of which were devoid of microvilli. At the bottom of the clefts, collagen fibrils related to the submesothelial membrane appeared (Fig 4), as confirmed by LM and TEM observations. The surfaces of surrounding cells were characterized

**Table 1—Cells (200 Specimens Observed)**

<table>
<thead>
<tr>
<th>Surface (sq μ)</th>
<th>Max Diameter (μ)</th>
<th>Min Diameter (μ)</th>
<th>SD ±</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visceral pleura</td>
<td>83.22</td>
<td>12.14</td>
<td>2.3</td>
</tr>
<tr>
<td>Costal pleura</td>
<td>69.16</td>
<td>10.01</td>
<td>2.08</td>
</tr>
<tr>
<td>Mediastinal pleura</td>
<td>84.28</td>
<td>11.58</td>
<td>2.27</td>
</tr>
<tr>
<td>Diaphragmatic pleura</td>
<td>56.01</td>
<td>9.33</td>
<td>1.92</td>
</tr>
</tbody>
</table>

**Table 2—Microvilli (200 Specimens Observed)**

<table>
<thead>
<tr>
<th>Length μ</th>
<th>Number per Cell</th>
<th>Number Per (sq x)</th>
<th>SD ±</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visceral pleura</td>
<td>1.42</td>
<td>207</td>
<td>60.26</td>
</tr>
<tr>
<td>Costal pleura</td>
<td>1.23</td>
<td>145.50</td>
<td>11</td>
</tr>
<tr>
<td>Mediastinal pleura</td>
<td>1.27</td>
<td>283</td>
<td>107.06</td>
</tr>
<tr>
<td>Diaphragmatic pleura</td>
<td>0.75</td>
<td>161</td>
<td>12.72</td>
</tr>
</tbody>
</table>

*Figure 1. Scanning electron microscopic photograph of mediastinal pleura (original magnification, ×2,000). Low magnification of the mesothelial surface showing the regular shape of cells (BAR = 10 μ). All specimens are gold coated.*

*Figure 2. Scanning electron microscopy, original magnification, ×23,000, of parietal pleura. High density of micro pores is evident in a zone showing scarce microvilli (BAR = 1 μ).*
by smaller and sparse microvilli. This feature was mostly recognized on the diaphragmatic pleura though also present on the parietal, visceral and mediastinal surfaces.

It was possible to see a few steep and narrow infoldings of the surface of two neighboring cells. These holes did not always display the same pattern. The cell borders appeared deepened and no contact between two contiguous cells was seen. The internal walls of these holes, as far as we would observe, showed sparse microvilli. The bottom of the holes was not always evident. Sometimes it was possible to see a series of holes between two or three cells, divided by narrow ribbons of cell membranes or bridging microvilli. At the bottom of these holes either the surface of another cell or the internal fibrils of the submesothelial membrane were evident (Fig 5). We do not have information sufficient to permit us to attribute these features to artifacts of fixation or to the specialized activity of different pleural sites. Neither cilia nor blebs were observed in our human specimens.

**DISCUSSION**

According to classic observations, the basic struc-

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21571/)

**FIGURE 3.** Visceral pleura, SEM, original magnification ×10,000. Notice long and dense microvilli (BAR = 2 μ).

ture of the resting pleura, similar to the lining of other celomic cavities, consists of a layer of flat mesothelial cells, basal lamina, a layer of loose connective tissue with collagen fibrils, capillary loops, fibroblasts, lymphocytes, macrophages, and occasionally mast cells, eosinophils and plasma cells; the deep connective tissue varies considerably in thickness and adheres firmly to either ribs or muscles underneath.²⁻⁴

The mesothelial layer is either bumpy or flattened, usually reflecting the rigidity of the structures underneath; bumpy areas are present on most of the visceral pleura, on the mediastinal and subcostal regions and pleural recess; the flattened areas are mainly present over the diaphragm.⁴

A bulging corresponding to the nucleus and most of the cytoplasmatic organelles is present in the center of the mesothelial cells, as observed in previous experimental reports.¹⁻⁴

Overlapping of cellular margins is evident both on the visceral and parietal pleura and may represent the response of the nonelastic mesothelium to the elastic recoil of the underlying reticular membrane during the movement of respiration.⁴ The parietal pleura shows either overlapped intercellular junctions or narrow linear contacts between cellular margins.⁴

With scanning electron microscopy, the most characteristic feature found on the pleural surface is the abundance of microvilli; in experimental animals their concentration is generally very high in the caudal portion of the visceral pleura and in the ventral and caudal mediastinal pleura;⁴⁻⁶ microvilli decrease over the muscular portion of the diaphragm and on the lower intercostal muscles, and become rare on the ribs and tendinous portion of the diaphragm.

In human subjects, as well as in experimental animals,¹⁻⁴ microvilli in some areas are irregularly distributed, so that even adjacent cells differ highly for density of microvilli.
Regional differences are more consistent among experimental animals and may have some functional significance.\textsuperscript{1,4} The rate of microvilli reflects the rate of cellular absorption\textsuperscript{4} and may also reflect the number of adjacent blood vessels. In fact, their number increases in pleural effusions, where submesothelial vessels also are engorged.\textsuperscript{7} In addition, blood vessels are very close to the pleural surface in zones with abundant microvilli.\textsuperscript{2,8}

Areas with many microvilli, such as in the lower visceral pleura, have thus been attributed a high absorption activity;\textsuperscript{8,10} our observations, demonstrating high concentration of microvilli also over the parietal pleura, do not substantiate the hypothesis of expressive regional differences in man.

