Biomarker Detection Using Mass Spectroscopy

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Outline

- Introduction to Biomarkers
- Introduction to MS
- Detailed Introduction to ICP-MS
- Biomarker Detection using ICP-MS
- Summary
- References
Biomarkers

- The hottest new term in Biotech industry: BIOMARKERS
- Defined as a characteristic objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic indication.
- Biomarker indicates a change in expression or state of a protein that correlates with the risk or progression of a disease.
Need for Biomarker Detection

- Drug Discovery and Development*
- Indicate drug efficacy and toxicity
- Reduce cost and duration of clinical trials
- In Biotechnology and Pharmaceutical industry

* FDA critical path initiative
Biomarker discovery process

Discover, validate & identify predictive protein biomarker panels

- On-chip profiling
- High throughput detection
- Biomarker pattern recognition
- Purification & biomarker identification
How to detect Biomarkers?

- Comparative Proteomics of Biofluids
- Immunohistochemistry Staining
- Spectroscopy-based Techniques
  - Surface Enhanced Raman Spectroscopy
  - SELDI-TOF (Surface Enhanced)
  - ICP-MS
- Sensing systems
  - Automatic Microfluidic Detection System
  - Carbon Nanotubes Immunosensors
  - Newly developing technologies such as arrayed imaging reflectometry, Porous silicon biosensors, etc.
Mass Spectroscopy

MECHANISM

1. inject sample
2. heater to vapourise sample
3. electron beam ionises sample
4. particles accelerated into magnetic field
5. magnetic field separates particles based on mass/charge ratio

Detector

lightest

heaviest

charged particle beam

magnet
Different kinds of MS

- Laser Microprobe Mass Spectrometry
- Inductively coupled Plasma Mass Spectrometry (ICP-MS)
- Glow Discharge Mass Spectrometry (GDMS)
- Secondary ion Mass Spectrometry
- Electrospray Mass Spectrometry
ICP-MS

Different Parts of ICP-MS:

- Principle of Ion Formation
- Sample Introduction
- Plasma Torch
- Interface region
- Ion focusing
- Mass separation
- Ion Detection
- Sampling accessories
Generation of ions in the plasma

- Sample is introduced in liquid state

- Series of events: Sample pumped into the sample introduction system → Sample emerges as aerosol → Sample Injector → Base of plasma → Analytical zone of plasma

- We Get: Liquid Sample → Solid Sample → **Gaseous sample** → Atoms and ions

- ICP-MS is predominantly used for detection of positive ions, negative ions are also produced in plasma
Sample Introduction System

- Also named as the “ACHILLES HEEL” of ICP-MS
Droplet Selection

**Why:** Large droplets can’t be dissociated by the plasma discharge. Hence, the allowance for small droplets

- Liquid in the drain is kept at positive pressure
- Small droplets move between the outer wall and the central tube
- Tiny droplets go to the sample injector of the plasma torch

**Double Pass Spray Chamber**

**Droplet Size:**
- Large droplets: $>\sim 10 \, \mu m$ in diameter
- Small droplets: 5-10 $\mu m$ in diameter
The ICP-Plasma Torch

- Argon is used to form plasma
- Flow rate of Ar $\sim 12-17 \text{ L/min}$
- Auxiliary gas rate $\sim 1 \text{ L/min}$
- Nebulizer gas rate $\sim 1 \text{ L/min}$
- RF coil is grounded to keep the potential of interface region $\sim 0$
Formation of ICP Discharge

(a) Quartz torch

(b) Electromagnetic field

(c) High voltage spark

(d) Collision-induced ionization of argon

(e) Sample introduced through sample injector

Formation of inductively coupled plasma
Mechanism

- Tangential Flow of Argon passes between the outer and the middle tube
- RF power is applied to the load coil ➔ Intense electromagnetic field
- High voltage spark produces free electrons
- Free electrons get acceleration by the RF field ➔ Collision and ionization of Argon
- ICP forms at the open end of the torch
The Interface region

