Published in final edited form as:

Anal Chem. 2018 September 18; 90(18): 11078–11085. doi:10.1021/acs.analchem.8b02927.

T-Probe: An Integrated Microscale Device for Online In Situ Single Cell Analysis and Metabolic Profiling Using Mass Spectrometry

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Abstract

The exploration of single cells reveals cell heterogeneity and biological principle of cellular metabolism. Although a number of mass spectrometry (MS) based single cell MS (SCMS) techniques have been dedicatedly developed with high efficiency and sensitivity, limitations still exist. In this work, we introduced a microscale multifunctional device, the T-probe, which integrates cellular contents extraction and immediate ionization, to implement online in situ SCMS analysis at ambient conditions with minimal sample preparation. With high sensitivity and reproducibility, the T-probe was employed for MS analysis of single HeLa cells under control and anticancer drug treatment conditions. Intracellular species and xenobiotic metabolites were detected, and changes of cellular metabolic profiles induced by drug treatment were measured. Combining SCMS experiments with statistical data analyses, including Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) and two-sample *t*-test, we provided biological insights into cellular metabolic response to drug treatment. Online MS/MS analysis was conducted at single cell level to identify species of interest, including endogenous metabolites and the drug compound. Using the T-probe SCMS technique combined with comprehensive data analyses, we provide an approach to understanding cellular metabolism and evaluate chemotherapies at the single cell level.

Graphical Abstract

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Author Contributions

All authors have given approval to the final version of the manuscript.

The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.analchem.8b02927](https://doi.org/10.1021/acs.analchem.8b02927).

Experimental details, supporting tables, and supporting figures (PDF).

Cell, as a basic component of living organisms, contains rich biological information such as expression of genes,¹ interaction with other cells,² and metabolic response to altered microenvironment.³ Numerous techniques have been dedicatedly developed to study cellular constituents and reveal the biological principles of cellular metabolism.^{4–7} Mass spectrometry (MS), a rapidly developing technique to analyze ion species with high accuracy and sensitivity, stands out as a powerful tool to achieve efficient and reliable analysis of cell extractions.^{8–10} Conventional MS-based methodologies, such as the analysis of cell lysate using LC (liquid chromatography)-MS, have been broadly adopted in studies of populations of cells and, consequently, resulted in averaged chemical information from cell populations analyzed.^{11–14} In fact, cells are unique individuals that present heterogeneity due to intrinsic (e.g., genetic information) and extrinsic (e.g., surrounding microenvironment) factors.¹⁵ To overcome drawbacks of the conventional methods and obtain a biological signature of individual cells, a number of MS-based techniques have been developed to conduct single cell MS (SCMS) analysis. Depending on the sampling mechanisms, these methods can be generally classified as ion beam-based, laser-based, probe-based, and other methods. Ion beam-based SCMS methods primarily refer to secondary ion MS (SIMS), which provides high special resolution, allowing for subcellular analysis.^{16,17} Laser-based SCMS methods include but are not limited to matrix assisted laser desorption/ionization (MALDI) MS, laser ablation electrospray ionization (LAESI) MS, and laser desorption/ionization on porous silicon (DIOS) MS. MALDI MS is a popular method to achieve tissue level and single cell level analyses with high resolution and throughput.^{18–20} LAESI MS, as an ambient SCMS method that allows for studies of live cells with minimum sample preparation, has been successfully applied to analyze a large variety of cell lines in situ.^{21–23} DIOS MS is an alternative ambient SCMS method, which allows cells to be cultured directly on porous silicon substrates followed by laser ablation and MS detection.²⁴ Probe-based ambient SCMS methods include live single cell video MS,^{25,26} probe electrospray ionization (PESI) MS,²⁷ pressure probe electrospray ionization (PPESI) MS,²⁸ induced nanoelectrospray ionization (InESI) MS,²⁹ and the single-probe MS methods.^{30,31} In addition to these nonseparative methods, separation techniques, such as capillary electrophoresis (CE) and ion mobility separation (IMS), have been coupled with MS to study left–right patterning of single blastomeres in frog embryos^{32,33} and obtain metabolic signatures of single human hepatocytes.³⁴

