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Piezoelectric inkjet assisted rapid electrospray ionization mass spectrometric analysis of metabolites in plant single cells via a direct sampling probe

Zhan Yu,*[a] Lee Chuin Chen,[b] Satoshi Ninomiya,[b] Mridul Kanti Mandal,[c] Kenzo Hiraoka *[c] and Hiroshi Nonami[d]

Direct sampling probe mass spectrometry (DSP-MS) enables fast and direct profiling of metabolites in biological samples. However, because the solvent amount for the online dissolution of acquired analytes is difficult to control, the detection sensitivity is not satisfactory. In this study, we present a modified version of DSP-MS system for direct mass spectrometric profiling of metabolites in plant single cells. Two major improvements are introduced in this work, including a pointed-tip probe with high surface wetting property, which is ten times finer than the previous version, and a piezoelectric inkjet system working as the auxiliary solvent delivery means. The probe tip can be controlled to insert into a cell through the cell wall. Metabolites loaded on the tip surface can be extracted by the auxiliary solvent and electrosprayed after applying a high direct current voltage. The unique features such as low cost, disposability and versatility make this technique a competitive tool for single cell analysis.

1. Introduction

Single cells are the minimal functional units of living organisms. Most of cellular chemical/biological researches are performed on cell population, which contains a number of cells due to the assumption that cells from the same type are identical in both chemical components and biological behaviors[1]. However, more and more experimental evidence has shown the cell-to-cell variability within the same cell population[2]. Conventional cell research methods using population-level cells for analysis can obscure the cell-to-cell heterogeneity and cause misunderstanding of functions of the cell population. Thus, it is of great importance to develop methods for single cell analysis to disclose the cell-to-cell difference, which will be helpful for better understanding of the physiological state of cells subjected to environmental stresses. Techniques for individual cell analysis contribute greatly to the detection and identification of cell-based diseases in clinical diagnostics either[3].

It is a great challenge to analyze substances from a single cell due to its small volume and the absolutely low number of target molecules inside[4]. Owing to improvements of scientific instrumentation and methods, many techniques find a role in this field. Recent reviews[1, 3, 5, 6] have highlighted achievements in analysis of single cells by using modern instrumental methods. However, many analytical methods either require a specifically labeled compound (e.g., radioactive tracing and fluorescence) or cannot provide enough information on molecular specificity (e.g., Raman spectroscopy and electrochemistry). Considering the ability of detection and simultaneous identification of a broad range of individual cellular compounds with excellent sensitivity and specificity, no matter they are known or heretofore unknown, mass spectrometry (MS) is a well-established alternative method for analysis at the single-cell level[7]. Although imaging of subcellular substances (e.g., lipids) in a single cell has been well demonstrated by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) and secondary ion mass spectrometry (SIMS) [3, 8], profiling of metabolites in a single cell under its native conditions is still increasingly favorable because the physical and chemical characteristics inherent can be revealed[9]. Applying MS techniques to chemical profiling of single cells often requires elaborate and rapid sampling methods, including *in situ* capillaries[10, 11] and laser ablation[12-15].

In 2007, Hiraoka and coworkers introduced probe electrospray ionization (PESI) [16], where a sharp solid probe working for both sampling and electrospraying was utilized to replace the capillary in the conventional version of electrospray ionization (ESI). It has been demonstrated that solid ESI emitter can convey some unique features, such as no sample preparation

requirements, low sample consumption and high tolerance to salts[17, 18]. By following this philosophy of design, we proposed a simplified version of PESI, direct sampling probe (DSP)[19], consisting of a disposable solid probe with hydrophilic surface modification and an auxiliary vapor generator. After direct sampling from a variety of real-world samples, analytes on the DSP tip can be extracted by the condensed solvent vapor from an auxiliary vapor generator and then electrosprayed. Because the solvent was pumped at a flow rate of several microliters per minute, sensitivity of DSP-MS is unsatisfactory[19].

As presented previously at a scientific conference[20], we found the sensitivity of DSP-MS can be enhanced by decreasing the solvent volume on the probe tip, which indicates the applicability of this method to single cell analysis. Recently, Gong et al[21] reported an interesting approach to direct analysis of onion single cells by applying a tungsten probe and a solvent spray. Possibly due to the smooth surface, their sampling probe has to be kept inside the cell for 30 s for the enrichment of analytes. As to increase the sensitivity and rapidity of analysis by DSP-MS, we herein present an improved version consisting of a sampling probe with enough sharpness and surface hydrophilicity and a piezoelectric inkjet system as the solvent provider. We demonstrate that metabolites, including carbohydrates, flavonoids, amino acids, etc., contained in less than 1 pL biofluid from a single cell can be analyzed rapidly by this method. We select a variety of plant single cell samples, including rhododendron petals, *geranium carolinianum* leaves and soybean sprouts, to show the feasibility of this method.

