Available online: April 21, 2019

Commun.Fac.Sci.Univ.Ank.Series C
Volume 28, Number 1, Pages 1-9 (2019)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/tr/pub/communc/issue/45050/562240

GENERAL OVERVIEW OF THE STUDIES WITH MALDI-TOF

EVRIM KOCAMAN, E. SUMER ARAS

ABSTRACT. In the 1980s, the discovery of a variety of soft ionization techniques provided the technical basis for the first time mass spectrometric detection and analysis of free, chemically polar, variable biopolymers and their degradation products. In recent years, Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has become a well-liked and multifaceted method to perform analyses of macromolecules which is the biological origin. This review provides a general overview of the areas of use of MALDI-TOF MS for biotechnological researches. MALDI-TOF MS is a way that supplies well-directed, affordable, and speedy identification of fungi, viruses, bacteria in clinical microbiological laboratories. Newly, this method has been successfully applied to DNA sequencing (genotyping) as well as mutation screening. Also, this way has the potential to become a conventional method for high throughput genotyping of single nucleotide polymorphisms (SNPs), both for biotechnology laboratories and for clinical applications. When it is compared with conventional and molecular identification methods, it makes a major contribution to minimizing the mortality rate and hospitalization period of the patients. It is more effective on per sample costs and elapsed time on working.

1. INTRODUCTION

MALDI-TOF-MS is a considerable method that is used for the characterization of biomolecules and macromolecules from biological origin. This system allows analysis of DNA (Deoxyribonucleic Acid) and RNA (Ribonucleic Acid) fragments at high speed, accuracy and can be used for clinical diagnosis by the reason of its sensitivity and measurement characteristics. One of the most important advantages is that it enables multiple sample analysis simultaneously. In addition to this feature that has great importance for genotyping and haplotyping analyzes, it is also possible to study allele-specific gene expression and epigenetic modifications. A further advantage is that it allows for the reproduction of a large number of DNA

Received by the editors: February 23, 2019; Accepted: March 30, 2019.
Keywords and phrases: MALDI-TOF, protein, microbiology, genome screening, haplotype, SNP, disease-specific.

/ gene regions in a single reaction with a very small sample volume and as little as 15 ml sample is sufficient for this system. These advantages reduce the cost of the research and save time [1].

Koichi Tanaka and John B. Fenn were considered worthy the Nobel Prize for developing techniques using mass spectrometry to determine the molecular mass of proteins and peptides [2].

2. What Can Be Done By Using Maldi-Tof?

Genotyping Single Nucleotide Polymorphisms

The single nucleotide polymorphisms (SNPs) on the deoxyribonucleic acid molecule are our genetic properties, which are the basis of our sensitivity to environmental conditions, diseases, medications and other environmental factors, as well as many physical differences between individuals. For this reason, SNP-genotyping is important for personalized medical [3].

The SNPs analyzes with MALDI-TOF-MS is on the different basis of in mass formed by a single base change. The most important advantage is the simultaneous analysis of hundreds of SNPs. The DNA region selected for this purpose is amplified by PCR (Polymerase Chain Reaction). Following the PCR, the reaction products are cleaned and subjected to an extension reaction (homogeneous mass extend-hME) using a primer designed specifically for the SNP region to be analyzed. The extension is terminated by the addition of the complementary base to the primer in accordance with the SNP in that region. The differences of the base added to the primer due to the allele difference in individuals result in a mass difference, allowing SNP identification [1].

SNP-Mutation Analysis

DNA sequence analysis is an indispensable method to illuminate the genetic basis of hereditary diseases. However, the sequence analysis of large and numerous genes is an expensive and time-consuming approach. For this reason, many pre-screening techniques are used to reduce the cost of the research and save time.
The MALDI-TOF-MS system makes it possible to determine the variations in single nucleotide changes that may occur in the DNA sequence, such as insertion

and deletion type differences, in a short time. Following the multiplication of the gene of interest, PCR products are *in vitro* transcribed with primers containing T7-promoter tags. The single chain RNAs generated are subjected to the base-specific fraction. The resulting fragments by MALDI-TOF-MS are analyzed in parallel of the reference sequence. According to the reference sequence, different signals indicate the difference in the region [4].