Despite the broad applications reported, the majority of existing SCMS methods are classified as offline SCMS methods, because they generally involve multistep, offline

sample preparation procedures. However, cellular metabolites shift immediately (in seconds) upon changes to the surrounding microenvironment,⁹ and therefore, rapid real-time SCMS techniques are obligatory to analyze live single cells. We have previously developed a probe-based SCMS method, the single-probe MS, and demonstrated its applications in single cell analysis,^{30,35} MS imaging of brain tissues,^{36,37} and MS analysis of live multicellular tumor spheroids.³⁸

Here, we report the design and application of a novel miniaturized, integrated sampling and ionization device, the T-probe, that can be coupled to MS for online in situ live single cell analysis and metabolic profiling. As shown in Figure 1A, the T-probe has three major components: a sampling probe, a solvent-providing capillary, and a nano-ESI emitter. These capillaries are sandwiched by two polycarbonate (PC) substrates engraved with T-shaped grooves, and bound through thermal binding process. Designed for sampling individual mammalian cells with averaged size of $\sim 10\ \mu\text{m}$,^{9,27} the sampling probe has a sharp tip that is small enough ($5\text{--}8\ \mu\text{m}$) to be inserted into a target cell and subsequently extract cellular contents (Figure 1A, inset).

When the T-probe is used to conduct SCMS experiments, the solvent-providing capillary delivers sampling solvent (e.g., methanol (50%)/water (50%) with 0.1% formic acid) from a syringe. A DC voltage is applied to the conductive union and transmitted through the solvent to provide an ionization voltage at the nano-ESI emitter (aperture size $\sim 20\ \mu\text{m}$). Using the optimized solvent flow rate (e.g., $0.2\ \mu\text{L}/\text{min}$) and ionization voltage (e.g., $\sim 4\ \text{kV}$), a stable electrospray can be observed at the nano-ESI emitter without forming solvent droplets at the sampling probe tip. Our experiments demonstrate that a suction force is generated at the sampling probe tip to withdraw cellular contents. At the T-junction, the solvent is mixed with cellular species, and the mixture is subsequently delivered to the nano-ESI emitter for ionization and MS detection (Figure 1B). Although the exact mechanism of generating such suction force is unclear, this self-aspiration process is likely due to the continuous consumption of solution inside the nano-ESI emitter, leading to a liquid flow toward the nano-ESI emitter through the capillary action. Switching off the ionization voltage immediately resulted in a paused liquid suction from the sampling probe, until the ionization voltage was turned on again. Similar phenomena of solvent self-aspiration have been observed in other ambient MS methods such as liquid microjunction surface sampling probe (LMJ-SSP),³⁹ nanodesorption electrospray ionization (nano-DESI),⁴⁰ and the single-probe.³⁰ Under the optimized experimental conditions, the T-probe has no efflux of solvent from the sampling probe during the analysis of a target cell, minimizing the perturbation of the microenvironment of nearby cells to be analyzed. The perturbation of cell's microenvironment in some other sampling methodologies, in which the solvent, heat, or matrices is needed, can potentially alter the native biological states of live cells. In addition, the sampling probe (with one channel) of the T-probe can be further minimized (e.g., $<1\ \mu\text{m}$), and potentially used for subcellular-level analysis of mammalian cells. To provide additional evidence and shed light on the sampling process of the T-probe, we carried out experiments using a prepared solution, and estimated the fraction of liquid introduced from the sampling probe to that from the solvent-providing capillary.