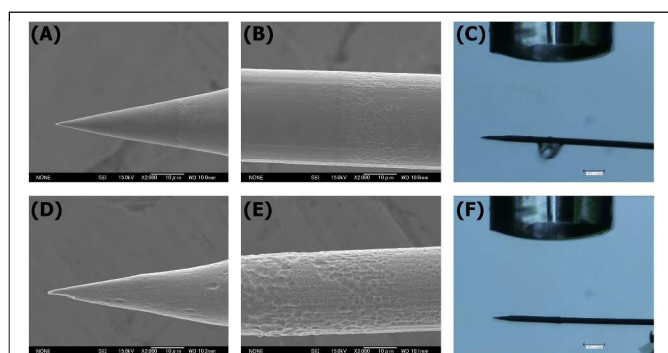.

2. Experimental

2.1 Materials and Chemicals

Fig.1 SEM pictures of etched probes: (A), the tip with untreated surface; (B), the part close to the tip with untreated surface; (D), the tip with hydrophilic surface; and (E), the part close to the tip with hydrophilic surface. (C) and (F) show the snapshot pictures of electrospray generated on the etched probes with untreated and chemically modified surfaces, respectively, when a Microjet piezoelectric inkjet system was applied as the online solvent delivery means. The high DC voltage between the probe and the counter electrode is 1.7 kV. Videos are available in the online supplementary materials.

Stainless steel (SS) wire (SUS304, o.d. 30 μm, purity 99.95%) and platinum wire (o.d. 0.5 mm, purity 99.98%) were purchased from Nilaco (Tokyo, Japan). Reserpine was purchased from Sigma-Aldrich (St Louis, MO, USA). HPLC grade acetonitrile and ethanol, H_2SO_4, HNO_3, $KMnO_4$, $K_2Cr_2O_7$, HCl (>36.5%, w/w) and oxalic acid were obtained from Kanto Chemicals (Tokyo, Japan). Water was purified and deionized by a Milli-Q system (Millipore, Bedford, MA, USA). All reagents and solvents used in this work were of analytical grade or higher and were used directly without any further purification.

Rhododendron petals and *geranium carolinianum* leaves were collected in the campus of the University of Yamanashi. Dry soybean seeds were purchased from a local grocery. Soybean sprouts were cultivated in a dark place by keeping soybean seeds in vermiculite with adequate water for three days.

2.2 Fabrication of the DSP

The tip-pointed probe was prepared by following a previous published electrochemical etching protocol[22] with certain modifications. An $H_2O/EtOH$ solution (1:1, v/v) containing 6M HCl was prepared as the etchant. A 3.5mm in diameter platinum wire loop (the cathode) was placed on the surface of the echant. A 1~1.5 cm long SS wire was sonicated in a 30% HNO_3 solution for 20 minutes to remove the passive layer. Then this SS wire (the anode) was positioned centrally within the cathode loop and dipped into the etchant for about 1 mm. The DC voltage between the two electrodes was set at 100 V and the stop current was 50 mA. After etching, a sharp tip (≈150 nm) could be observed. This wire was then rinsed with deionized water for cleaning.

The hydrophilic surface modification was performed by putting tip-pointed probes into a 30% (w/w) H_2SO_4 solution containing $KMnO_4$ (30 mg/mL) and $K_2Cr_2O_7$ (15 mg/mL). This solution was heated at 80-100 °C for 1 hour. After this oxidative treatment, the probe was sonicated sequentially by a 20% oxalic acid solution and deionized water to remove the attached reduction products of the oxidants. Finally, this probe was heated at 120 °C for 1 hour for activation.

Scanning electron microscopic (JSM-6500F, JOEL, Tokyo, Japan) images of the etched probes before and after surface modifications were shown in Fig. 1.

2.3 Direct Sampling from Single Cells

In this work, sampling from single cells was performed under a digital long focus microscope (VH-5500, Keyence, Osaka, Japan) and recorded to videos by a DVI2USB 3.0 video grabber (Epiphan Systems Inc., Ottawa, Canada). Videos of direct sampling of single cells are available in the online supplementary materials. As shown in Fig. 2(A), the biological sample was mounted on a glass slide which was fixed on the

