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Application News

No. B108

Probe Electro Spray Ionization

Evaluation of Differentiated/Undifferentiated State of iPS Cells Using DPiMS™-2020 and eMSTAT Solution™

The differentiated/undifferentiated state of pluripotent stem cells is a critical evaluation item in research and development and quality control in the field of regenerative medicine. Because the conventional method for evaluation of the differentiated/undifferentiated state required fractionation and pretreatment of some cultured cells, quick analysis was difficult, and it was necessary to use part of the valuable cultured cells. If data on the differentiated/undifferentiated state could be obtained from a small sample by simple pretreatment, quick evaluation with minimal loss of cells would be possible.

This article introduces an example of quick and simple measurement of the components contained in iPS cell culture medium with a DPiMS-2020 mass spectrometer (Fig. 1) using the probe electro spray ionization method, which is a new ionization method. This article also introduces an example in which grouping of the culture media of differentiated/undifferentiated cells was possible by using eMSTAT Solution statistical analysis software.

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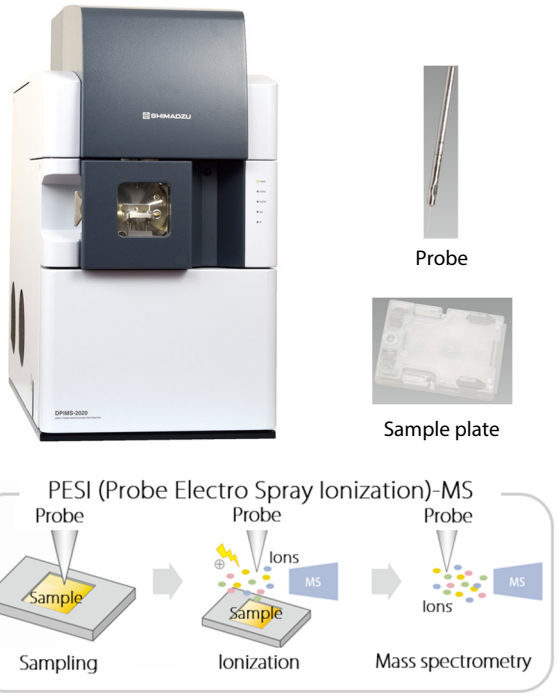

Fig. 1 DPiMS™-2020 and Principle of Probe Electro Spray Ionization
The probe is thrust into the sample on the sample plate, and the molecules of the sample are ionized by applying a voltage to the sample adhering to the probe surface.

Preparation and Analysis of Analysis Samples

The iPS cells used in the analysis were cultured under the conditions shown in Table 1. The relative expression level of the differentiated/undifferentiated marker was compared at the time intervals (days) of culture shown in Table 1 by using qPCR on each sample, and progression of cell differentiation in the order of undifferentiated spheroid, differentiated spheroid, and adherent differentiated cell was confirmed.

Samples were prepared by mixing the supernatant of each culture medium sample and ethanol at a ratio of 1 : 1. 10 µL of each sample was then dripped on a dedicated sample plate of the DPiMS-2020 for liquid samples, and a measurement was conducted in the scan mode. Fig. 2 shows an example of a mass spectrum.

Table 1 List of Culture Medium Samples

Culture medium sample	Culture medium	Cell culture time (days)	Sample name
Undifferentiated spheroid culture medium	Essential8™	2	Undifferentiated cell culture medium (D2)
		7	Undifferentiated cell culture medium (D7)
Differentiated spheroid culture medium	Essential8™ + FBS	2	Differentiated cell culture medium (D2)
		7	Differentiated cell culture medium (D7)
Adherent differentiated cell culture medium	Essential8™ + FBS + Gelatin coat	2	Adherent differentiated cell culture medium (D2)
		7	Adherent differentiated cell culture medium (D7)

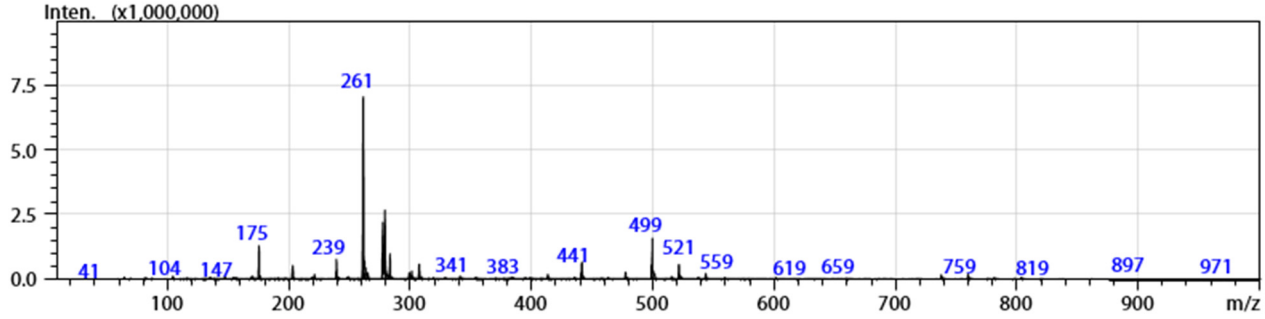

Fig. 2 Example of Mass Spectrum of iPS Cell Culture Medium Measured by DPiMS-2020

Grouping by eMSTAT Solution

Fig. 3 shows the result of a multivariate analysis by eMSTAT Solution of the mass spectrum data obtained by the analysis described above. As shown in Fig. 3a, grouping is possible by dividing the differentiated cell culture media and undifferentiated cell culture media based on the difference of the components contained in the culture media.