- What happens here:
  Transportation of ions efficiently, consistently, and with electrical integrity from the plasma to the analyzer region
Mass Separation

- Ions of selected mass pass through the rods of the detector while others are ejected from the quadrupole on application of dc current on one pair of rods and radio frequency field on the other pair.
Application of ICP-MS in analysis of biological samples

- For quantification of toxic elements
- Study of metal transport
- Adsorption and metabolic studies of metals and metallo-drugs
- Affinity products having metal tag are used for ICP-MS immunoassays e.g. Antibodies raised against antigens of interest and tagged with metal
- Means of detection by fluorochromes and radioisotopes suffer from limited dynamic range and limited ability to detect multiple proteins simultaneously So, this provides a good alternative
Metal tagging to $2^0$ antibodies

1. Sample IgG 1 nmol - 150µg
2. Concentration and buffer exchange
3. Incubate 37°C 30'
4. Centrifuge for buffer exchange
5. Bis-maleimido-Polymer-Ligand [n]
6. Incubate 37°C 30' for labeling
7. Ln$^{3+}$ + MESNA (mercaptoethanesulfonate used to quench)

TCEP - alkylphosphine used to reduce disulfide bonds; MESNA - mercaptoethanesulfonate used to quench
Advantages of ICP-MS in enhancing performance of immunoassays

- High Precision
- Low detection limits
- Large dynamic range, both for each antigen and between antigens
- Lower matrix effects from other components of biological sample
- Lower background from plastic containers and plates
- Independence of non-specific background and analytical response from incubation or storage times
- Large Multiplexing Potential
- Better Spectral Resolution
Immunolabeling Cellular Components

- Three Basic Stages
  - Harvesting of cells of interest → Separation from contaminants → React with specific primary antibodies
  - Addition of secondary antibodies with the metal tag directed against $1^0$ antibody specifically labeling the biomarker
  - Quantification by ICP-MS

- ICP-MS used in:
  - Surface antigen detection
  - Concordance of ICP-MS elemental signal to cell numbers
  - Simultaneous cell surface and intracellular detection
  - Multiplex identification
ICP-MS detection of a surface biomarker

- **Target:** Myeloid cell surface antigen CD33 (biomarker)
- **Antibodies considered:**
  - Anti-CD33 antibody A (BD biosciences)
  - Anti-CD33 antibody B (Immunotech Inc.)
  - Anti-mouse-Au
- **Three cell systems:**
  - Cells stained with antibody A (3 cell populations)
  - Cells stained with antibody B (3 cell populations)
  - Control cells stained with IgG1 (3 cell populations)
Results

- Anti-CD33 Antibody B shows higher response as compared to Anti-CD33 Antibody A

- Black bars: Antibody A
- Grey bars: Antibody B
Simultaneous cell surface and intracellular biomarker detection

- **Target:** 2 cell surface biomarkers (CD33 and c-Kit) and 2 intracellular biomarkers (BCR and p210BCR/Abl)

- **Antibodies:**
  - Anti-CD33
  - Biotinylated-anti-c-Kit
  - Anti BCR

- **Metal tagged reagents:**
  - Anti-mouse - Au
  - Streptavidin - Tb
  - Anti-rabbit - Eu
Results

- The signal obtained are comparable in cases where the secondary tags were added all at once as to sample “All” and where they are added separately.
- So there is no interference from each metal tag when used in a multiplex assay.
Summary

- Biomarker basics
- Introduction to MS and ICP-MS
- Detailed description of ICP-MS
- ICP-MS in Biomarker detection
- Various studies
References

- A beginners guide to ICP-MS, Robert J. Thomas
  - Part I
  - Part II: The Sample-Introduction System
  - Part III: The Plasma Source
  - Part IV: The Interface Region
  - Part V: The Ion focusing system
  - Part VI: The Mass Analyzer
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