Coupled to a mass spectrometer, the T-probe allows for online in situ MS analysis of live single cells in real time with minimal sample preparation. Due to its high sensitivity, the T-probe was applied to the detection of a variety of species present in single cells such as endogenous cellular metabolites, environmental stimulus (i.e., anticancer drug), and xenobiotic metabolites. Among those, we performed online MS/MS analysis for abundant species of interest to confirm their chemical identities. In addition, we established a comprehensive MS data analysis approach, including multivariate and univariate methods, to perform metabolic profiling at single cell level. As a result, we illustrated cellular response to drug treatment and discovered cellular species sensitive to such microenvironmental alternation.

EXPERIMENTAL SECTION

Fabrication of the T-Probe.

We developed a semi-automated fabrication protocol to manufacture T-probes with high reproducibility and efficiency, as demonstrated in Figure 2. The fabrication workflow includes five major steps. (1) We engraved multiple sets of T-shaped grooves (width $\sim 150\ \mu\text{m}$) on one PC substrate using a computer numeric control (CNC) microengraver. (2) A mini electric cutting saw was used to cut the engraved PC substrate into individual pieces (each piece contains one set of T-grooves). The pristine PC substrates (i.e., not engraved) were also cut into pieces with identical dimensions. Both engraved and pristine PC substrates were ultrasonically cleaned in *iso*-propanol to remove any residual debris within the grooves and contamination on surfaces. (3) Those cleaned PC substrates were dried and then treated with bis[3-(trimethoxysilyl)propyl]amine (Bis-TPA), a siliconization reagent, to increase hydrophilicity of PC surfaces and enhance PC–PC binding in the following thermal binding step.⁴¹ (4) Three capillaries (i.e., a solvent-providing capillary, a sampling probe, and a nano-ESI emitter) were produced from the same type of fused silica capillary (O.D. = $150\ \mu\text{m}$, I.D. = $75\ \mu\text{m}$). Among them, the sampling probe and the nano-ESI emitter were pulled using a laser micro pipet puller (Model P-2000, Sutter Instrument, Novato, CA). To fabricate a T-probe, these three capillaries were carefully positioned in grooves on an engraved PC substrate. The solvent-providing capillary and the nano-ESI emitter were aligned in-line, whereas the sampling probe was vertically placed so that they formed a T-junction. A pristine PC slide was used to sandwich these three capillaries to form a unit. To retain the positions of the capillaries and PC slides, we used two glass slides and applied pressure using two paper clippers. (5) Lastly, we kept the sandwiched assembly in an oven at $110\ ^\circ\text{C}$ for 30 min, and two PC slides were bound to form a T-probe through thermal binding. With enhanced PC–PC binding strength, solvent leakage from T-probes was rarely observed during the following SCMS experiments. Moreover, the employment of CNC micromachining promotes our manufacture reproducibility and efficiency. These standardized T-probe fabrication protocols can be readily adopted by other laboratories or industrial manufacturing facilities. In addition, the relevant techniques (e.g., micromachining, hydrophilic treatment of PC surface, and thermal binding process) are valuable for studies using plastic microfluidic devices.

SCMS Experiments Using the T-Probe.

The experimental setup for SCMS analysis using the T-probe was adapted from our single-probe SCMS setup (Figure S1). The T-probe was attached to a manual XYZ-manipulator for position adjustment. The nano-ESI emitter was ~2 mm away from the mass spectrometer inlet, and the solvent-providing capillary was connected to a syringe through a conductive union, where the ionization voltage was applied. A glass coverslip containing live HeLa cells and culture medium (see “SCMS Sample Preparation” in Supporting Information) was placed onto a motorized XYZ-translational stage system controlled by a LabView package.

⁴² Two microscopes were utilized to monitor the working status of the T-probe: a lateral microscope focusing on the nano-ESI emitter of the T-probe to monitor its electrospray conditions, and a stereo microscope focusing on the sampling probe tip to monitor the cell sampling process. During SCMS experiments, the sampling solvent was continuously delivered through the solvent-providing capillary, and stable signals of solvent ions were obtained without solvent dripping from the sampling probe by carefully adjusting the solvent flow rate and ionization voltage. We then selected a target single cell by moving the motorized XYZ-translational stage so that both the cell and the sampling probe tip were clearly observed under the stereo microscope (Figure 1C). By gradually lifting the stage (at an increment of 0.1 μm), the sampling probe tip was first submerged into culture medium above cells, and then penetrated the cell membrane with a continuous suction. Cellular contents along with the surrounding culture medium were subsequently withdrawn into the sampling probe and immediately ionized by nano-ESI emitter for MS analysis.

