Real-time connection of Mass Spectrometry with Medicine and Surgery

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Clinical Chemistry, Medicine & Surgery

Metabolomics
Lipidomics

1913
Mass spectrometry
Stable isotopes

Fundamental physics
Nuclear weapons

Enzymology

NIH

Genomic sequencing
Gene cloning

Transcriptomics
Proteomics

Metabolomics
Lipidomics

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Knowledge that will change your world

GBS 724
March 18, 2016
Dissociative research

• Samples are collected and stored for analysis at a “later” time
• “Later” can be months or years after sample collection
  – Of little direct benefit to the patient
  – Although may influence the community of patients
  – True of many analyses

Real time analysis

• Existing, familiar applications
• Gases!
• The iknife
  – GI surgery
  – Cancer margins
  – Pathology
  – Bacterial masses
• DESI
• CARS
Real-time analysis

- We see the real-time use of MS when we go through security checks at the airport
  - Checks for ion signatures of explosives

- Other devices are used to check for specific volatiles in the breath

Noses and smell – real time analysis

The superior metabolite detector
Gases produced in the GI tract

• H₂, CO₂ and CH₄ from carbohydrates
  – Firmicutes
  – From pyruvate and NAD(P)H/FADH₂
  – H₂ used by sulfate-reducing bacteria (SRBs), methanogenic Archaea, and acetogens

• SRBs produce H₂S

• NO from nitrates

Methods for measuring gases

<table>
<thead>
<tr>
<th>Technology</th>
<th>Operation mode</th>
<th>Target intestinal gas</th>
<th>Detection limit</th>
<th>Cross-sensitivity</th>
<th>Response time</th>
<th>Life time</th>
<th>Estimated cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-MS</td>
<td>Off line</td>
<td>All gases</td>
<td>ppt to ppb</td>
<td>Low</td>
<td>&lt; Several minutes</td>
<td>Long</td>
<td>&gt; US$300k</td>
</tr>
<tr>
<td>IMS</td>
<td>Real time</td>
<td>All gases</td>
<td>ppb</td>
<td>Low</td>
<td>&lt; 1 min</td>
<td>Long</td>
<td>&gt; US$100k</td>
</tr>
<tr>
<td>PTR-MS</td>
<td>Real time</td>
<td>All gases</td>
<td>ppt</td>
<td>Low</td>
<td>&lt; 1 min</td>
<td>Long</td>
<td>&gt; US$400k</td>
</tr>
<tr>
<td>SIFT-MS</td>
<td>Real time</td>
<td>All gases</td>
<td>ppb</td>
<td>Low</td>
<td>&lt; 1 min</td>
<td>Long</td>
<td>&gt; US$400k</td>
</tr>
<tr>
<td>LS</td>
<td>Real time</td>
<td>Most gases except H₂</td>
<td>ppt to ppb</td>
<td>Low</td>
<td>&lt; 1 min</td>
<td>Long</td>
<td>&lt; US$50k</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>Real time</td>
<td>H₂, H₂S, NO, and CO₂</td>
<td>ppm</td>
<td>Medium</td>
<td>&lt; 30 s</td>
<td>Short</td>
<td>US$100k</td>
</tr>
<tr>
<td>Calorimetric</td>
<td>Real time</td>
<td>H₂, CH₄, and CO₂</td>
<td>ppt</td>
<td>High</td>
<td>&lt; 10 s</td>
<td>Medium</td>
<td>US$100k</td>
</tr>
<tr>
<td>NDIR</td>
<td>Real time</td>
<td>CO₂, CH₄, and VOCs</td>
<td>ppm to ppt</td>
<td>Low</td>
<td>&lt; 20 s</td>
<td>Long</td>
<td>&lt; US$300k</td>
</tr>
</tbody>
</table>

Jian Zhen Ou et al., Trends Biotech, 2015
Device for measuring fecal gas production

Jian Zhen Ou et al., Trends Biotech, 2015

Fecal gas production (ex vivo)

Jian Zhen Ou et al., Trends Biotech, 2015
Real-time *in situ* monitoring gas production

- The device is swallowed
- Completes full mouth-to-anus transit, reporting data as it goes
- Also provides positional information
- Operates at 405, 433, and 915 MHz
- Uses Lithium batteries

Jian Zhen Ou et al., Trends Biotech, 2015

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Real time intestinal gas production

Jian Zhen Ou et al., Trends Biotech, 2015
The Challenge for Mass Spec

How to get the mammoth into the gas phase for analysis?

Droplet principle of electrospray

Droplet spray
- Sneeze
- Lung motion
- Surgical knife
- Other vapors
iKnife device

Need to rebuild the interface

Mass spectrum of canine stomach

Predominantly phospholipids

Balog et al
Anal Chem
82:7343, 2010

Fatty acid profiles of bacteria and other microorganisms

- Gas chromatography of fatty acid methyl esters distinguishes organisms
  - Used in Pathology Labs
- In 1990-93 my lab supported early engineering work on the International Space Station (subcontract from Boeing Corp.)
  - To determine the microorganisms that grow in a water treatment unit in microgravity

Phospholipid patterns are characteristic of cells and tissues

- Single items are not sufficient as biomarkers
- The classes of phospholipids and their fatty acid composition contain pattern discriminators
- In the absence of known classifiers, principal components analysis looks for groups of components that have the larger sources of variation
  - An individual sample’s contributions to these groups are plotted in a 2D or 3D manner
Principal components analysis of ions from surgical “smoke”

Balog et al
Anal Chem
82:7343, 2010

Differentiation of brain tumors
Changing lipids across cancer margin

Laser-driven direct mass spectrometry

Differentiation of tissues

Computer-driven, Rapid Evaporative Imaging MS (REIMS) for tissue sections
Examining tissue (slices) by REIMS

Modes of data acquisition for REIMS
Optimizing data acquisition for REIMS

PCA analysis of REIMS data from tissue sections

Golf et al., Anal Chem 2015
Desorption electrospray ionization (DESI)

- Works by directing an electrical fine spray at a tissue target

The IDH story of brain and other tumors

- IDH1 (isocitratedehydrogenase) is mutated in position 132 in a GWAS study of patients with glioblastomas
- IDH1 catalyzes the conversion of isocitrate to alpha-ketoglutarate ($\alpha$KG) which is a two-step reaction
- Mutant IDH1 catalyzes the first step – to 2-hydroxyglutarate (2HG), but not the second one to $\alpha$KG
- 2HG is considered to be an oncometabolite
Whither 2-hydroxyglutarate?

Tissue expressing wild type IDH1

MSMS of m/z 147 - not 2HG

Tissue expressing mutant IDH1

MSMS of m/z 147 is 2HG

Value of exact mass – “147” vs “147”

Santagata et al. PNAS 2014
Tumor xenograft imaging and 2HG

Application to human glioblastoma

Santagata et al. PNAS 2014
Comparative imaging of normal-tumor tissue transition

Distinguishing bacterial populations

Golf et al., Anal Chem 2015
Use of Raman spectroscopy
Real-time imaging of metabolites in skin

- [http://bernstein.harvard.edu/research/cars-why.htm](http://bernstein.harvard.edu/research/cars-why.htm)

Sunny Xie, PhD - Harvard

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The future of medicine and surgery

[http://www1.imperial.ac.uk/phenomecentre/](http://www1.imperial.ac.uk/phenomecentre/)