

Efforts to Develop New Biomarkers – e.g. Proteomics

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Biomarkers: Definition and State of Field

- Physical, functional or biochemical indicators of a physiological or disease process that has diagnostic and/or prognostic utility
- Novel and innovative technologies for biomarker discovery and validation are rapidly replacing more traditional methods

Growth of Interest in Biomarkers

- Increasing application in epidemiologic, demographic and biomedical research
- May overcome various reporting biases and inaccuracies that are increasingly questioned (and which can't be fully overcome by behavioral approaches)
- May provide more information
- Technological advances make application of biomarkers more feasible than before

Rapid Adoption of Biomarkers

- In clinical research (e.g. as surrogate endpoints)
- In survey work
- In contraceptive and STI fields, e.g. to assess condom effectiveness more objectively and in future, to evaluate microbicide use
- Likely gains from such work, as well as profound challenges

Advantages of Biomarkers

- Relatively easy to investigate (in theory)
- Can obtain data quickly and relatively inexpensively (in theory)
- More objective than self-reports
- May yield new insights
- Multiple applications, e.g. diagnostic, prognostic, predictive, monitoring

Problems in the Biomarkers field

- Not all biomarkers are created equal
- Interpreted results from studies remain controversial
- Biomarkers may provide ambiguous data
- Biomarkers may waste time and money (but so can all types of research)
- There is much use and misuse of biomarkers

Search for New Biomarkers

- Continued need for unique, reliable, quantifiable, easily measured, relatively inexpensive, noninvasive biomarkers
- Need to test the validity of candidate biomarkers and to develop more reliable assays
- Until recently, the search for new biomarkers was very slow. This is changing.

Approaches to Biomarker Discovery

- 2 philosophically different but parallel approaches
 - 1). Hypothesis-driven approaches
 - 2). Open-ended 'discovery-based research', using technologies such as genomics, proteomics and other high-throughput approaches

The Proteome

- A rich source of biological information because proteins are involved in almost all biological activities
- Comparisons to the genome- different in quantity and statics
- Only a small percentage of the thousands of proteins in human cells have been sequenced or identified

Proteomics

- Many definitions; concern is with the study of proteins in a cell, tissue or organism
- Increasingly used as it allows (1) rapid identification of protein patterns in living organisms; (2) protein characterization
- Proteomics technology is being explored for potential uses in cancer and other disease fields, including research for proteins that may serve as biomarkers
- But there are many technical challenges to proteomic research (and cost of technology)

Link between 'Standard' Biomarker Research and Proteomics

- Discovery of disease markers has included quantitative measurement of disease-specific proteins in body fluids
- New technology for high resolution, high sensitivity detection and analysis of such proteins
- Procedure: remove most interfering proteins from body fluids, and separate and display remaining low abundance proteins as a map; analyze such maps to construct database

Proteomic-based Development of Biomarkers

- Major push in multiple fields, e.g. cancer, CVD disease and many others, although some of these disease types have still no or very few biomarkers
- Advent of novel proteomic approaches permits new insights into these multi-factorial diseases
- There is much proteomic analysis on readily available bodily fluids, but nothing on semen or vaginal fluid (except for present study)

Proteomic analysis (continued)

- Used to identify new biomarkers; multiple applications in the discovery of new diagnostic, prognostic and therapeutic targets
- We may need multiple biomarkers
- It may still be somewhat premature to expect substantial results from proteomic analysis applied to sexual activity, but some successes already exist in various clinical areas

Some Caveats

- Science is still not at full potential
- As always, need to determine validity of proposed biomarkers and to establish more reliable assays
- Need to standardize specimen collection, selection of appropriate technology platforms
- Costs and complexity of research
- There are limitations to current hopes and strategies

Identification of Potential Biomarkers of Semen Exposure

- These may provide more reliable ways to measure condom use and unprotected intercourse
- May also help assess validity of self-reported data
- Some of this work has been pioneered by forensic medicine
- PSA is most promising biomarker to date
- other possible biomarkers ?
- biomarkers of exposure to vaginal fluids ?

