Elevated Pressure Improves the Extraction and Identification of Proteins Recovered from Formalin-Fixed, Paraffin-Embedded Tissue Surrogates

Carol B Fowler1, Cedric D Moore2, Timothy J O’Leary3, and Jeffrey T Mason1

1Baltimore VA Medical Center, Baltimore, MD, USA; 2Johns Hopkins University, Baltimore, MD, USA; 3Veterans Health Administration, Washington, DC, USA

OVERVIEW

- We demonstrate the utility of elevated hydrostatic pressure for improved protein recovery from formalin-fixed paraffin-embedded (FFPE) tissue.
- As a model for FFPE tissue, a multi-protein FFPE tissue surrogate comprised of lysozyme, carbonic anhydrase, ribonuclease A, bovine serum albumin, and myoglobin (55:15:15:15:10 wt%) was developed.
- Mass spectrometry of the FFPE tissue surrogates retrieved under elevated pressure showed that both the low and high-abundance proteins were identified with sequence coverage comparable to that of the surrogate mixture prior to formaldehyde treatment.
- In contrast, non-pressure-extracted tissue surrogate samples yielded few positive and many false peptide identifications.

RESULTS

Table 1. LC-MS analysis for a 5-protein FFPE tissue surrogate extracted under atmospheric and elevated hydrostatic pressure.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Peptide Coverage</th>
<th>Sequence Coverage</th>
<th>Peptide Score</th>
<th>Protein Peptide Auto-Indian Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) native, unfixed tissue surrogate mixture</td>
<td>57%</td>
<td>26%</td>
<td>10.5</td>
<td>n.d</td>
</tr>
<tr>
<td>B) FFPE tissue surrogate retrieved at 100 ± 7 psi</td>
<td>75%</td>
<td>39%</td>
<td>14.7</td>
<td>n.d</td>
</tr>
</tbody>
</table>

Figure 4. Quality comparison of MS profiles of native protein mixture and tissue surrogate extracts. A) native, unfixed tissue surrogate mixture; B) FFPE tissue surrogate retrieved at atmospheric pressure (14.7 psi), pH 4

CONCLUSIONS

- Extracting FFPE Tissue Surrogate with Elevated Pressure: and Heat;
- Improves total protein recovery by 4-fold.
- Both the low and high-abundance proteins were identified by LC/MS with sequence coverage comparable to that of the native, unfixed protein mixture.
- The false protein identification rates by MS for the pressure extracted multi-protein tissue surrogate samples were comparable to the native protein mixture.
- The false identification rate for the non-pressure-extracted tissue surrogate was 42% to 100%.
- There was a rapid decrease in protein aggregate size with increasing hydrostatic pressure (Fig. 1). Recognition of monomeric protein, as shown by SDS-PAGE (Fig. 2), indicates the decrease in aggregate size corresponded to the reversal of formaldehyde-induced protein cross-links.
- These results suggest that elevated hydrostatic pressures improve the recovery of proteins from FFPE tissue surrogates by hydrating and promoting solubilization of the protein aggregates, allowing for the subsequent reversal (by hydration) of formaldehyde-induced protein cross-links.

REFERENCES