Microvilli holding conglomerate mucopolysaccharides also act as a buffer zone lessening the friction between the constantly moving lung and the chest wall.\textsuperscript{2,4,11}

Allen,\textsuperscript{12} Dibkowsky\textsuperscript{1} and Hedenstedt\textsuperscript{13} described the presence of stomata in the mesothelium, and Wang\textsuperscript{14} and Albertine and colleagues\textsuperscript{2} confirmed this by SEM on the pleura. According to these authors, they range from 2 nm to 6 nm or more, are round or slit-like in shape and are present, less than 1 per mm\textsuperscript{2} on the mediastinal and parietal pleura, but not on the visceral pleura, though lymphatic vessels are presented therein; red blood cells, macrophages and irregularly shaped noncellular structures like crystallized fibrin, were frequently associated with stomata.

The exact functional significance of the stomata is uncertain: their number is too low to be physiologically important; in addition, stomata seem too small to admit cells, especially macrophages with material engulfed.\textsuperscript{14}

It has been postulated that flexible cells and other particles are sieved through the stretched stomata into the lymphatic lacunae, when the walls of the lacunae are pulled apart by the outward drag of the intercostal muscles and the inward drag of the elastic recoil of the lung and pleura; retrograde blow is barred by the valves in the lymphatic channels and in the narrowed stomata, contracted and elongated at expiration.\textsuperscript{6}

However, if we consider as "stoma" a direct continuity of the endothelial cells of the lymphatic vessels with pleural mesothelial cells, in man we did not see any structure considered a stoma. The bottom of the clefts between two cells clearly show the underlying basal membrane, thus excluding any direct continuity with lymphatic vessels or lacunae. In samples taken from normal tissues, this aspect is due to a tangent traction applied to the mesothelial surface; this traction appears to arise from the pattern assumed by the sample after dehydration. On the other hand, these infoldings may only be considered as the thinnest row of mesothelial cells, and not as a direct continuation of lymphatic vessels. Probably metabolic exchanges with the lymph and blood streams are facilitated in these zones, for a shorter pathway connects the pleural cavity and the lumina of the vessels.

Some authors have described microscopic structures on the pleural surface constituted by modified mesothelial cells by a cuboidal appearance, named Kampmeier foci.\textsuperscript{4,6,16} In our observations on human samples we did not identify any structure to be considered a Kampmeier focus. In fact, cell bulge was fairly uniform within the same specimen.

In our previous experimental studies on pigs, we have observed the presence of blebs on the surface of mesothelial cells lining the parietal pleura, especially in the microvilli-free areas and close to intercellular junctions.\textsuperscript{1}

With further increasing magnification, micropores have been observed; they seem to be invaginations of the cell membrane and suggest micropinocytotic activity.\textsuperscript{1,4}

Wang\textsuperscript{4} has described the presence of cilia on the pleural surface, and Ishihara et al\textsuperscript{17} have also reported this finding when they studied the pericardium by SEM and MET. In our previous and present observations, both in man and in animals (pigs and rabbits), we did not see any membrane process to be considered by its morphologic features as a cilium.

In conclusion, the surface of the human pleura appears to be on the whole rather homogeneous in all different sites. The secretion and absorption activities are therefore not to be regarded as accomplished by different topographic zones of the pleura. In fact, at the level of the same cell or of two neighboring cells we observed both microvilli and micropores, offering evidence of the accomplishment of both functions in the same pleural area.

Absorption of high weight molecules and small cells may be facilitated at the level of the discontinuities between cellular junctions, which, however, we would not consider as "stomata," according to our anatomic definition of such structures.

\textbf{References}

6. Courtice FC, Simmonds WT. Physiological significance of lymph drainage of the serous cavities and lungs. Physiol Rev 1954; 34:419-49
7. Legrand M, Pariente M, Andre J, Chretien J, Bronet G. Ultra-
8 Casey-Smith JR. An electron microscopic study on the passage of ions through the endothelium of lymphatic and blood capillaries and the mesothelium. Quart J Exp Physiol 1967; 105-13
9 Agostoni E. Mechanics of the pleural space. Physiol Rev 1972; 52:57-128
12 Allen L. The peritoneal stomata. Anat Rec 1936; 67:89-103
14 Wang NS. The preformed stomas connecting the pleural cavity and the lymphatics in the parietal pleura. Am Rev Respir Dis 1975; 111:12-20

24th Annual Arizona Chest Symposium

The Tucson Medical Center will sponsor this symposium March 17-19 at the Doubletree Hotel, Tucson. For information, contact Susan Thornton, RN, MS, PO Box 92195, Tucson 85733 (602:327-5461).

Seventh Annual Tuberculosis Symposium

This seventh annual symposium will be held at the Pasadena Hilton Hotel, Pasadena, California, February 4 and 5, sponsored by the American Lung Association of Los Angeles County and Center for Diseases Control. For information, contact ALA of Los Angeles County, 5858 Wilshire Blvd, Los Angeles 90036-0926 (213:935-5864).