Epigenetic Modifications Analysis

Epigenetic modifications are effective on gene regulation (gene activation and silence), gene expression and genome stability. DNA methylation can be effective in many hereditary diseases as well as can be an important factor in the sensitivity of the individual to complex diseases. The systems with high speed and accuracy are required to investigate the epigenetic factors and the effects of these factors on the etiology of the disease. MALDI-TOF-MS is a method that is used out of this purpose and allows the simultaneous detection of methylation levels in different regions with a single reaction. The operability basis in this system is relying on the sodium bisulfite-treated DNA and the formation of fragments with the base-specific fraction. The alterations in the methylation profile of the site cause the formation of various fragments and these fragments are analyzed by MALDI-TOF-MS [5].

SNPs Allele Frequencies Analyzes

Increased number of samples in allele frequency studies increases the accuracy of the results. Therefore, it is important for population studies that the number of samples is high, as well as the analysis, can be done in a short time and with high accuracy. MALDI-TOF-MS is particularly advantageous in terms of reducing the time and cost in population-based studies [6].

To determine the allele frequency, primarily a DNA pool is created, and the desired DNA region propagated by PCR. Following the PCR, the reaction products are cleaned and subjected to an extension reaction (hME) using a primer designed specifically for the SNP region to be analyzed. The extension is terminated by the addition of the complementary base to the primer in accordance with the SNP in that region. After a final cleaning phase, the samples are analyzed by MALDI-TOF-MS system and the frequency of alleles are determined with specially developed software [7].

Quantitative Analysis of Gene Expression

Today, quantitative studies of DNA and RNAs are particularly important in investigating the effects of genetic changes on gene products. The knowledge gained from such studies is important in clarifying the basic mechanisms of hereditary diseases. MALDI-TOF-MS enables the allele-specific quantitative analysis of gene expression at high speed and accuracy. The basic operating principle of the system is based on the co-duplication (PCR) of the synthesized cDNA with the synthetic DNA molecule, which is different from the single nucleotide itself. The single nucleotide difference mimics two different alleles in the reaction system. Following the PCR, the reaction products are cleaned and subjected to an extension reaction (hME) using a primer designed specifically for the SNP region to be analyzed. The extension is terminated by the addition of the complementary base to the primer in accordance with the SNP in that region. Quantitative analysis of the obtained peak areas is done with the help of special software [8].

Haplotype Analysis

Haplotype analysis is another area of use for MALDI-TOF-MS. The effect of a particular SNP profile (haplotype) on the gene or protein is more than the effect of each SNP creates separately. Such studies are particularly important in disease susceptibility, epidemiology, and pharmacogenetic studies. The basic operating principle of the system is based on the analysis of allele-specific PCR-generated fragments. For this purpose, DNA regions of a certain size are amplified (4kb) and the products are processed according to GOOD assay protocol and analyzed in MALDI-TOF-MS [9-1].

Disease-Specific Genome Screening

A considerable statistical difference between the patient and control groups necessitates pretty much sample analysis and SNPs, also this situation brings about to rise of time and cost. Therefore, the MALDI-TOF-MS system became a common alternatively way which is used, instead of the routine biomolecular identification methods in parallel of this. This way also has been developed for use of in clinical as an essential examination tool to characterize many diseases and allergy protein markers of disease or susceptibility to disease. In recently practices in oncological methods and especially colorectal cancer have conducted in show

off clinical availability of MALDI-TOF-MS. Another reason for the availability and preference of MALDI-TOF-MS is that it allows for the analysis of multiple samples with high precision and accuracy [10].

Protein Identification

Quantitative determination of proteins and identification of disease-causing protein markers is another area of use of MALDI-TOF-MS and this identification process is based on the principle of forming peptide mass maps and peptide mass fingerprints**.** For this purpose protein-specific amino acid sequences are generated by proteolytic treatment. MALDI-TOF-MS allows the analysis of these fragments due to the mass difference. The peptide criteria that appear in this system are special to the relevant protein and are unique. Therefore, protein is known as fingerprints. Screening of the database according to the obtained mass data makes it possible to identify much protein [11].

Another approach developed in this context is to identify protein markers that play a key role in disease. This system enables the identification of protein markers in tissue or serum samples (by comparing patient and control) in cancer research, the synovial fluid characterization in serum. The sensitivity of the system makes it possible to detect markers in very few samples. With MALDI-TOF-MS it is possible to visualize chemically modified proteins from a very low volume body fluid, such as CSF (Cerebro Spinal Fluid), quickly and with high performance. Another important advantage of MALDI-TOF-MS is that it can determine macromolecules/biomolecules in a triturate with buffer and salt. This situation eliminates the purification steps, which lead to sample loss in each step when analyzing biological samples [11].

