The Role of Fiber Analysis in Asbestos Induced Lung Disease: TEM vs. SEM. Is There Controversy
Elizabeth N. Pavlisko, M.D., Department of Pathology, Duke University Medical Center

I. Introduction to Fiber Analysis in the Lung

II. Producing Analytical Data, Microscopy and Analytical Procedures

III. SEM vs. TEM pros and cons

IV. Fiber Morphology and Disease

V. Concluding Thoughts: SEM vs. TEM revisited

Introduction to Fiber Analysis in the Lung

Occupational and environmental exposure to asbestos fibers is known to cause fibrosing and neoplastic pulmonary diseases, including asbestos airway disease, asbestosis, mesothelioma and lung cancer (1, 2). Asbestos bodies can be found in the lungs of disease free individuals, more so in industrialized areas (3). Thus, the analysis of tissue mineral fiber content and its relation to the aforementioned pathologies is of increased interest as occupational safety standards are defined and redefined as well as in the implication of a fiber source, or lack thereof, in the medico-legal context.

The different methods available for quantification of asbestos include bright field light microscopy (LM), phase contrast light microscopy (PCLM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The latter two will be a focus of this discussion.
Qualitative analysis to determine asbestos fiber type requires the coupling of electron microscopy with energy dispersive x-ray analysis (EDXA) or selected area electron diffraction (SAED). Asbestos fibers are forms of hydrated silicates which can be broadly classified into commercial amphiboles (amosite and crocidolite), noncommercial amphiboles (tremolite, actinolite and anthophyllite) and serpentine fibers (chrysotile). The most potent, in terms of pathogenicity, is crocidolite followed by amosite (4). There is little evidence to support chrysotile as a cause of mesothelioma (5, 6).

**Producing Analytical Data**

Early work by Langer and Pooley led the way for analysis of lung tissue for mineral fiber content using ultrastructural microscopy (7-12). The technique for isolation of lung mineral fibers involves 3 main steps: 1. dissolution of organic matter (wet chemical digestion and low or high temperature plasma ashing), 2. recovery and concentration of mineral fibers, and 3. fiber content analysis by microscopy (3). Our laboratory uses the sodium hypochlorite digestion technique as has been previously described (13). As there is some variability in fiber content within the lung, generous sampling is emphasized with autopsy, pneumonectomy or lobectomy specimens providing more accurate data; however, 0.1 gram or less of wet tissue can be used. We prefer samples of lung parenchyma (one from each lobe if available) subjacent to visceral pleura and weighing approximately 0.3 grams. Areas of fibrosis or neoplasia should be avoided due to their dilutional effect on the concentration of fibers in lung parenchyma (13). Alternatively, paraffin embedded tissue may be used following a process of deparaffinization; however, a correction factor must be applied to the determined asbestos fiber concentration.
The recovery of fibers following digestion involves care such that no sample contamination occurs. Methods include collection/filtration onto acetate or polycarbonate filter (pore size of 0.2 -0.45 µm). Error through loss of fibers can be introduced if the pore filter size is too large. Filters can then be used for LM, SEM or TEM.

**Microscopy**

There are several different microscopy methods available for analysis for lung mineral fiber content. The least complex method is conventional bright field light microscopy. Asbestos bodies can be counted on filters prepared from digested sections of lung tissue at a magnification of 200x (whole filter) or 400x (requires at least 2 asbestos bodies on two perpendicular passes at greatest diameter and requires calculation). The results are reported as asbestos bodies per gram of wet lung tissue. There is excellent interlaboratory correlation using this method (14). The down side to this form of analysis is that a large number of fibers are beyond the resolution of light microscopy and identification of fiber type is not possible (15).

PCLM has the benefit of resolving fibers with a diameter of 0.2 µm or greater and can detect uncoated fibers, unlike light microscopy. A majority of asbestos fibers are <0.2 µm in diameter and thus are missed using PCLM.

SEM is used by some for the quantification of tissue mineral fiber content. At low magnification (1000x) SEM can detect coated and uncoated fibers yielding results similar to that of PCLM. Higher magnifications (10-20,000x) allow for detection of fibers not visible using
PCLM. TEM is the microscopy technique preferred by many in analyzing lung fiber mineral content as it has superior resolution; detecting the smallest fibers. SEM and TEM can be coupled with energy dispersive x-ray analysis for qualitative analysis and TEM can more easily be coupled with selected area electron diffraction (SAED) for pattern analysis (3).

**Analytical Procedures**

Counting rules of various sorts must be applied when quantitatively and qualitatively analyzing lung parenchyma for mineral fiber content. Pathogenic asbestos fibers are defined as particles with an aspect ratio of at least 3:1, parallel sides and have a length of 5µm or greater. This is based on prior studies which looked at fiber morphology and pathogenicity (5, 16). For our studies, using SEM, 100 fields at 1000x magnification or 200 fibers are counted, whichever comes first. The first 20 uncoated asbestos fibers and first 10 asbestos bodies are analyzed by EDXA.