Correspondingly, we observed dramatic changes of mass spectra profiles from the solvent background through culture medium to the mixed cellular constituents and culture medium. Upon finishing the measurement of a cell, we lowered down the translational stage to remove the sampling probe tip out of the cell. Meanwhile, due to the loss of cytoplasmic contents, a significant change of cell morphology was observed under the microscope, and the cell was unlikely viable. To eliminate residues (carry-overs) from the sampled cell, we rinsed the T-probe by gently flushing it with the sampling solvent while monitoring the MS signal change. Such process ensured cleanness of the T-probe prior to the analysis of the next cell. A Thermo LTQ Orbitrap XL mass spectrometer was employed in our SCMS data acquisition process for high sensitivity and mass resolution. The experimental mass analyze parameters are listed as follows: mass resolution 60000 at m/z 400, mass range 200–1500, +4 kV ionization voltage, 1 microscan, 100 ms max injection time, and AGC (automatic gain control) on.

SCMS Data Analysis.

A detailed procedure of SCMS data analysis is described in the Supporting Information. Generally, ion signals corresponding to cellular metabolites were extracted from the raw data, whereas background signals (from exogenous species such as cell culture medium and sampling solvent) and instrument noise were removed. We carried out Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA)⁴³ in combination with univariate analysis (e.g., two-sample t -test) to study changes of cellular profiles induced by drug treatment. We determined cellular species with significant abundance change after drug treatment, which are of potential interest of clinical and pharmaceutical studies. Lastly, by referring to available online metabolome database such as METLIN (<https://metlin.scripps.edu>)⁴⁴ and

HMDB (<http://www.hmdb.ca>),⁴⁵ we accomplished tentative labeling for detected species, and further identified (through online MS/MS at single cell level) abundant species by comparing their fragmentation patterns with in silico data from online database.

RESULTS AND DISCUSSION

Estimation of Mixing Ratio at the T-Junction.

To gain a better understanding of the working mechanism of the T-probe, we estimated the volume mixing ratio of cellular contents withdrawn by the sampling probe to the sampling solvent delivered from the solvent-providing capillary. Considering the viscosity of cell cytoplasm (2–3 cP),⁴⁶ we prepared 250 nM deuterated irinotecan (d10-irinotecan) solution in *iso*-propanol (viscosity = 2.86 cP) in a vial located underneath the T-probe, whereas regular irinotecan with the same concentration was prepared in the sampling solvent (methanol (50%)/water (50%) with 0.1% formic acid). In the following experiment, the sampling solution was continuously delivered through the solvent-providing capillary at the flow rate used in our SCMS experiment (0.2 $\mu\text{L}/\text{min}$). Upon applying the ionization voltage, the protonated irinotecan ($[\text{irinotecan} + \text{H}]^+$) was observed. We then submerged the sampling probe tip into the vial containing d10-irinotecan, and observed both $[\text{irinotecan} + \text{H}]^+$ and $[\text{d10-irinotecan} + \text{H}]^+$. The volume mixing fraction was estimated by calculating the relative ion intensities of $[\text{irinotecan} + \text{H}]^+$ to $[\text{d10-irinotecan} + \text{H}]^+$. Such a process was repeated five times, and we found $41.8\% \pm 1.4\%$ (mean $\pm$ standard deviation) volume of solution was drawn through the sampling probe. Our experimental results indicate that a sufficient suction is produced at the tip of the sampling probe, and a dilution of extracted cellular contents with the sampling solvent occurs at the T-junction, which promotes detection sensitivity.⁴⁷ However, it is worth noting that the volume mixing ratio may vary among individual T-probes in SCMS experiments, and further studies, such as computational fluid dynamics (CFD) simulations, need to be carried out in future work.