Mini-DNA Sequence Analysis

In MALDI-TOF MS, high accuracy short (35-100b) sequence analysis can be performed without the need for gel electrophoresis. The use of SPC-ddNTP (solid phase capturable dideoxynucleotides) is one of the most effective approaches. In this system, the sequence reaction is performed using biotin added ddNTP. The reaction products are passed through a streptavidin-coated solid surface and the biotinylated ddNTP-terminated fragments are removed from the reaction residues while retaining the surface. These fragments are then separated from the solid phase surface using ammonium hydroxide and formamide. The resulting DNA sequence fragments are analyzed in MALD1-TOF MS. The most important

advantage of MALD1-TOF MS in DNA sequence analysis is that it allows the characterization of frame-shift mutations even in heterozygous individuals [12-13].

MassARRAY

MassARRAY (Sequenom) platform is used in high-throughput genetic analysis applications. The platform, which is specifically designed for SNP and nucleotide analysis, works with the MALDI-TOF principle. MALDI-TOF mass spectrometry technology was developed for the protein analysis in beginning. In the MassARRAY system, an innovative step is added to this technology and this approach is used in the nucleotide analysis. DNA and RNA analysis can be performed on a MassARRAY device, that can be considered as an important R & D product in the field of biotechnology [12].

3. Discussion

In recent years, many studies have been performed to indicate reliable and rapid results in the diagnosis of bacterial and fungal infections with MALDI-TOF [14-15-16].

In a study published in 2014 by Kim et al., 26 *Candida spp.* isolates identified as *C. famata* with VITEK-2 were studied with the MALDI-TOF-MS system, and then a validation study performed with gene sequence analysis for compared obtained results. The strains identified as *C. famata* by VITEK-2 demonstrated 100% homogeneity with *C. guilliermondii* in the gene sequence analysis. Although MALDI-TOF MS correctly identified 21 of the strains, 4 of the isolates could not be identified at acceptable levels [15].

MALDI-TOF MS not only performs better than conventional methods in terms of sensitivity in organism identification but also in terms of speed, performs better than those methods. Therefore, another advantage associated with MALDI-TOF MS would be to decrease the duration of the results reporting to researchers and physicians [17].

Cherkaoui et al. reported that MALDI-TOF-MS was able to detect 10 batched isolates in less than 15 minutes. Furthermore, the identification period determined by the MALDI-TOF-MS in the forward-looking analysis of 952 microbial isolates

yielded a definitive identification of an average of 1.45 days earlier compared to standard methods [17-18]. This development in the identification period is even greater in the case of difficult or slow-growing organisms. Thus, it has been suggested that physicians may target antimicrobial treatment in advance and that patient care may be affected positively for patients who continue their clinical course [19].

At the same time, some MALDI-TOF-MS approaches can provide the characterization of biomarkers in untreated biofluids. Although these approaches allow for high throughput analysis, and the biomolecules, which are quite abundant for targeted biomarkers, constitute a boundary [20].

Although the results obtained with this technique are not better than the conventional methods, they are preferred when compared with the results obtained with other time consuming and expensive microbial identification methods. MALDI-TOF MS is a considerable, rapid, affordable, and reliable technique for the identification of microorganisms. The insufficient and limited size of database and the inadequacy of the existing software used to differentiate between the related types are some of the current challenges of this technology [21].

4. Conclusion

MALDI-TOF-MS is a system used in the biomolecule characterization, which enables the analysis of DNA and RNA fragments at high speed and accuracy, which can be used for clinical diagnosis because of its sensitivity and measurement properties, and one of its most important advantages is the simultaneous analysis of multiple samples. These advantages ensure save time and reduce costs in researches.