Background levels of asbestos are determined by our reference population. Examination of 20 disease free control lungs between 1981 and 2001 demonstrated between 0 and 22 asbestos bodies per gram wet lung tissue as determined by light microscopy (17, 18) and <500 commercial amphibole fibers (coated and uncoated) as determined by scanning electron microscopy(3, 18). In terms of asbestosis, we use the 5th percentile of fiber content of all asbestosis cases seen at our institution as a cutoff for distinguishing a causative relation to asbestos exposure.
SEM versus TEM

SEM can resolve fibers to as small as 0.3 µm long and 0.05 µm diameter. As pathogenic fibers are defined as ≥ 5 µm length, this is adequate resolution for mineral fiber analysis. At 1000x asbestos bodies and uncoated fibers can be counted with similar results to phase contrast light microscopy (PCLM). Higher magnification (10-20,000x) allows for identification of fibers not visible to PCLM. SEM can be coupled with energy dispersive x-ray analysis (EDXA) for fiber type identification (3). The possibility of automation also exists though not employed. SEM lacks the ability to identify the smallest of fibers and some have criticized it as being slow (1). There is no way around electron microscopy being a time consuming and expensive process.

TEM is a widely used method for determination of mineral fiber content in tissue digestion preparations. It provides superior resolution and can be coupled with both EDXA and SAED for qualitative purposes. SAED can be helpful when 2 fibers have similar chemical composition as with talc and anthophyllite, both of which are magnesium silicates. The preanalytical process prior to TEM analysis of lung mineral fiber content is more complex and thus there is more opportunity for error through loss of fibers, contamination, and lack of a representative grid in selection for analysis.

Fiber Morphology and Disease

Prior studies have proven that fibers ≥ 5µm in length with an aspect ratio of 3:1 are pathogenic (5, 16). Studies have shown low fibrogenic and carcinogenic potential for short asbestos fibers (<5µm in length) (19-22). The most important aspect of fiber analysis is the
search for fibers that are pathogenic. Crocidolite and amosite (commercial amphiboles) are the most potent fibers in terms of disease causing capability. Tremolite, a noncommercial amphibole, is frequently found as a contaminant in talc, vermiculite and chrysotile. Chrysotile, the sole serpentine asbestos fiber, is easily fragmented and cleared rapidly from the lung. As mentioned previously it is frequently contaminated with amphiboles (1, 23-26). There is little evidence that pure chrysotile causes disease (23, 27, 28). Thus, if we focus our energy and attention to the short fibers we may miss longer fibers which studies have shown are more fibrogenic and carcinogenic.

Concluding Thoughts: SEM versus TEM Revisited

Lung fiber burden analysis is important in the implication of a source and can help make decisions on eliminating health hazards. It is well documented in studies that amphibole fibers are the most potent in terms of fibrogenicity and carcinogenicity. Chrysotile is frequently contaminated with amphibole asbestos. There is no evidence to suggest that pure chrysotile causes mesothelioma in humans. The most important part of fiber burden is assessing the quantity and make-up of disease causing fibers. While TEM is clearly superior to SEM in resolution; being able to detect the smallest particles, these small fibers are overshadowed by the potency of larger amphiboles in terms of disease producing potency. With resolution off the table, SEM and TEM are similar in terms of their benefits. Both provide the ability to detect fibers ≥ 0.5 µm in length with an aspect ratio of 3:1, and both can be coupled with EDXA to determine fiber composition. SEM has the ability (only with great difficulty) to provide information on crystalline structure (SAED) which becomes important when 2 fibers have a
similar chemical composition. This can be performed much more readily with TEM. Both methods are expensive and laborious. TEM has a more complex preparative process and thus there is more opportunity for fiber loss or contamination. When considering the reproducibility and precision of SEM and TEM there is considerable variability between laboratories, largely due to sample preparation (whole filter mount versus a select portion). Thus, comparison of light microscopy data is superior between laboratories (14). While both techniques are equally capable, SEM has a bias for identification of amphiboles while TEM does for chrysotile. While acknowledging that TEM has superior resolution for detecting chrysotile, several studies in human lung mineral fiber analysis have shown correlation with mesothelioma and commercial amphiboles (18, 23, 27, 30).
### SEM/TEM Revisited

<table>
<thead>
<tr>
<th><strong>SEM Pros</strong></th>
<th><strong>TEM Pros</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Resolution to 0.3 µm long and 0.05 µm diameter - At low mag (1000x) asbestos bodies and uncoated fibers can be counted with similar results to PCLM - At high mag (10-20,000x) identification of fibers not visible to PCLM</td>
<td>1. Preferred method by most for detection of environmental mineral fiber content*. - TEM is used by AHERA 1986</td>
</tr>
<tr>
<td>2. Can be coupled with EDXA for qualitative analysis of fiber chemical composition</td>
<td>2. Highest resolution (to 0.5 nm) for identification of the smallest particles</td>
</tr>
<tr>
<td>3. Possibility for automation</td>
<td>3. Can be coupled with EDXA for qualitative analysis of fiber type</td>
</tr>
<tr>
<td>4. SAED can be performed in conjunction with TEM providing information on the crystalline structure. This is helpful when 2 fiber types have similar chemical composition.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>SEM Cons</strong></th>
<th><strong>TEM Cons</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lacks highest resolution capabilities</td>
<td>1. More complex preparative steps - increased opportunity for error via loss of fibers or contamination</td>
</tr>
<tr>
<td>2. Time consuming</td>
<td>2. Only a small portion of the filter is mounted for TEM. Is it truly representative?</td>
</tr>
<tr>
<td>3. Expensive</td>
<td>4. Time consuming</td>
</tr>
<tr>
<td>5. Expensive</td>
<td></td>
</tr>
</tbody>
</table>