Limit of Detections (LODs) of the T-Probe.

To evaluate the sensitivity of the T-probe in MS experiments, we used both the standard nano-ESI ion source and the T-probe device to measure the prepared solutions and determine their LODs, which are defined as the minimum concentrations allowing for MS detection with obvious ion signal intensities (i.e., signal-to-noise (S/N) > 3). We selected four standard compounds relevant to our SCMS experiments, including cellular lipids 1-oleoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (PC(18:1/16:0)) and 1,3-dihexadecanoyl-2-(9Z-octadecenoyl)-glycerol (TG(16:0/18:1/16:0)), anticancer drug irinotecan, and a peptide example leucine enkephalin, to conduct LODs determination. Our results (Table 1) indicate that the T-probe device exhibits comparable LODs as the standard nano-ESI method. In addition, the LOD of the T-probe is comparable to that of the single probe (e.g., the LOD of PC(18:1/16:0) is 5 nM).³¹

SCMS Experiments of Cells under Control Condition.

The T-probe was used to obtain cellular profiles of randomly selected HeLa cells under control condition (without drug treatment). The ion signals of cellular species were observed within seconds upon the insertion of the sampling probe tip into a cell. Typically, the ion

signals from one cell could last for >15 s before a rapid decrease, providing sufficient time to conduct online MS/MS analysis of abundant species. Due to the robustness of the T-probe, multiple cells can be consecutively measured using one T-probe without changing the device (Figure S2), which paves the way toward high throughput SCMS analysis. We summarized our SCMS detection results in Table S1 and tentatively labeled the detected cellular species based on their accurate m/z values. According to the tentative labeling, a broad range of cellular metabolites, including organic acids, cholesteryl esters (CE), phosphatidylcholines (PC), phosphatidylethanolamides (PE), and triglycerides (TG) in their protonated, sodiated, and potassiated forms (Figure 3A) were present in single cells. It is worth noting that these tentative labels are based on the accurate m/z measurement. They are not regarded as identifications and, therefore, can only be used as the reference to screen for molecules of interest. Compared with previously published studies of single HeLa cells, ^{30,31,48} our current results show a comparable number of lipid species at a higher mass range (i.e., m/z 600–900). While at a lower mass range (i.e., m/z 200–600), our method provides more coverage of cellular metabolites (e.g., organic acids) than those obtained using other methods (e.g., the single-probe MS and MALDI-MS). For ions with high abundances, such as [PC(34:1) + Na]⁺ (m/z 782.5676) and [PC(36:2) + Na]⁺ (m/z 808.5832), online in situ MS/MS spectra were obtained through collision-induced dissociation (CID) tandem mass spectrometry (MS/MS) to confirm their molecular structures at single cell level (Figure S3). Due to the extremely small volume of one HeLa cell (1.2–4.3 pL)⁴⁹ and the influence of matrix effect on ionization (e.g., culture medium extracted along with cellular species suppresses the ionization efficiency), it is very challenging to acquire a large number of abundant cellular species for MS/MS analysis. Improvement of the current technologies, such as combining with microseparation (e.g., capillary electrophoresis) or postionization separation (e.g., ion mobility separation) methods, is needed to enhance both the coverage and identification of metabolites.

SCMS Experiments of Cells under Drug Treatment Condition.