A multivariate analysis was done using only the data for the differentiated cell culture media. The results are shown in Fig. 3b. Because D7 for both the adherent differentiated cell culture media and the differentiated cell culture media were plotted further to the left side than D2, indicating that the plot positions are correlated with the degree of progress of differentiation. Moreover, the fact that the groups of D2 for the adherent differentiated cell culture media and D7 for the differentiated cell culture media largely overlap suggested that the degree of progress of cell differentiation of the two groups is similar.

Conclusion

By using the DPiMS-2020, it was possible to obtain the data necessary for a multivariate analysis in a time of approximately 3 minutes/sample, including pretreatment. Use of eMSTAT Solution enabled grouping not only by the differentiated or undifferentiated state of the cells, but even by the degree of progress of cell differentiation.

These results suggested that the differentiated/undifferentiated state of cells and the degree of progress of cell differentiation can be evaluated quickly and simply by combining DPiMS-2020 and eMSTAT Solution. Therefore, this is expected to become a useful analytical method in cell quality control and research in the field of regenerative medicine.

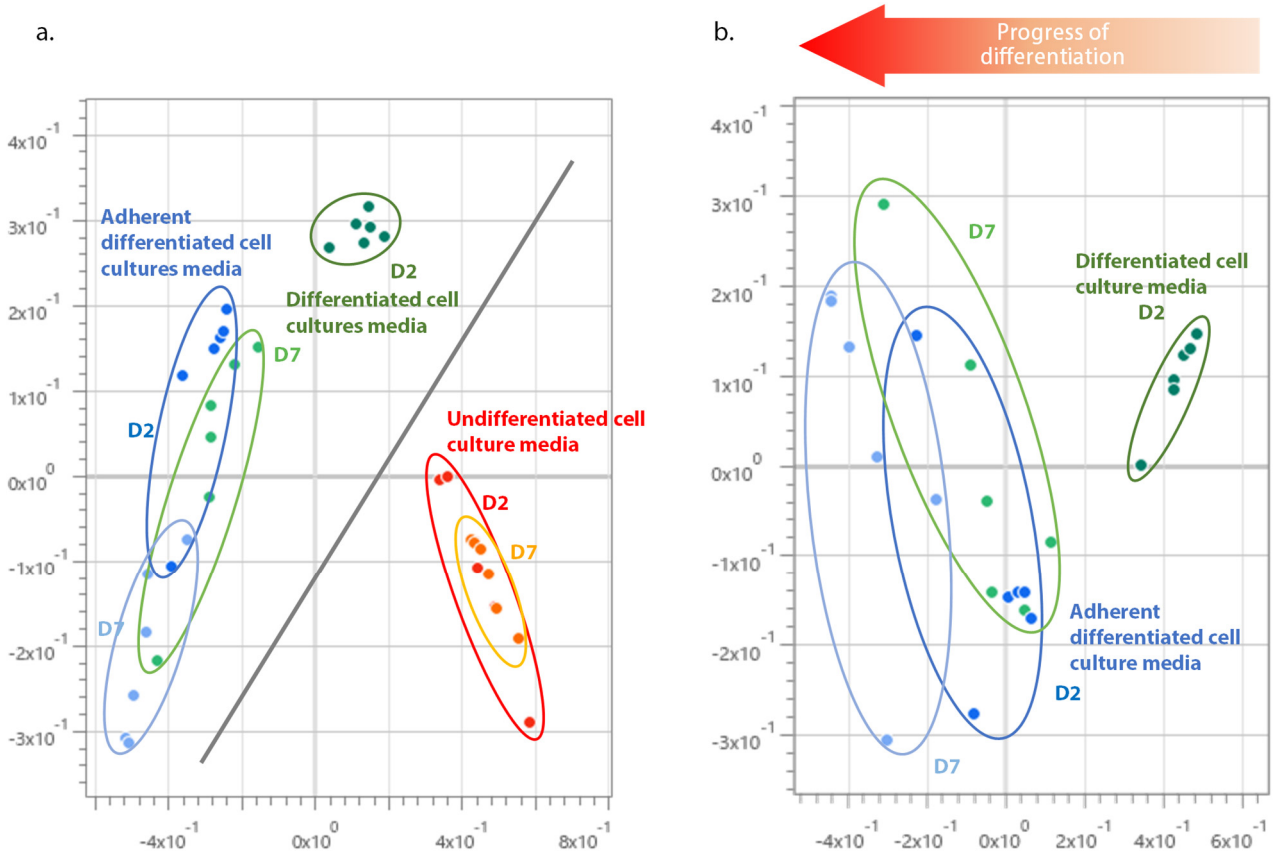

Fig. 3 Multivariate Analysis Results of iPS Cell Culture Media (Score Plots)
a. Grouping of Differentiated Cell Culture Media and Undifferentiated Cell Culture Media
b. Grouping of Adherent Differentiated Cell Culture Media and Differentiated Cell Culture Media

The products described in this document are not devices that have been approved and certified based on Japan's Pharmaceuticals and Medical Devices Act. The analysis technique described herein may not be used for the purpose of medical diagnosis.

First Edition: Feb. 2020

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