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Application News

MALDI-TOF Mass Spectrometer MALDI-8020

Intact-Cell Mass Spectrometry for Monitoring of Stem Cells Cultures

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User Benefits

- Uncover hidden unfavorable changes within the cell ahead of microscope.
- Unbiased, feasible and time-efficient approach for monitoring cell cultures.
- Quick method using bench-top MALDI-TOF MS and statistical software

■ Introduction

Matrix-Assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry (MALDI-TOF MS) is a simple and quick analytical method to obtain qualitative and quantitative information from diverse types of samples. Intact cell MALDI-TOF MS provides spectral information generated from eukaryotic cells spotted directly on the target plate without preceding lysis, fractionation or separation of particular molecular species or biomolecules. Here, we demonstrate a novel application of MALDI-TOF MS in quality control and monitoring of routine stem cell cultures.

Human embryonic stem cells (hESCs) are promising tools for cell therapy, bio-industry or drug development. However, long-term cultured hESCs inevitably develop unwanted and potentially hazardous changes in phenotype, that may prevent safe application.

These changes remain unnoticed if cell morphology is not altered, or in case of genetically or karyotypically silent changes that appear over time in long-term cell cultures.

hESCs differentiation to clinically relevant cell types is a gradual process, from immature stages to the final phenotype. However, the substantial heterogeneity in the complex differentiation process produces cells with unwanted properties, such as lack of functional phenotype, or propensity to cancer growth.

Intact cell MALDI-TOF MS allows feasible and unbiased discrimination between normal and aberrant hESCs.

■ Sample preparation

Cultured hESCs were harvested (Fig. 1a), washed with isotonic MS-compatible buffers, mixed with sinapic acid matrix solution acidified by trifluoroacetic acid and spotted on the target plate. Scanning electron microphotograph (SEM) of a target spot documents that cells keep their integrity after spotting (Fig. 1b).

MS analysis was then performed using MALDI-8020 instrumentation (Fig. 1c) followed by a statistical analysis with eMSTAT Solution™ software (Fig. 1d).

■ Statistical analysis of mass spectra reveals alterations in morphologically identical hESCs

While the morphology, genotype or stemness-associated phenotype do not change in prolonged cultures (Fig. 2), the cells may acquire hazardous properties such as chromosomal aberrations, or resistance to apoptosis. In addition, standard molecular or microscopic methods may often fail and undesired hESCs clones may be selected over time.

Interestingly, mass spectra are informative enough to reveal alterations in the molecular profiles of short- and long-term cultured hESCs. Such differences between the early and late passage number can be visualized by cluster or classification analysis embedded in software like eMSTAT Solution (Fig 1d). Spectral fingerprints can thus provide as unbiased tool for identification of stem cells with potentially hazardous phenotype.

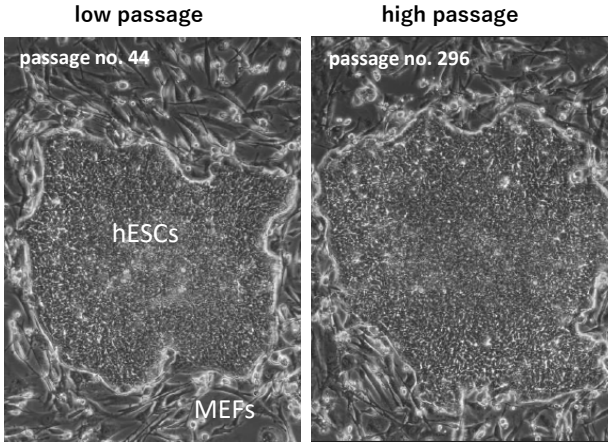

Fig. 2 Morphology of hESCs cultured on layer of mouse embryonic fibroblasts (MEFs) for short or long time, indicated by passage number.

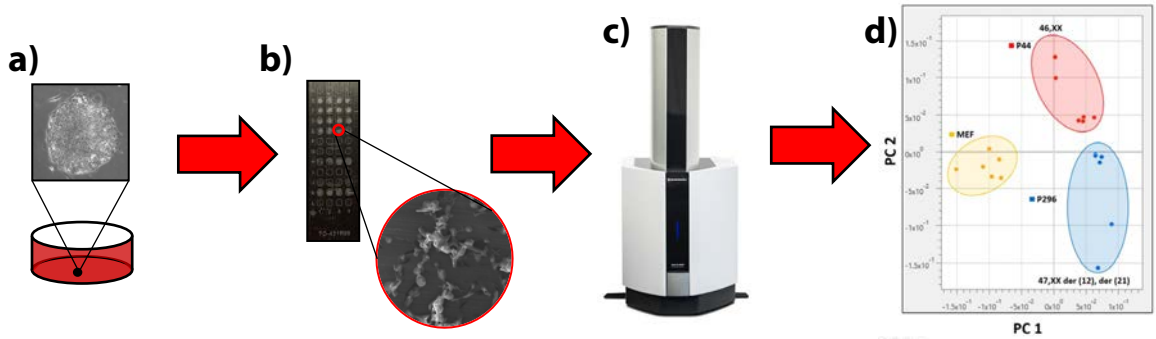

Fig. 1 Workflow: a) Harvest cultured hESCs → b) Wash, mix with matrix and spot on target → c) Analyze in MALDI mass spectrometer → d) Statistical analysis and interpretation