Protein Profiling and Biomarker Discovery with SELDI-TOFMS

- SELDI-TOFMS is a useful proteomic approach that has facilitated the discovery of disease-specific protein profiles
- It is a mass spectrometry (MS) technique that produces a mass spectral fingerprint that can distinguish differences in protein expression levels between diseased and normal samples
- Well-suited for high-throughput protein profiling

Basic idea of SELDI-TOFMS approach

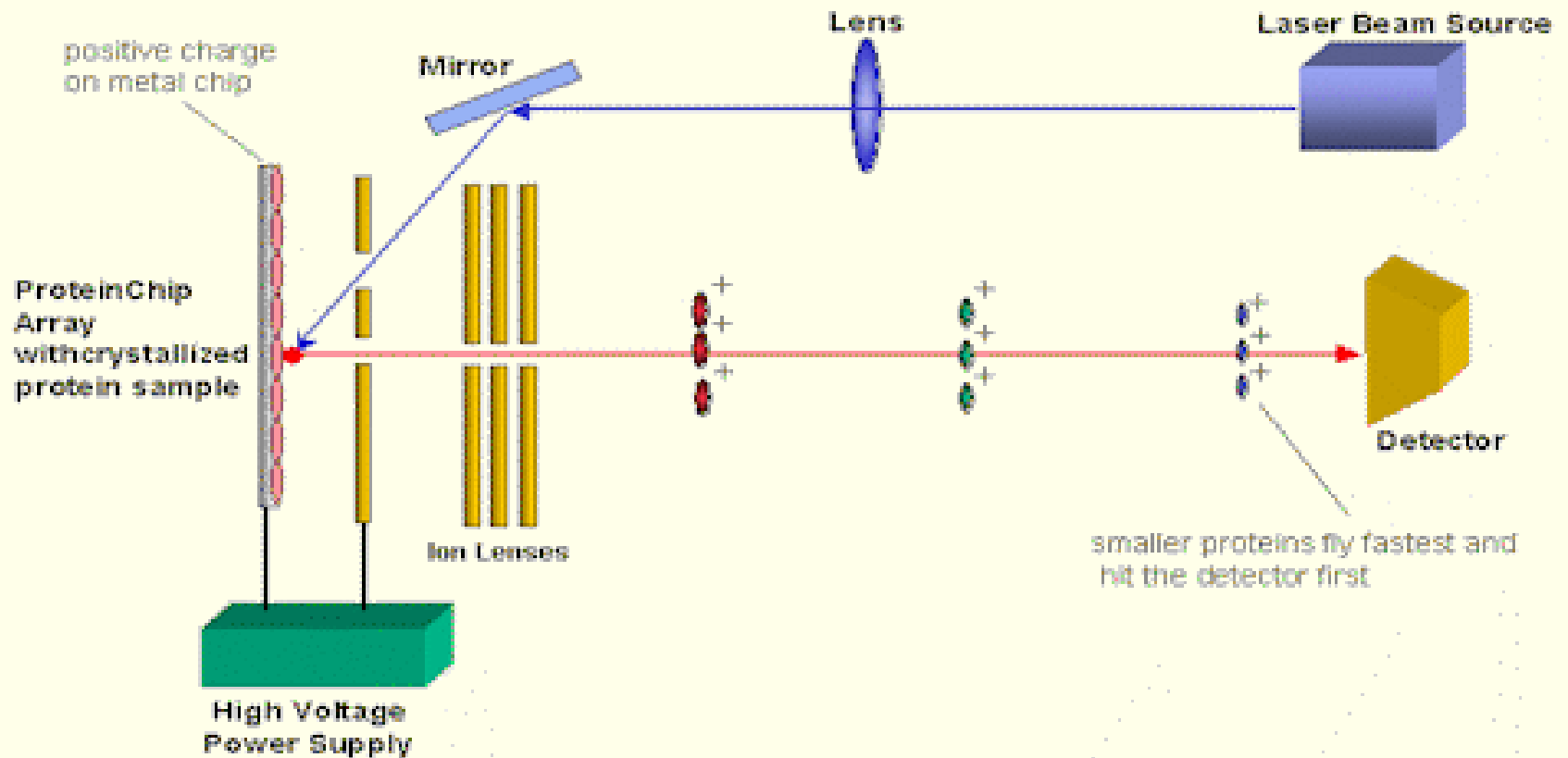
- Bodily fluids can provide much information on presence/absence of diseases
- Molecular markers in bodily fluids can be used to identify patients with specific cancers by looking for specific protein patterns ('fingerprints')
- New approaches developed for early detection of disease based upon protein patterns found in bodily fluids
- SELDI-TOFMS can be used to identify, at an early stage, individuals with specific cancers (e.g. ovarian, endometrium, cervical, prostate)