The SCMS experiments were also carried out using cells treated with irinotecan (7-ethyl-10-[4-(1-piperidin-4-yl)-1-piperidinyl]-1H-carbazole-9-carboxylic acid), which is known as a Topoisomerase I (Topo-I) inhibitor that damages the replication and transcription processes of single-strand DNAs and ultimately induces cell apoptosis.⁵⁰ In our experiments, HeLa cells were treated with irinotecan under a series of concentrations (i.e., 10 μ M, 1 μ M, 100 nM, and 10 nM) for 1 h, and then rinsed using fresh cell culture medium to remove drug molecules in extracellular medium. We performed SCMS analysis and detected the protonated species, [irinotecan + H]⁺ (m/z 587.2870), in all drug-treated single cells (Figures 3D and S4). Furthermore, the detection of this drug target was confirmed by online MS/MS analysis (Figure S5) at single cell level. As we decreased the treatment concentration of irinotecan, the ion intensity of this drug target markedly decreased. For example, [irinotecan + H]⁺ detected from cells treated with 10 nM irinotecan presented a low signal intensity (S/N $\sim$ 4) that is slightly above its LOD (Figure 3D). We then explored cellular xenobiotic activities in response to drug stimulus. In particular, we detected related drug metabolites including decarboxyl-irinotecan (m/z 543.2971), dehydro-irinotecan (m/z 585.2713), and hydroxyl-irinotecan (m/z 603.2819) from single cells (Figure S6). Our findings are in good agreement with previous results, as those metabolites were reported in

other studies of multicellular spheroids treated using the same drug compound.^{38,51} It is worth noting that these drug metabolites were observed from single cells treated using 10 μ M irinotecan, but they were not detected from cells under lower treatment concentrations (i.e., 1 μ M, 100 nM, and 10 nM). The missing drug metabolites from single cells under low-concentration treatment conditions are likely attributed to multiple factors, including limited uptake of drug molecules with lower initial dosage,⁵² insufficient treatment time to develop xenobiotic activities,⁵³ and matrix effect (i.e., due to mixed cellular metabolites and species in the culture medium) in MS measurements.⁵⁴

Changes of Metabolic Profiles after Drug Treatment.

We conducted SCMS data analysis to gain biological insights into cellular response to the altered microenvironment due to drug treatment. Generally, we obtained SCMS data sets from both the control (9 cells) and the drug treatment group (11 cells, treated using 1 μ M irinotecan for 1 h) and performed multivariate and univariate analyses (see “SCMS Data Analysis” in the Supporting Information). Typically, we applied OPLS-DA to illustrate group discrimination and visualize high dimensional cellular profiles in lower dimensional space, as shown on the OPLS-DA score plot (Figure 4A). The statistical rationale behind this approach is that the between-group variation (i.e., differences of cellular profile between control and treatment group) is represented by the predictive component T[1], whereas the within-group variation (rendered by cell-to-cell heterogeneity) is represented by the orthogonal component To[1].⁵⁵ A decent separation between cells from two groups can be achieved, indicating cellular profiles were significantly altered by drug treatment. The visualized separation obtained from the OPLS-DA model was well explained by the variation of each orthogonal component ($R^2X(\text{cum}) = 0.705$, $R^2Y(\text{cum}) = 0.991$) with high predictive ability ($Q^2(\text{cum}) = 0.704$). We further performed permutation tests⁵⁶ for the established model to evaluate the potential for model overfitting (Figure S7). Our results suggest that irinotecan treatment can induce a rapid change of cellular profiles (e.g., within 1 h upon treatment),⁵⁷ and our model has no overfitting.

In addition to the shifted metabolic profiles, we further discovered a number of cellular species with both high variable magnitude ($p[1]$) and high reliability ($p(\text{corr}[1])$) in the corresponding loading plot (S-plot, Figure 4B). These species located on both “wings” of the loading plot have major contributions to the observed groups separation,⁵⁸ and therefore, they are species sensitive to drug treatment and can be regarded as potential biomarkers reflecting the treatment efficacy.⁵⁹ We performed two-sample t -test for each metabolite and we found 17 species with testing p -values < 0.05 (as listed in Table S2 and marked in Figure 4B), indicating statistically significant change of abundance due to drug treatment. Among those, the protonated pyridoxamine-5'-phosphate ($[\text{PMP} + \text{H}]^+$, m/z 249.0640, shown in Figure 3B) and sodiated nicotinamide adenine dinucleotide ($[\text{NAD} + \text{Na}]^+$, m/z 686.0989, shown in Figure 3C) were detected along with other common cellular species. They were tentatively assigned with relatively high confidence using the combined information such as accurate mass, isotopic distribution, and database search (see details in Supporting Information and mass spectra in Figure S8). PMP is a precursor of pyridoxal-5'-phosphate (PLP), a coenzyme regulating a variety of intracellular pathways, and it was reported as a biomarker of cancer risk.⁶⁰ NAD, as a coenzyme that maintains energy homeostasis and

