Review Article

Surface-Enhanced Laser Desorption/Ionization (SELDI): A New Comer Into Diagnostic Medicine

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Abstract

The completion of the Human Genome Project has marked the official start of the post-genomic era. Due to limitations obstructing DNA and RNA studies, the advent of proteomics, the large-scale analysis of proteins, is considered a crucial consequence and a chief player of post-genomic initiatives. The immediate goal of proteomic studies is understanding proteins including their expression, function, interaction, and structure. The final aim of proteomics is discovering protein biomarkers that can be used in the detection, prognostication, and treatment of diseases. However, the challenge of studying complete sets of proteins in cells, or proteomes, is driving the development of newer technologies. In this review, a discussion of one proteomic biotechnology, Surface-Enhanced Laser Desorption Ionization (SELDI) mass spectrometry, and its clinical applications is offered. This instrument has been successfully utilized in analyzing human samples for the discovery of biomarkers and in disease diagnosis. Recent advancement in proteomics has added, and will continue to add, valuable information to our knowledgebase of the human biological system.

Keywords: Proteomics, 2D-PAGE, Laser Capture Microdissection, Mass Spectrometry, Surface-Enhanced Laser Desorption/Ionization, Protein microarrays.

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Introduction

The completion of the Human Genome Project has generated tremendous excitement in the scientific community with the many opportunities it provides (for more information on the Human Genome Project, please refer to www.genome.gov). Among the primary goals of the Human Genome Project have been to elucidate the human genes, link genes to diseases, and understand genetic variations in humans. However, many deficiencies exist in regard to the basic knowledge of human genes.

Very importantly, the expression pattern of the 20,000-30,000 genes that make up the human genome has yet to be determined in different cells and tissues.

Major modern scientific research efforts are aimed towards "molecular profiling", or global measurements of mRNA and proteins levels in biological systems. [1, 2] This strategy is based on information provided by the Human Genome Project and aided by the development of biotechnologies for molecular analysis of diseases.

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Current biotechnologies used in molecular profiling studies generate enormous amount of data points in a short period of time; accordingly, it is termed "high-throughput." High-throughput studies allow the concurrent investigation of thousands of genes in order to enhance the discovery of novel genes and to elucidate the molecular and cellular interactions. Due to the relative biochemical consistency of RNA molecules and the ease of mRNA manipulation in contrast to proteins, molecular profiling studies and technological developments have mainly been driven towards the determination of mRNA levels.

Although RNA-based studies are promising in identifying gene products linked to diseases, such studies suffer from serious shortcomings. First, it is important to recognize that mRNA molecules are transit products in the process of making proteins, the primary functional player in cells. In addition, RNA transcript levels may not correlate to matched protein levels due to differences in regulation and stability. This is illustrated in previous studies where levels of mRNA molecules and their corresponding proteins poorly correlate.[3, 4] Moreover, proteins may undergo extensive modification such as proteolysis, glycosylation or phosphorylation, generating different isoforms each with distinct function. The same protein may also differ in its localization in physiological and pathological conditions influencing its function. Finally, proteins are the main target of therapeutic agents accounting for more than 98% of drug targets. [5] Due to the biological significance of proteins, the field of "proteomics" has launched.

Many attempts have been made to define the term, "proteomics." A simple definition can be stated as "high-throughput analysis of proteins" involving hundreds to thousands of proteins. Among the many goals of proteomics is understanding all the aspects of proteins including their expression, function, interaction, and structure.

It is hoped with proteomic analyses to discover novel disease biomarkers that can be utilized in the detection, prognostication, and treatment of diseases. The challenge of studying complete sets of proteins in cells, proteomes, has been driving the development of new technologies. Continuous improvement of older instruments has facilitated the execution of prominent proteomic studies. In addition, newer and powerful instruments have also been developed. Among the older proteomic instruments is Mass Spectrometry (MS), which has been tremendously improved and then has become a method of choice in high-throughput proteomic studies. A newer instrument that has diverged out of MS is the Surface-Enhanced Laser Desorption Ionization (SELDI). In this review, the basic concept of both MS and SELDI is clarified. In addition, the significance of SELDI in the clinic will be highlighted.

What Is Mass Spectrometry (MS)?

A mass spectrometer can measure the masses of small molecules by converting them into ions and sorting them, via a stream of electrical fields, according to their mass/charge (*m/z*) ratio. MS instruments are composed of three components: an ionization source, a mass analyzer, and an ion detector.