What is SELDI-TOFMS ? (1)

- **S**urface **E**nhanced **L**aser **D**esorption/**I**onisation
Time **o**f **F**light **M**ass **S**pectrometry
- involves on chip separation of complex mixtures together with mass spectrometry
- able to rapidly analyse samples containing vast amounts of proteins
- generates patterns that these proteins produce
- shows differences between these patterns for proteins expressed in different tissues, or in tissues during different disease states

SELDI-TOFMS, 2

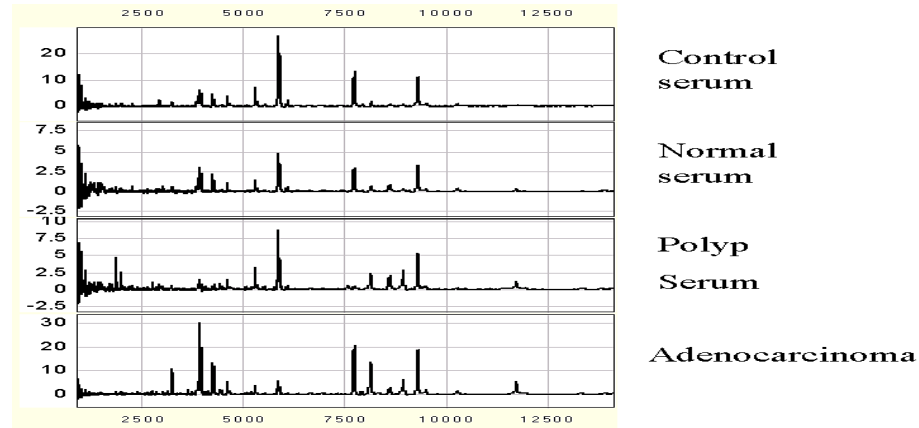
Schematic of ProteinChip Reader



SELDI-TOFMS, 3

- Laser desorbs the proteins on the chip, causing them to be launched as ions
- The time-of-flight (TOF) of the ion before detection by an electrode is a measure of the m/z value of the ion
- Peptides with a larger m/z move more slowly down the flight tube and therefore have a longer TOF

Example of How the Spectra of Proteins in Different Serum Vary



UAB Study and Team

- PI: Andrzej Kulczycki, PhD
- Co-PIs: William Grizzle, PhD and Jane Schwebke, MD
- Recruiting 40 couples to further elucidate the PSA decay curve
- Subset used to assess feasibility of SELDI for studying indications of semen exposure
- We will also provide an initial correlation of PSA with SELDI results

Promise of SELDI-TOFMS

- Pilot component to this study has small sample size, but will collect first SELDI-TOFMS data on exposure to vaginal or seminal fluids
- May help develop an alternative biomarker for measuring semen in vaginal fluids post-coitus, & a biomarker for measuring vaginal fluids in semen post-coitus
- We expect to detect differences b/w genders corresponding to distinct profiles of vaginal & seminal fluid
- UAB has built up experience with SELDI-TOFMS method in other research areas

Facilitating Biomarker Development in the Future

- Biomarkers are being steadily adopted and integrated into research
- Proteomics can be a tool for biomarker development
- It may yet change the field of biomarkers for semen exposure, but as yet, this is only a promise
- Our goal is to develop and propose such markers, developing methods using the new proteomics technology of SELDI and related approaches