Elemental imaging via LA-ICP-MS
New opportunities in the life sciences

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• MRI contrast agents
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LA-ICP-MS Overview

- Elemental surface analysis technique.
- Laser used to vaporise sample.
- Ablated material is transferred to ICP-MS.
- Selected elements are detected/ measured in a time resolved mode.
Multiple Line Rastering Across Tissue Surface

- Specific time points will relate to laser position (x,y coordinate) on sample.

Sealed Sample Chamber

Rasters across Sample

Thin Tissue Section

Ablated material

Laser

Ar

4500 ICP-MS

Time resolved ion-counting
Distribution Map Construction

LA-ICP-MS Distribution Map

Gd157

GdDOTA-Liposome, Treated Tumour

Histological Section

Gd line-raster ion-responses

Line Raster 25

Line Raster 17

Line Raster 4
MRI Contrast Agents

• Used in MRI to enhance **image contrast**
• MRI measures relaxation of nuclei in a magnetic field following RF pulse
• **Paramagnetic** atoms (Fe, Gd, Mn) affect proton relaxivity
• Contrast imaging
  
  **Tumours**
  
  Neural pathways and brain activity
  
  **Liver and kidney**
  
  Gastrointestinal tract
  
  Heart ischemia and blood vessel constrictions
MRI Contrast Agents

• Three main elements: Mn(II), Gd(III), Fe(III)

Gd(III) chelates

• Most commonly available contrast agents: Magnevist, MultiHance, Omniscan, Gadovist, etc.

• Main applications as extracellular (e.g. tumour detection) and blood pool (vascular structure) agents.

• Novel targeted treatments currently under research.
The Need for Quantitative Information on Contrast Agents in Tissue

- To enable better understanding of cellular chemistry of CA and how this correlates with MRI response.

- Better understanding of effective agent dose for *in vivo* experiments.

- ICP may be a valuable tool in understanding CA tissue concentration and distribution
Calibration standards for LA tissue imaging must demonstrate:

- Similarity with biological matrix (matrix-matching)
- Comparable section thickness (amount of material sampled)
- Homogenous elemental distribution

The following strategies were explored:

a) Homogenised spiked tissue
b) Spiked blood standards in drilled teflon
c) Sectioning spiked blood standards in tygon tubing
d) Sectioning lyophilised spiked serum in CMC blocks
Quantitation – Gd Spiked Serum

- Adapted from quantitative whole body autoradiography
- 2% carboxymethylcellulose (CMC) solution is frozen (-20°C) into a block
- Block machined with series of holes
- Serum/blood, spiked with Gd (ICP standard) solutions
- Solutions added to holes and frozen

8 mm

Frozen CMC block

• Section onto tape

• Place on Teflon disc

ng g⁻¹

0 1 10 100 200
Ion-time Response For Gd Standards

(10, 100 and 200 ng g⁻¹)

Tumour Sample Analysis (rasters omitted)

Average peak heights plotted against elemental concentration…
Quantitation – Gd Calibration Graph

Average Ion Signal Intensity / Counts

Concentration / ng g$^{-1}$

$y = 0.8548x + 0.0089$

$R^2 = 0.9924$ (value for all data points)
Gd-tagged VDA - Tumour Therapy

• Novel therapeutic molecule with MRI contrast

• Experiments performed in collaboration with MRI research group (ICL)
Husbandry and Dosing Regimen

• $5 \times 10^6/0.1$ ml OVCAR-3 cells inoculated into the flank of 6-8 weeks old Balb/c nude mice

• When tumors reached $\sim 7-10$ mm$^2$, mice tail vein cannulated for the administration of either:
  • $200$ mg/kg ($22.64$ mmol) Gd.DOTA.Colchicinic acid
  • $200$ µl saline (control)

• MRI scanning at 2, 8 and 24 hours post injection.

• After the final scan mice were sacrificed, the tumor excised and snap-frozen.
Gd Tumour Therapy Raw Raster Data

Ion Intensity / Counts

Time / s

μg/g

Gd157
Quantitative distribution Gd Contrast Agent

μg/g 157Gd 66Zn

H&E Histology Section
### LA-ICP-MS Parameters For Analysis of Tumour and Standards

<table>
<thead>
<tr>
<th>Laser Ablation Unit: New Wave MACRO (266nm) Nd:YAG</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Beam diameter</td>
<td>100µm</td>
</tr>
<tr>
<td>Raster spacing</td>
<td>200µm</td>
</tr>
<tr>
<td>Scan rate</td>
<td>50µm/s</td>
</tr>
<tr>
<td>Energy,</td>
<td>0.7mJ</td>
</tr>
<tr>
<td>Freq</td>
<td>10Hz</td>
</tr>
<tr>
<td>Runtime</td>
<td>~ 4.5 hours</td>
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</table>
Control Sample

Saline Treated (Control) Tumour

Counts

H&E Histology Section

12mm

14mm

Cetac LSX-200 (266nm) Nd:YAG

Beam diameter 50μm, Raster spacing 100μm

Scan rate 50μm/s,

Energy 0.6mJ, Freq 10Hz,
Comparative imaging – MRI and LA-ICP-MS

Study involving Gd-tagged nanoparticle delivery in mouse brain (developing treatment for dementia), collaborating with UCL

LA-ICP-MS

<table>
<thead>
<tr>
<th>Time Post-Dose</th>
<th>Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>6hrs post-dose</td>
<td>![Image]</td>
</tr>
<tr>
<td>24hrs post-dose</td>
<td>![Image]</td>
</tr>
<tr>
<td>48hrs post-dose</td>
<td>![Image]</td>
</tr>
</tbody>
</table>

MRI
Benefits of combining LA-ICP-MS with MRI

- Signal quantitation
- Affords superior spatial resolution (~5µm) and sensitivity
- Also affords detection of multiple elements

Particularly useful in determining sources of MRI contrast e.g. MRI image below demonstrates a ‘false positive’ result in a control sample

MRI TR=200

Fe\textsuperscript{57}          Gd\textsuperscript{157}
Conclusions

• Studies show ultimate fate of Gd in selected tissues
• Signal quantitation achieved
• Benchmarking to MRI implemented
• Unique benefits of LA-ICP-MS utilised to complement MRI studies

Further Work

• Ongoing studies will involve 2nd generation Gd nanoparticle formulations (UCL collaboration)
• Advance pharmacokinetic-like studies, determining elemental (Gd, Pt) quantitation and distribution over serialized time points
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