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BIOMEDICAL AND ENVIRONMENTAL APPLICATIONS OF SECONDARY ION EMISSION MICROANALYSIS

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Résumé - Le SIMS est appliqué à la caractérisation de contaminations dues au milieu ambiant et à l'étude cytopathologique de particules respirables dans les macrophages alvéolaires. La quantification des données SIMS provenant d'échantillons biologiques est en partie limitée par des irrégularités de surface dues au rétrécissement différentiel de l'échantillon pendant sa déshydratation et par une vaporisation hétérogène ou différentielle des divers constituants tissulaires. Dans les études préliminaires on a comparé les effets morphologiques du décapsulation par plasma ou par bombardement ionique sur différentes préparations.

Abstract - The application of SIMS to the characterization of environmental contaminants is described, including the study of the cytopathology of respirable particles in alveolar macrophages. Quantification of SIMS data of biological specimens is limited in part by surface topography introduced by differential shrinkage during specimen dehydration and by non-uniform or differential sputtering of various tissue components. Preliminary studies are discussed comparing the morphologic effects of radiofrequency plasma etching vs. ion beam sputtering on various tissue preparations.

The development of analytical techniques for the morphochemical characterization of trace and/or toxic elements in various environmental or biological media is a current subject of interdisciplinary research. The elemental coverage, detection sensitivity, and spatial resolution of secondary ion emission microanalysis (SIM) offer the promise of unique analytical capabilities in this applications area (1,2). Our current collaborative SIM studies involving trace or toxic element characterization in biological specimens include: lung tissue pathology, atherosclerosis, neuronal tissues impacted by Alzheimer's disease, and in vitro studies of cell monolayers.

One specific subject under investigation in our laboratory has been the SIM study of the surface chemistry of various airborne pollutant particles. At issue is the role of the particle surface in mediating both heterogeneous atmospheric reactions and particle toxicity subsequent to deposition via inhalation or ingestion. Particles produced in high temperature industrial processes frequently exhibit surface enrichments of sorbed inorganic and organic compounds (2). A few examples of SIM results for authentic pollutant materials are listed in Table 1.

To investigate the role of surface enriched components on particle toxicity, experiments have been initiated involving in vitro studies of particle interactions with alveolar macrophages. General techniques involving sample preparation and correlative microscopic examination have been described previously (3-5). The distribution of phagocytosed Pb from various lead oxide coated particles (including coal fly ash) has been
Table 1. SIM of the Surface Layers of Airborne Particulate Pollutants.

<table>
<thead>
<tr>
<th>Element</th>
<th>Coal Fly Ash</th>
<th>Automobile Exhaust</th>
<th>Steel Blast Furnace Dust</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>3.3</td>
<td>-</td>
<td>0.3</td>
</tr>
<tr>
<td>Pb</td>
<td>11.0</td>
<td>2.0</td>
<td>0.5</td>
</tr>
<tr>
<td>S</td>
<td>7.7</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Tl</td>
<td>10.0</td>
<td>6.8</td>
<td>-</td>
</tr>
<tr>
<td>Fe⁺</td>
<td>0.8</td>
<td>0.7</td>
<td>0.8</td>
</tr>
</tbody>
</table>

a. secondary ion intensity change observed while sputtering approximately the outer 1000Å of the particle surface with O₂⁺.

b. Fe (as iron oxide) is a principal bulk or matrix component of all three particle types.

established using TEM in conjunction with electron and ion microprobe analysis (4, 5). Dissolution and migration of Pb from the surfaces of phagocytosed particles resulted in the liberation of intracellular Pb, reprecipitation of Pb as P and Ca - containing complexes throughout the cell, and cytopathic changes including swelling of the mitochondria and nuclear membranes.

One limiting factor in the quantitative interpretation of SIM data in the above study was the apparent non-uniform or differential sputtering of various cell components (6). This is a phenomenon of general concern in the SIM characterization of biological specimens. For alveolar macrophages, secondary electron images (Fig. 1A, 1B) suggest that the nucleolus, nuclear envelope and plasma membrane are more resistant to sputter removal by the primary ion beam (O₂⁺). However, these features also are observed to protrude from the surfaces of unsputtered sections of pelletized cells (7) as

Figure 1. Secondary electron images of C coated rabbit alveolar macrophages (width=15µm).

A) Snap frozen, freeze-dried cell
B) Cell after 10keV O₂⁺ bombardment
C) Snap-frozen, freeze-dried 0.2µm thick section of pelletized cells.

Single Arrow = nucleolus; Double Arrow = nuclear envelope
the apparent consequence of differential shrinkage processes during drying (Figure 1C). Such structures, therefore, may have inherently greater mass, density and thickness in the dehydrated tissue. Consequently, the interpretation of apparent sputtering artifacts is complicated by the effects of sample preparation. Future cold stage SIM on frozen, hydrated tissues may be a considerable advantage in light of these potential sputtering or preparative artifacts.

A preliminary compilation of the various effects of ion sputtering or radiofrequency plasma etching on tissue morphology is shown in Table II.

Table II. Etching Effects on Biological Tissues.

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Source</th>
<th>O₂ rf plasma</th>
<th>2keV Ar⁺ beam</th>
<th>10keV O₂ beam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear heterochromatin</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclear envelope</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Nucleolus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lipid droplet</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lysosomes</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M lines (skeletal muscle)</td>
<td>+</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Z lines (skeletal muscle)</td>
<td>+</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = preserved during etching  
- = preferentially etched  
ND = not determined

The results of Ar⁺ and O₂⁺ ion beam bombardment are comparable suggesting that sputtering processes reflect collisional momentum transfer largely independent of primary beam composition. The chemical "microincineration" induced by the O₂ rf plasma, however, appears to have a substantially different selective etching behavior relative to the O₂⁺ ion beam.

References