mediates cell signaling pathways, was reported to be a pharmaceutical target for cancer treatment.^{61,62} Our findings suggest that cellular metabolites, such as PMP and NAD, can be closely monitored upon irinotecan treatment at single cell level, and they may be potential biomarkers to indicate drug efficacy (inset of Figure 4B and Figure S9).

CONCLUSION

We designed and fabricated a microscale multifunctional device, the T-probe, to facilitate online in situ SCMS analysis and metabolic profiling at single cell level in ambient conditions. We demonstrated high sensitivity of the T-probe in SCMS analysis, and interrogated cellular contents using our in-house developed platform. Metabolic profiles of single HeLa cells under control and a series of drug treatment conditions were obtained and compared. In addition, online MS/MS analyses were performed to identify abundant species of interest (i.e., cellular metabolites, drug target). We observed that metabolic profiles of cells were significantly changed by the treatment of anticancer drug irinotecan, and discovered cellular species sensitive to drug treatment using statistical analyses. Those species, among which are PMP and NAD, can be potentially used as early prognostic indicators for treatment efficacy, and arouse a great interest in biological and pharmaceutical studies. In the future, the T-probe can be potentially coupled with broader types of ambient MS instruments to study fundamental cell biology, perform single cell metabolomics, and develop novel chemotherapies at single cell or even subcellular level.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This research was supported by grants from National Institutes of Health (R01GM116116 and R21CA204706).

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Figure 1.

Utilizing the T-probe for the SCMS experiments. (A) Photo of a T-probe. Inset: a zoomed-in photo of the sampling probe tip. (B) Illustration of the working mechanism and fluid flow directions in the T-probe. (C) Photo illustrating the insertion of the T-probe tip into a cell.

Figure 2.

Semiautomated fabrication workflow of the T-probe. (1) T-shaped grooves are engraved on a polycarbonate (PC) substrate using computer numeric control (CNC) micromachining. (2) PC slides are cut and (3) subjected to surface silconization treatment. (4) Three capillaries are positioned within grooves and sandwiched by a pristine PC piece. (5) Sandwiched assembly is heated in an oven for thermal binding.

Figure 3.

Mass spectra obtained from SCMS experiments using the T-probe device. (A) Zoomed-in view of tentatively labeled cellular species in a mass range of m/z = 700–900. Other example species, such as (B) [PMP + H]⁺, (C) [NAD + H]⁺, and (D) drug target [irinotecan + H]⁺ (cell treated using 10 nM concentration for 1 h), were also shown.

Figure 4.

Multivariate analysis of metabolic profiles of single cells using OPLS-DA. (A) Score plot of OPLS-DA model shows group separation between the control and drug treated single cells ($T[1]$) as well as intrinsic cell-to-cell heterogeneity ($To[1]$). (B) Loading plot (S-plot) of OPLS-DA model indicates cellular species significantly changed by drug treatment (primarily located on two “wings” of the S-plot). These species were revealed using two sample t -test and numbered as 1–17. Inset: box plots of two tentatively assigned species (PMP and NAD) present significant abundance changes (t -test p -value < 0.05) upon drug treatment.

Table 1.

LODs (nM) of Standard Compounds Detected by Nano-ESI and the T-Probe

compound	nano-ESI	T-probe
irinotecan	0.1	0.1
leucine enkephalin	0.8	1.0
PC (18:1/16:0)	5.0	10.0
TG (16:0/18:1/16:0)	0.1	0.1