top of a manual XYZ stage (Sigma Koki Co., Ltd., Saitama, Japan). The DSP was mounted firmly on the injection holder of a piezo micro-manipulator (PMM-150HJ, Prime Tech. Ltd., Ibaraki, Japan). The tip of the DSP can be well controlled to touch and then insert into the cell wall precisely. Due to the plant cell inner pressure, biofluid in a single cell would come out and attach on the hydrophilic surface of the DSP tip. Both the micro manipulator and the XYZ stage were located on the top of a desktop vibration isolator (AVT-0405N, Meiritz Seiki Co., Ltd., Yokohama, Japan).
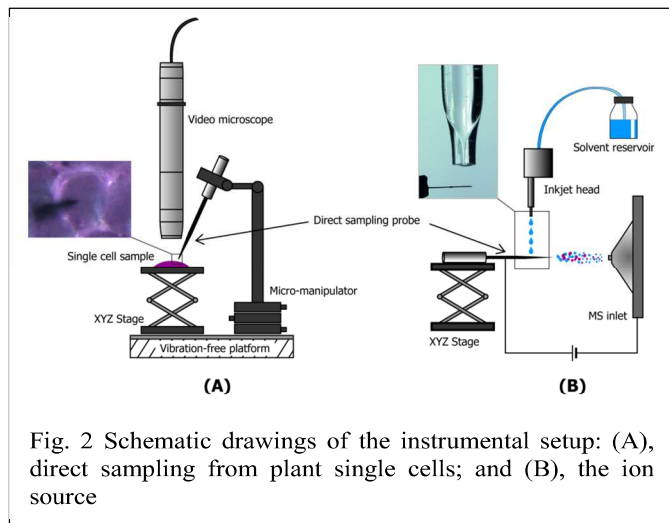

Fig. 2 Schematic drawings of the instrumental setup: (A), direct sampling from plant single cells; and (B), the ion source

2.4 Setup of the Ion Source

After sampling, the DSP was aligned horizontally and coaxially towards the ion sampling orifice of a time-of-flight (TOF) mass spectrometer. The distance between the probe tip and the apex of the ion sampling orifice was set as 2 mm. A piezoelectric inkjet system (IJK-200HS, Microjet Inc., Tokyo, Japan) equipped with an inkjet head (AD-K-501) was used in this work as the auxiliary solvent droplets generator. The nozzle diameter of the inkjet head is 30 μm. Parameters for this apparatus were: acceleration voltage, 100 V; operation frequency, 50 Hz; pulse time, 100 μs. Typically, a mixture of acetonitrile and pure water (50/50, v/v) was stored in the reservoir as the solvent. Because the droplets were generated discontinuously, the solvent delivery apparent flow rate was estimated at about 40 nL/min by assuming the generated droplet has the same radius as the inkjet head nozzle. The glass capillary of the inkjet head was placed perpendicularly to the DSP tip. The distance from the DSP tip to the inkjet head nozzle was adjusted to about 1 mm. When falling on the DSP surface, solvent droplets would spread and become an extended thin liquid film due to the high surface wettability. Analytes attached on the DSP tip would be extracted and then electrosprayed when a typical high direct current (DC) voltage of 1.7 kV was applied. Videos of electrospray by using surface-untreated and surface-modifed DSPs are available in the online supplementary materials, while snapshots are presented in Fig.1(C) and 1(F), respectively.

2.5 Mass Spectrometry

Mass spectrometric recording was carried out on an orthogonal time of flight mass spectrometer (AccuTOF, JEOL, Tokyo, Japan). The original ESI source was removed and the interlock was overridden. Typical parameters for the AccuTOF were: ion sampling orifice temperature, 150 °C; inlet orifice, 100 V; ring electrode, 10 V; first skimmer voltage, 5 V; ion guide rf amplitude, 1000 V; and the multichannel plate detector, 2500 V. All data were acquired and recorded under positive ionization mode by using the analogue-to-digital converter (ADC)/continuous averaging ion detection system.

2.6 Safety Considerations

The whole procedure of fabrication of DSPs was carried out in a well-ventilated fume hood. Both the etchant solution and the solution for surface modification are highly corrosive. Special care is necessary for handling and disposal of these solutions. Because high voltages were present at the DSP source and the inlet part of the mass spectrometer, electrically insulating gloves were used to prevent electrical shock.

3. Results and Discussions

3.1 Optimization of the DSP Ion Source

According to our previous work[19], several tens of pL fluid from biological samples can be loaded on the tip of a 300 μm (o.d.) DSP in a direct sampling process. Considering the size of a single plant cell varies from 10 μm to 100 μm, it is necessary to develop new strategy for less sample consumption when facing the task of analysis of single cells. In this work, a 30 μm (o.d.) probe and a less than 1 nL/s flow rate solvent delivery technique are both adopted to achieve less than 1 pL sampling and enough sensitivity.