Prior to protein analysis by mass spectrometry, the protein is specifically digested into smaller peptide fragments by a specific protease. The sample is then introduced into the mass spectrometer for ionization. A common type of ionization method is termed Matrix-Assisted Laser Desorption/Ionization (MALDI), by which the samples are embedded into specific matrix molecules. The matrix absorbs the ionizing laser beam and transfers the energy into the analyzer converting it into ions. These ions travel through a mass analyzer, which focuses the ions to impact the detector as for measuring the mass/charge value. A mass analyzer known as TOF (Time-Of-Flight) is commonly combined with MALDI instruments, hence, known as MALDI-TOF.

In TOF, the ions are accelerated from the ionizing source down into a flight tube until they impact the ion detector at the other end of the tube. Since all ions carry the same amount of energy but have different masses, smaller ions travel faster and reach the detector earlier than larger ones. Based on the time needed for the ion to reach the detector, the masses on ions can be calculated generating a "Peptide Mass Fingerprint" (PMF) of the analyzed protein. Since proteins would differ in their proteolytic digestion generating their unique PMF, the protein identity can be determined. This is done by comparing the mass spectra of the analyzed protein with the theoretical mass spectra of other proteins in a sequence database. Figure 1 illustrates the mechanism of MALDI-TOF MS. For more information on the concept of MS and the different types of MS instruments, refer to Hirsch et al. [6] and Domon and Aebersold. [7]

Surface-Enhanced Laser Desorption Ionization (SELDI)

SELDI is a new technology that is a modification of MALDI-TOF mass spectrometry. [8] A major difference is that a sample is applied on the surface of a chip rather than being mixed with a matrix molecule. The chip surface is made of a defined chemical property (e.g. hydrophobic, cationic and anionic) allowing certain classes of proteins to adsorb. The chip is then placed in a vacuum chamber of the SELDI instrument where proteins and peptides are ionized and travel towards a detector inversely according to their masses, similar to a MALDI-TOF. The Time-Of-Flight of ions, in particular of peptides below 20 kDa, can be viewed as MS spectra, a chromatogram, of gel-like bands (Figure 2).

Due to its speed, high sensitivity, ease of use, and reproducibility, SELDI is a true high throughput proteomic instrument. Two major advantages of SELDI are; the ability to analyze highly complex samples, and the low volume needed for analysis, which can be as low as 0.5 μl. SELDI is also versatile where, instead of a chemical surface, the chip can be coated with antibodies to capture even more specific antigens as has been reported earlier in measuring prostate-specific antigen and prostate-specific membrane antigen. [9-11]

SELDI has also been utilized in search for biomarkers for Alzheimer's disease [12,13] as well as cancers of the prostate, [14, 15] bladder, [16] colon, [17] and breast. [18] Although direct determination of the proteins represented as mass peaks is not possible, different means can be utilized to reveal the identity of specific peaks. In a recent report, SELDI analysis of Cerebro-Spinal Fluid (CSF) samples of patients with multiple sclerosis reveals the presence of a differential peak when compared to subjects with other diseases. [19] A differential peak is observed between control and multiple sclerosis samples. This protein has been identified by further MS analyses as cystatin C, an inhibitor of the lysosoaml cysteine protease cathepsin B. Although burdensome and elaborate, proteins represented by specific SELDI spectra peaks can be identified by a series of liquid chromatography fractionation as has been illustrated by Diamond et al., [20] Sanchez et al. [21] and Yang et al.[22]

In one study, proteins extracted from prostatic normal and tumor cells have been analyzed by SELDI. The mass spectra patterns of the proteins reveal several remarkable alterations as compared to those of matched normal samples. [23] However, due to the dynamic heterogeneity of proteomes even within the same individual, consistent detection of differential peaks is not feasible. This complexity has prompted the group of Petricoin and Liotta to integrate an artificial neural network algorithm to search for "hidden" patterns. In a fascinating study, they have been able to differentiate ovarian cancer patients from normal subjects and patients with other ovarian diseases with unprecedented sensitivity of 100% and specificity of 95%. [24]

These Results are significant accomplishment considering that; first, the 5-year survival rate is 11% for stage IV in ovarian cancer patients, in comparison to up to 93% for early-stage patients, second, there are no superior biomarkers for detecting early-stage ovarian tumors, and, therefore, two-third of ovarian cancers are detected in an advanced stage. [25] Very importantly, the SELDI analysis is performed using a small volume (a few microliters) of unfractionated serum samples. The latter study has initiated similar studies on numerous diseases, ending with promising findings in detecting a variety of disorders. [26-29]

The Future

Despite the many challenges, it is certainly an exciting time through which the scientific research is going. Completion of the Human Genome Project has surely been a major factor in increasing the understanding of many aspects of biological systems. Similarly, knowledge of human proteomes will contribute significantly to elucidating our genetic and proteomic makeup. This knowledge will help us understand physiological and pathological conditions and, therefore, design better and more specific therapeutics. It is hoped that we can achieve the level of personalized medicine, where therapy is tailored according to the genetic and proteomic makeup of individuals. [30, 31] In order to achieve these goals, better interaction and collaboration are needed among clinicians and basic scientists.