References

[1] M.W. Duncan, Applications of MALDI Mass Spectrometry in Clinical Chemistry, Volume 62, Issue 1, (2016) 134–143.
[2] https://sandwalk.blogspot.com/2009/02/nobel-laureates-john-b-fenn-and-koichi.html, (2009), Erişim Tarihi: 29.01.2019.
[3] E.W. Ng, Advances in MALDI Mass Spectrometry in Clinical Diagnostic Applications, Chemical Diagnostics, Volume 336, (2013) 139–175.
[4] K. Matsuda, PCR-Based Detection Methods for Single-Nucleotide Polymorphism or Mutation: Real-Time PCR and Its Substantial Contribution

Toward Technological Refinement, Advances in Clinical Chemistry, Volume 80, (2017) 45–72.
[5] K. Jitendra, Epigenetic Activation of Antibacterial Property of an Endophytic *Streptomyces coelicolor* Strain AZRA 37 and Identification of the Induced Protein Using MALDI TOF MS/MS, PLoS ONE 11(2), (2016) 1–12.
[6] M. Tanaka, Genotyping of the ABCG2 gene using Matrix-Associated Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. Transfusion Medicine, 28(3), (2017) 255–260.
[7] K. Meyer, MALDI-TOF MS Genotyping of Polymorphisms Related to 1-Carbon Metabolism Using Common and Mass-Modified Terminators, Clinical Chemistry 55:1, (2009) 139–149.
[8] S. Paracchini, An Allele-specific Gene Expression Assay to Test the Functional Basis of Genetic Associations, Journal of Visualized Experiments, Issue 45, (2010) 1–5.
[9] S. Sauer, Full flexibility genotyping of single nucleotide polymorphisms by the GOOD assay, Nucleic Acids Research, Volume 28(23), (2000) 1–6.
[10] L. Marvin, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in clinical chemistry, Clinica Chimica Acta, Volume 337, Issues 1–2, (2003) 11–21.
[11] E.D. Zanders, Chemical Genomics and Proteomics Reviews and Protocols, Humana Press Publications, Royston, United Kingdom, (2012) 227–239.
[12] Y. Zhao, Yeast identification by sequencing, biochemical kits, MALDI–TOF MS and rep-PCR DNA fingerprinting, Medical Mycology, Volume 56, Issue 7 (2017) 816–827.
[13] J. Mengel-Jørgensen, MALDI-TOF mass spectrometric detection of multiplex single base extended primers. A study of 17 y-chromosome single-nucleotide polymorphisms, Analytical Chemistry 76 (20), (2004) 6039–6045.
[14] G. Pulcrano, Rapid and reliable MALDI-TOF mass spectrometry identification of Candida non-albicans isolates from bloodstream infections. Journal of Microbiological Methods, Volume 94, Issue 3, (2013) 262–266.
[15] S.H. Kim, Misidentification of Kandida guilliermondii as C. famata among Strains Isolated from Blood Cultures by the VITEK 2 System, BioMed Research International, Volume 2014, (2014) 1–6.
[16] C. Lacroix, Evaluation of two matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) systems for the identification of Candida species, Clinical Microbiology, and Infection, Volume 20, Issue 2, (2014) 153–158.

[17] T.C. Dingle, Mass Spectrometry for Microorganism Identification. Clinics in Laboratory Medicine, Volume 33, Issue 3, (2013) 589–609
[18] E. Bille MALDI-TOF MS Andromas strategy for the routine identification of bacteria, mycobacteria, yeasts, *Aspergillus spp*. and positive blood cultures, Clinical Microbiology and Infection, Volume 18, Issue 11, (2012) 1117–1125.
[19] K.E. Tan, Prospective evaluation of a matrix-assisted laser desorption ionization-time of flight mass spectrometry system in a hospital clinical microbiology laboratory for identification of bacteria and yeasts: a bench-by-bench study for assessing the impact on time to identification and cost-effectiveness. Journal of Clinical Microbiology, Volume 50, (2012) 3301–3308.
[20] Y.T. Cho, Biomarker Characterization by MALDI–TOF/MS, Advances in Clinical Chemistry, Volume 69, (2015) 209–254.
[21] P. Rahi, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass-Spectrometry (MALDI-TOF MS) Based Microbial Identifications: Challenges and Scopes for Microbial Ecologists, Frontiers in Microbiology, Volume 7, Article 1359, (2016) 1–12.

Current Address: EVRIM KOCAMAN: Ankara University, Faculty of Science, Department of Biology, Besevler, Ankara, Turkey.
E-mail : evrimkocaman@hotmail.com
ORCID: https://orcid.org/0000- 0002- 8498- 3395

Current Address: SUMER ARAS: Ankara University, Faculty of Science, Department of Biology, Besevler, Ankara, Turkey
E-mail : sumer.arasnz@gmail.com
ORCID: https://orcid.org/0000-0003-3474-9493