The SEM pictures of etched SS probes with untreated and chemically modified surfaces are shown in Figs. 1(A, B) and 1(D, E), respectively. After electrochemical etching treatments, the ends of SS wires were shaped to pointed tips of about hundreds of nm in diameter. As it has been reported[19] that the SS surface can be heavily oxidized after the removal of the initial passive chromium oxide layer, the macroscopic surface roughness can also be enhanced greatly by forming some micro walls, ditches and pores after the oxidative modification[19]. The exposed surface area was also increased apparently, which made it possible to load more analytes during a single sampling process in seconds.

Figs. 1(C) and 1(F) present the close-up photographs of electrospraying by using surface-untreated and surface-modified SS probes as solid emitters, respectively, when a piezoelectric inkjet system was used as the solvent droplets delivery means. Owing to the fact that the chemically modified surface is of greater liquid wettability than the untreated surface[19], liquid droplets impacted on the hydrophilic surface have a higher likelihood to spread and cover the probe surface

than to remain the drop shape. When a high voltage was applied to this hydrophilic-surface probe, stable electrospray could be observed at the probe tip. However, for the probe with untreated surface, electrospray was generated from the apex of the droplet trapped at the body of the needle. Because of its larger size, electrospray became unstable compared with that generated at the needle tip for the treated needle. We suppose this is due to the multiplicity of variously directed forces, namely the electric field force, the gravity and the surface tension.

According to Smith's equation[23], surface tension affects the ESI performance greatly. If a liquid drop is placed on the surface of a solid ESI emitter, the surface tension keeps the shape of the drop. The surface tension has the opposite direction to the Coulomb force brought by the electric field and can be taken as the major cause of the high onset voltage of ESI. Most researches focus on improving ESI performance by changing the solvent polarity. Only a few works have investigated the influences of the emitter surface wetting characteristic to the ESI performance[19, 24-26].

For a solid surface, the wetting property relies on the difference in surface energy between the liquid drop and the solid material. From a theoretical point of view[27], high wettability indicates strong liquid-solid interactions at the solid-liquid interface, which can lower the surface tension by forming a disjoining pressure and cause the liquid to spread along the surface. For the surface-modified probe shown in Fig. 1(F), due to the negligible interference from the surface tension, electric field force drives solvent droplets to move to the probe tip, where the solvent extraction occurs and Taylor cone is formed. But for the surface-untreated probe shown in Fig. 1(C), strong surface tension prevents the liquid drop from changing its shape, which indicates the analytes on the probe tip can hardly be dissolved. If the applied voltage reaches the onset voltage of ESI,
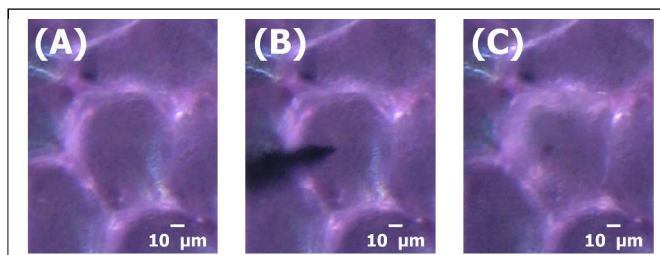

Fig. 3 (A) to (C) show the sequential images of a purple rhododendron petal cell before, in the middle of and after direct sampling by a surface modified probe.

electrospray will be generated at the position where the liquid drop locates. Thus in this case, few analytes will be ionized and transferred through the ion sampling orifice to the mass spectrometer. We think the chemical modification can bring at least two characteristics to the DSP: larger exposed surface area for better analyte accumulation from biological fluid and higher wettability for the formation of Taylor cone at the tip. When the surface-modified DSP is applied to a single cell, the tip can be well controlled to acquire metabolites in accordance to the order of contacting, pressing and piecing the cell wall. We assume the sampling volume is proportional to the surface area of the probe tip. Based on a previous observation[28], the sampling probe is estimated to load less than 1 pL biofluid from an individual cell. After sampling, there is a hole of a few μm in diameter left in the cell wall (shown in Fig. 3).