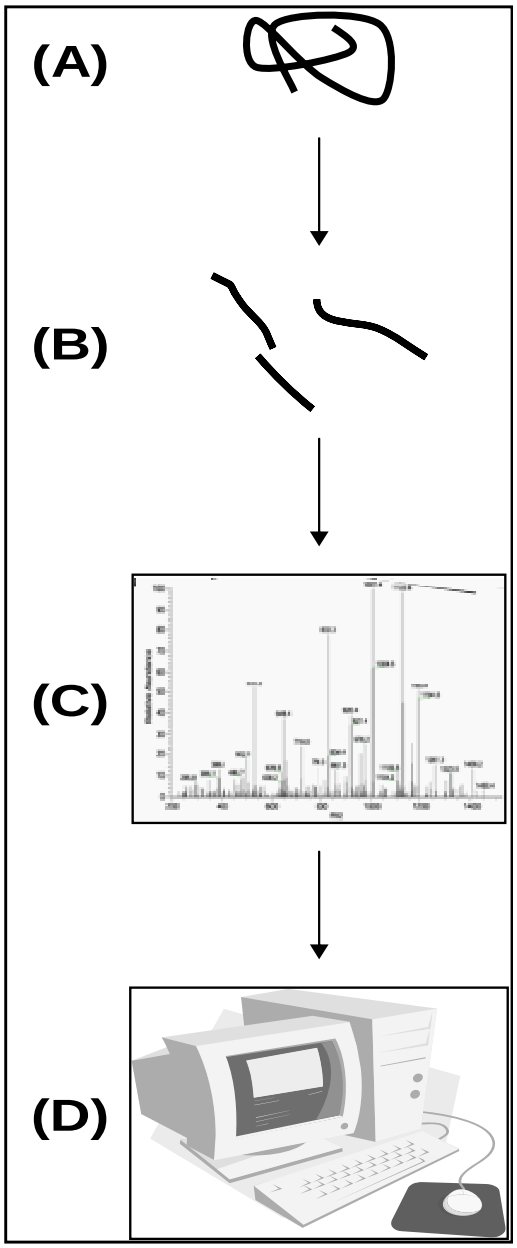

Figure 1: An outline of protein identification by MALDI-TOF MS. A single protein (A) is digested into smaller peptides by a protease at specific sites (B). The sample is injected directly into the mass spectrometer. Peptides are ionized and ions travel through the mass analyzer generating a peptide mass fingerprint (C), which is interpreted by computational methods (D) in order to determine the protein identity.