■ Differentiation towards early lung progenitors

Principally the same approach was used to monitor the differentiation of hESCs towards early lung progenitors (ELEPs). It enables to confirm the proper differentiation stage and cells with immature or cancer phenotype can be excluded. Spectral fingerprints from differentiating hESCs were recorded in the mass range between 2 and 10 kDa, representing mostly the peptidome or small proteome (Fig. 3).

The comparative analysis of the mass spectra highlighted changes. Statistical analysis using eMSTAT Solution software revealed clusters corresponding to differentiation stages from the parental hESC population, through stages D1-D10 corresponding to days of differentiation, to ELEPs (Fig. 4). The ELEPs represent a final functional cell entity in the differentiation route. The A549 lung cancer cell line was used for control purposes as a phenotypically distant, but still lung-associated cell type.

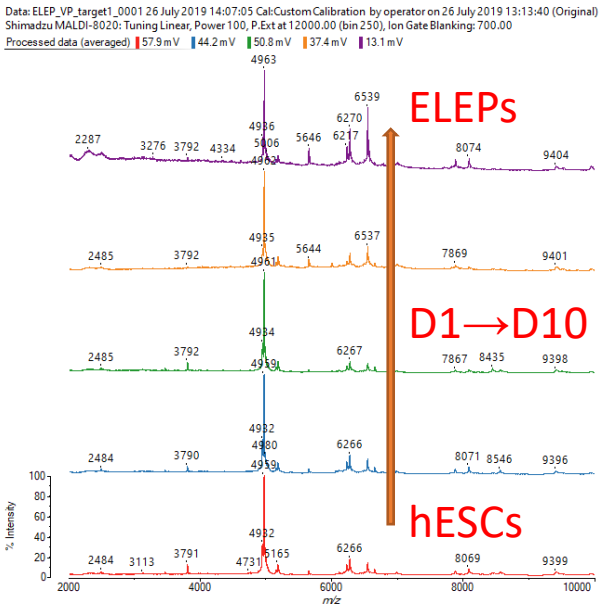

Fig. 3 Mass spectra documenting patterns of various low-mass molecular entities. hESCs were stimulated for differentiation towards ELEPs, harvested at indicated time intervals and processed for intact-cell MALDI TOF MS

■ Summary

This novel application shows the potential of the bench-top MALDI-8020 in combination with statistical analysis software eMSTAT Solution for intact cell mass spectrometry and quality control in clinical grade or bioindustrial stem cell cultures.

This method provides an unbiased, feasible and time-efficient approach for monitoring of hidden changes in long term cultures as well as in quality control of differentiation protocols, either in clinical or research-oriented scenarios.

The coupling with built-in statistical analyses helps to visualize the gradual changes in the molecular profile before these alterations becomes pronounced in the cell morphology or function.

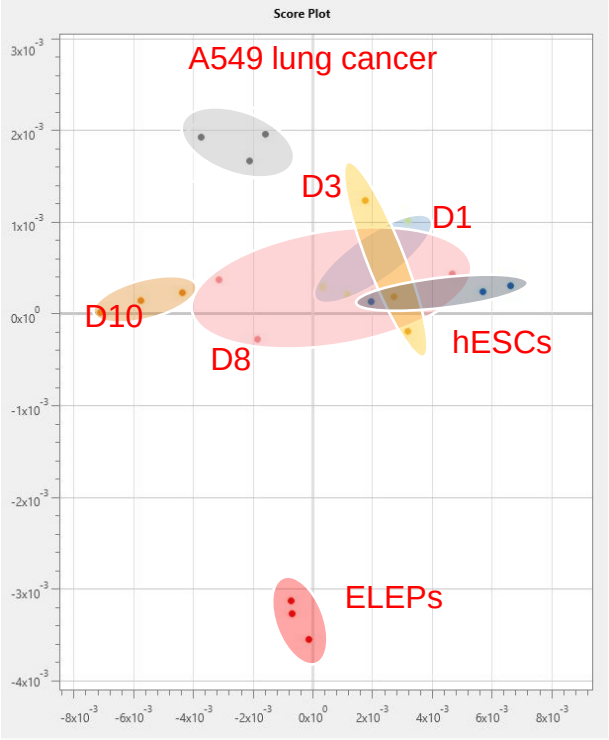

Fig. 4 Cluster analysis of hESCs differentiation to ELEPs as visualized by eMSTAT Solution. Each spot represents the spectral average of an individual biological sample measured in technical pentaplicate. Error ellipses indicate 95% confidence.

■ References

(1) Vaňhara et al., Stem Cells Transl. Med, 2018, 7 (1): 109-114.

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