In our previous work[19], an external vapor sprayer was adopted to generate pure solvent spray for dissolution the loaded analytes. Because the vapor dispersed in a wide angle, it is difficult to control the solvent amount deposited on the probe tip. Too much solvent vapor condensing on the probe tip will decrease the formed sample solution concentration and lead to poor sensitivity and reproducibility, especially for the fine
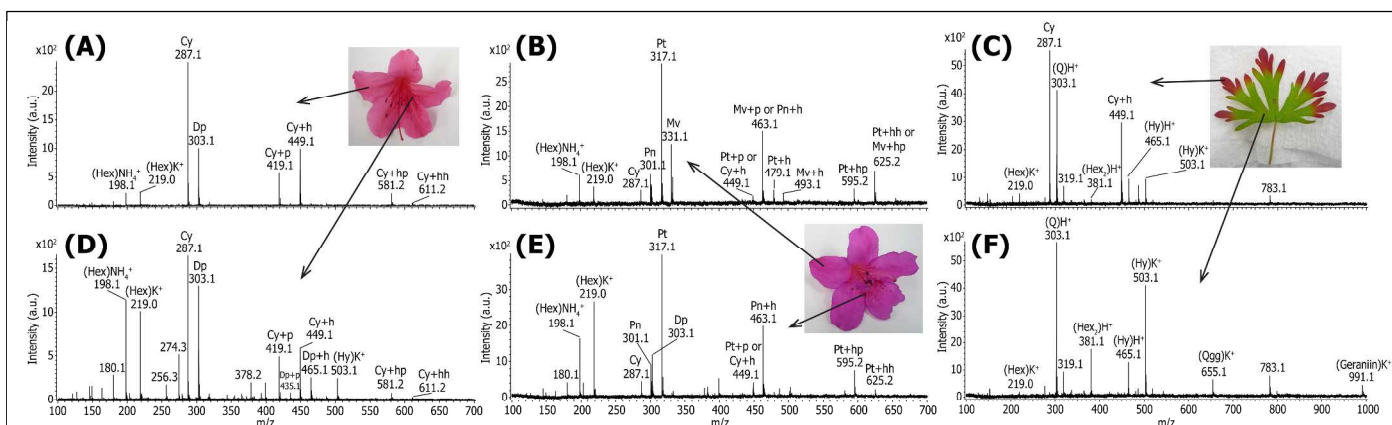

Fig. 4 Direct MS profiling of metabolites in plant single cells: (A), pink rhododendron petal; (B), purple rhododendron petal; (C), red part of a *Geranium carolinianum* leaf; (D), dark-colored spots in a pink rhododendron petal; (E), dark-colored spots in a purple rhododendron petal; and (F), green part of a *Geranium carolinianum* leaf. Notations are used throughout the paper. Hex$_n$, hexose oligomer, n indicates the number of hexoses; Cy, cyanidin; Dp, delphinidin; Pn, peonidin; Mv, malvidin; Pt, petunidin; Hy, hyperoside; Q, quercetin; QGG, quercetin-3-O-β-D-(6′-galloyl) galactoside; p, pentoside; h, hexoside; hp, hexosyl-pentoside; hh, di-hexoside.

probes with sharp tips. Nowadays piezoelectric inkjet is widely used in many fields due to its high accurate fluid delivery. Piezoelectric inkjet has also been demonstrated to combine with ESI-MS for generating pL level droplets[29]. Compared with the solvent vapor, droplets generated by the piezoelectric inkjet have higher linearity and volumetric accuracy, which indicates a better control of solvent delivery for the dissolution and electrospray of analytes.

In this work, a piezoelectric inkjet system was used as the external solvent delivery means. The solvent was ejected from a 30 μm in diameter nozzle discretely and linearly. A typical total ion chromatogram (TIC) diagram (shown in Fig. 5B) indicates that the process of solvent extraction and electrospray completes within 2 seconds. The reduction of solvent for the extraction of analytes increases the sensitivity. A series of reserpine solutions with different concentrations were used to evaluate the sensitivity of current method (data were shown in Fig. S1). The limit of detection for resepine using current DSP-MS was found at 5×10^{-6} mol/L.

3.2 Direct Mass Spectrometric Analysis of Plant Single Cells

Fresh flower petals are considered as good samples for single cell analysis due to their softness and water richness. The color of a flower pedal reflects the result of combination of anthocyanins. Rhododendron, one of the most diverse genera of over 1000 species, has a bi-colored corolla. Most species of rhododendron have spots of a contrasting (usually darker) color on their adaxial petals and bases of their upper lobes. The distribution of anthocyanins in the petal and the dark-color spots are also varied[30].