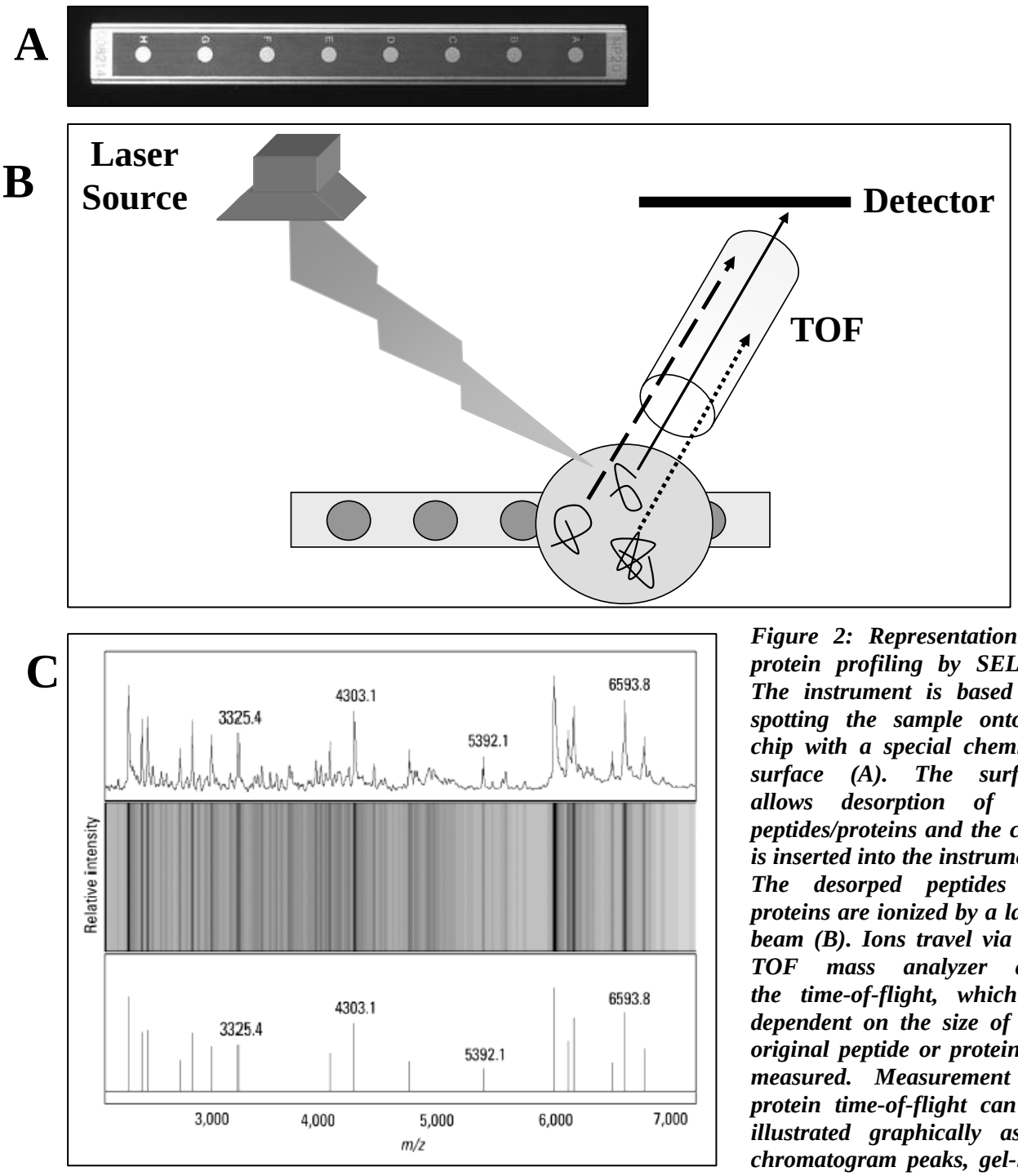

Figure 2: Representation of protein profiling by SELDI. The instrument is based on spotting the sample onto a chip with a special chemical surface (A). The surface allows desorption of the peptides/proteins and the chip is inserted into the instrument. The desorped peptides or proteins are ionized by a laser beam (B). Ions travel via the TOF mass analyzer and the time-of-flight, which is dependent on the size of the original peptide or protein, is measured. Measurement of protein time-of-flight can be illustrated graphically as a chromatogram peaks, gel-like bands or a mass spectra (C) with every peak, band or spectrum correspond to an ion.

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جهاز SELDI وتطبيقاته الواعدة في الطب التشخيصي

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الملخص

كان الانتهاء من خريطة الجينوم البشري بمثابة الإعلان الرسمي لمرحلة ما بعد دراسات الجينوم. وبسبب الحدود التي تتوقف عندها الدراسات المتعلقة بالحمض النووي منقوص الأكسجين والحمض النووي الريبي، فإن ظهور علم دراسات البروتيوم أو بمعنى آخر دراسات البروتينات على نطاق واسع يعد نتيجة مهمة ولاعباً رئيسياً في مبادرات ما بعد دراسات الجينوم. إن الهدف المباشر لدراسات البروتيوم هو فهم البروتينات، بما في ذلك إنتاجها ووظائفها وارتباطاتها الجزيئية وبنيتها. والهدف النهائي لدراسات البروتيوم هو اكتشاف وصمات بروتينية يمكن استخدامها في اكتشاف وتشخيص وعلاج الأمراض. ولكن التحدي في دراسة المجموع الكلي للبروتينات داخل الخلايا، أو ما يعرف بالبروتيوم، يحفز على تطوير تقنيات جديدة. من خلال هذه المراجعة، سيتم عرض إحدى هذه التقنيات وهي مقياس الكتلة الطيفي ذو التحفيز السطحي للتأين بالليزر وتطبيقاتها السريرية. تم استخدام هذه التقنية بنجاح في تحليل عينات إنسانية لاكتشاف وصمات بروتينية. التطورات الحديثة في دراسات علم البروتيوم أضافت وستستمر في إضافة معلومات قيمة إلى قاعدة معلومات الجهاز الحيوي الإنساني.

الكلمات الدالة: علم دراسات البروتيوم، مقياس الكتلة الطيفي، مقياس الكتلة الطيفي ذو التحفيز السطحي للتأين بالليزر.