Mass spectra of single cells from pink and purple rhododendron petals are shown in Fig. 4. According to our observation, rhododendron petal cells are of an average size ranging from 50 to 70 μm, corresponding to cell volumes of 35 to 83 pL, if these cells are assumed as semi-spheres. Due to the lack of MS/MS functionality, identification of each peak is based on the molecular weight information and previously reported data[30-35]. Metabolites such as anthocyanins, saccharides and flavonoids are found as major components. In Fig. 4(A), the dominant anthocyanins in the rhododendron petal cells are cyanidin (Cy) and its glycosides. Delphinidin (Dp) is observed either, which may contribute little to the color[30]. But in the case of the dark-colored spots cells, Dp and Dp glycosides, bluish red colored components, are detected accompanying Cy and Cy glycosides(shown in Fig. 4(D)). However, purple petal cells of rhododendron show a different pattern of anthocyanin distribution. As shown in Fig. 4(B) and (E), aglycones, including Cy, Dp, Pn, Mv and Pt, and their glycosides are detected in both the petal and the dark-colored spots with different relative contents. We think this difference makes the spotting pattern of rhododendron petals. This spotting pattern is of great importance for both the plant itself and pollinating insects. Dark-colored spots indicate insects a certain direction
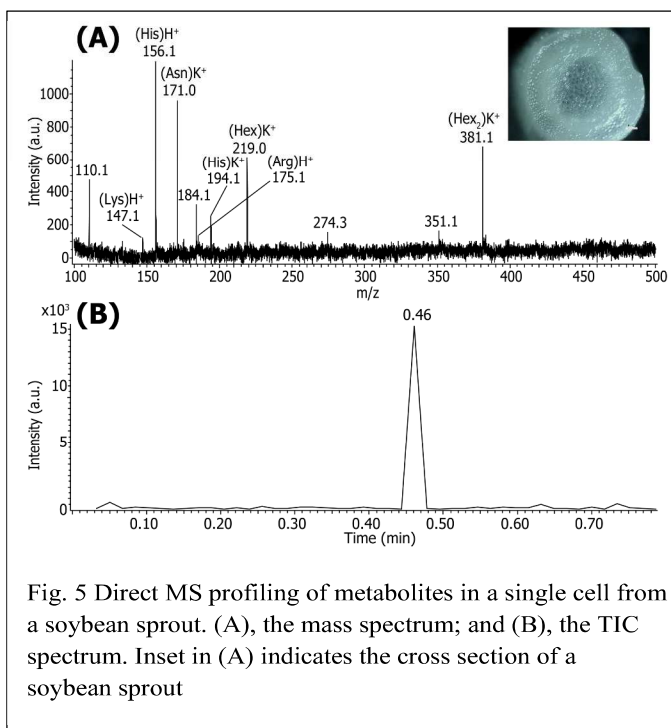

Fig. 5 Direct MS profiling of metabolites in a single cell from a soybean sprout. (A), the mass spectrum; and (B), the TIC spectrum. Inset in (A) indicates the cross section of a soybean sprout

for approaching and moving into, thus stamens and pistils will not be bypassed by these visitors[36].

Geranium carolinianum leaves are also selected to demonstrate the feasibility of DSP-MS for single cell analysis. Cells in the leaf are of an average size of 20 to 40 μm in diameter corresponding to 2 to 17 pL. Mass spectra of single cell analysis from the red-senescing and the green parts of a *Geranium carolinianum* leaf are shown in Figs. 4(C) and 4(F), respectively. Saccharides and flavonoids including Q and Hy, are observed in both the red-senescing and the green parts. Due to the senescence effect induced accumulation of anthocyanins[37], the red-senescing part gives strong signals of Cy and its glycosides. The most interesting is the detection of geraniin in the cells from the green part not the red-senescing part. This result is in coincidence with some early findings that the geraniin content varies with the leaf age[38, 39].

Fresh soybean sprouts are used as another source of single cells in this work. The cross section figure (inset in Fig. 5) indicates the multi-layer structure of the soybean sprout. Cells from the pith (in the center of the cross section) are selected, with sizes of 50-60 μm or volumes of 32 to 56 pL. Compared with leaf and petal cells, cells from soybean sprouts have tougher cell walls and more cytoplasma. DSP-MS result of single cells from the soybean sprout is shown in Fig. 5. Metabolites such as amino acids and carbohydrates are detected, which is in line with the early reported results[40, 41].

Conclusions

In this work, we introduce two major improvements to decrease the sample consumption and increase the sensitivity of DSP-MS, namely, a surface modified 30 μm in diameter sharp probe for direct sampling and a piezoelectric inkjet system for solvent delivery. Rhododendron petals, geranium carolinianum leaves and soybean sprouts were chosen to demonstrate the applicability of DSP-MS for direct MS profiling of phytochemicals in single cells. Metabolites such as sugars, anthocyanidins, flavonoids and amino acids from single plant cells can be loaded on the probe tip, extracted by the delivered solvent, electrosprayed and analyzed by the mass spectrometer.

The current research highlights the application of DSP-MS for direct analysis of single cells and could be extended to other plant cells. The method introduced here has some unique characteristics compared with other techniques, such as low sample consumption, disposability and simplicity in construction and operation. With further optimization, DSP-MS has the potential to be coupled with other techniques for high throughput single cell analysis. Further research will still be focused on applying DSP-MS for direct analysis of single cells. DSP-MS can also be applied as a monitoring tool for investigation of metabolism changes and changes of chemical content in plants cells subjected to environmental stresses.

Acknowledgements

Notes and references

a College of Chemistry and Biology, Shenyang Normal University, 253 Huanghe Street, Shenyang, Liaoning 110034, China. E-mail: yuzhan@synu.edu.cn

b Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, 4-3-11 Takeda, Kofu, Yamanashi 400-8511, Japan.

c Clean Energy Research Center, University of Yamanashi, 4-3-11 Takeda, Kofu, Yamanashi 400-8511, Japan. E-mail: hiraoka@yamanashi.ac.jp

d Plant Biophysics/Biochemistry Research Laboratory, Faculty of Agriculture, Ehime University, Matsuyama 790-8566, Japan.

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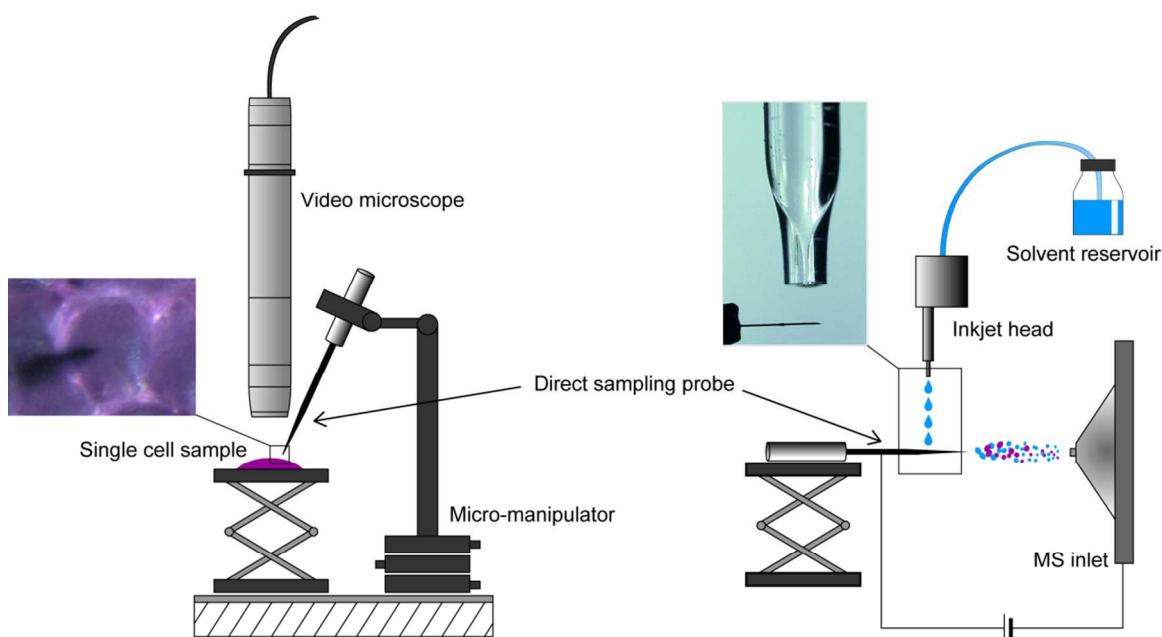
1   A. Schmid, H. Kortmann, P. S. Dittrich and L. M. Blank, *Curr Opin Biotech*, 2010, **21**, 12-20.
2. A. Ståhlberg and M. Bengtsson, *Methods*, 2010, **50**, 282-288.
3. Y. Lin, R. l. Trouillon, G. Safina and A. G. Ewing, *Anal Chem*, 2011, **83**, 4369-4392.
4. C. Schubert, *Nature*, 2011, **480**, 133-137.
5. K. Klepárník and F. Foret, *Anal Chim Acta*, 2013, **800**, 12-21.
6. D. Wang and S. Bodovitz, *Trends Biotechnol*, 2010, **28**, 281-290.
7. A. Svatoš, *Anal Chem*, 2011, **83**, 5037-5044.
8. E. J. Lanni, S. S. Rubakhin and J. V. Sweedler, *Journal of Proteomics*, 2012, **75**, 5036-5051.
9. P. Nemes and A. Vertes, *Trend. Anal. Chem.*, 2012, **34**, 22-34.
10. H. Mizuno, N. Tsuyama, T. Harada and T. Masujima, *J Mass Spectrom*, 2008, **43**, 1692-1700.
11. Y. Gholipour, H. Nonami and R. Erra-Balsells, *J Am Soc Mass Spectrom*, 2008, **19**, 1841-1848.
12. B. Shrestha and A. Vertes, *Anal Chem*, 2009, **81**, 8265-8271.
13. J. A. Stolee and A. Vertes, *Anal Chem*, 2013, **85**, 3592-3598.
14. P. L. Urban, T. Schmid, A. Amantonico and R. Zenobi, *Anal Chem*, 2011, **83**, 1843-1849.
15. B. N. Walker, J. A. Stolee and A. Vertes, *Anal Chem*, 2012, **84**, 7756-7762.
16. K. Hiraoka, K. Nishidate, K. Mori, D. Asakawa and S. Suzuki, *Rapid Commun Mass Spectrom*, 2007, **21**, 3139-3144.
17. L. C. Chen, K. Nishidate, Y. Saito, K. Mori, D. Asakawa, S. Takeda, T. Kubota, N. Terada, Y. Hashimoto, H. Hori and K. Hiraoka, *Rapid Commun Mass Spectrom*, 2008, **22**, 2366-2374.
18. M. K. Mandal, L. C. Chen, Z. Yu, H. Nonami, R. Erra-Balsells and K. Hiraoka, *J Mass Spectrom*, 2011, **46**, 967-975.
19. Z. Yu, L. Chen, M. Mandal, K. Yoshimura, S. Takeda and K. Hiraoka, *J Am Soc Mass Spectrom*, 2013, **24**, 1612-1615.
20. Z. Yu, L. C. Chen, S. Ninomiya, M. K. Mandal, K. Hiraoka and H. Nonami, Proceedings of the 61st Annual Conference on Mass Spectrometry, Tsukuba, Japan, September 10-12 2013, pp 200.
21. X. Gong, Y. Zhao, S. Cai, S. Fu, C. Yang, S. Zhang and X. Zhang, *Anal Chem*, 2014, **86**, 3809-3816.
22. Z. Yu, L. Chen, M. Mandal, H. Nonami, R. Erra-Balsells and K. Hiraoka, *J Am Soc Mass Spectrom*, 2012, **23**, 728-735.
23. D. P. H. Smith, *IEEE T Ind Appl*, 1986, IA-22, 527-535.
24. W. Shui, Y. Yu, X. Xu, Z. Huang, G. Xu and P. Yang, *Rapid Commun Mass Spectrom*, 2003, **17**, 1541-1547.
25. S. Su, D. Marecak and R. D. Oleschuk, *Rapid Commun Mass Spectrom*, 2008, **22**, 2053-2062.
26. Y. S. Choi and T. D. Wood, *Rapid Commun Mass Spectrom*, 2007, **21**, 2101-2108.
27. D. Bonn, J. Eggers, J. Indekeu, J. Meunier and E. Rolley, *Rev Mod Phys*, 2009, **81**, 739-805.
28. K. Yoshimura, L. C. Chen, D. Asakawa, K. Hiraoka and S. Takeda, *J Mass Spectrom*, 2009, **44**, 978-985.
29. C. Luo, Y. Ma, H. Li, F. Chen, K. Uchiyama and J.-M. Lin, *J Mass Spectrom*, 2013, **48**, 321-328.
30. B. Pecherer, *J Amer Rhodo Soc*, 1992, **46**, 200-202.
31. J.-M. Kong, L.-S. Chia, N.-K. Goh, T.-F. Chia and R. Brouillard, *Phytochemistry*, 2003, **64**, 923-933.
32. J. B. Harborne, in *Fortschritte der Chemie Organischer Naturstoffe*, ed. L. Zechmeister, Springer Vienna, 1962, **20**, pp. 165-199.
33. T. Reynolds, S. M. Smith and P. A. Thompson, *Kew Bulletin*, 1969, **23**, 413-437.
34. J. Li, Ph.D. Dissertation, Fudan University, 2008.

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35. J. Li, H. Huang, M. Feng, W. Zhou, X. Shi and P. Zhou, *Antivir Res*, 2008, **79**, 114-120.
36. E. E. Leppik, *Q Bull Am Rhodod Soc*, 1974, **28**, 70-89.
37. T. S. Feild, D. W. Lee and N. M. Holbrook, *Plant Physiol*, 2001, **127**, 566-574.
38. T. Okuda, K. Mori and T. Hatano, *Phytochemistry*, 1980, **19**, 547-551.
39. T. Tanaka, E. Sakai, M. Mizuno, T. Kawamura, Y. Hisata and Y. Noro, *Shoyakugaku Zasshi*, 1988, **42**, 48-50.
40. S. Lee and D. Chung, *J. Korean Agric. Chem. Soc.*, 1982, **25**, 75-82.
41. S. Kim, H. Hong, J. Lee and H. Choi, *J. Korean Soc. Agric. Chem. Biotechnol.*, 2000, **43**, 207-212.

Graphical Abstract

We report the combination of a piezoelectric inkjet and a surface modified sharp tipped probe for the direct sampling and rapid mass spectrometric analysis of metabolites in plant single cells.