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Highly Efficient Microscale Purification of Glycerophospholipids by Microfluidic Cell Lysis and Lipid Extraction for Lipidomics Profiling

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Abstract

This article presents a novel method for small-scale lipidomics of bacterial cells by integrating extraction of glycerophospholipids on microchip with a nanoelectrospray ionization quadrupole time-of-flight tandem mass spectrometer (nanoESI-Q-TOF MS/MS). The standard starting point for typical macroscale lipid analysis is a multiphase liquid-liquid extraction. Working with small populations of cells (1 to about 1000) requires a scaled down process in order to minimize dilution and facilitate the interface with microscale separation methods for sample cleanup and introduction to mass spectrometry. We have developed a microfluidic system that allows for lysis of bacterial cells, capture of lipids, and elution of captured lipids from a solid phase for microscale purification of lipids. The best on-chip extraction efficiency for glycerophospholipids was as high as 83.3% by integrating silica beads as the packing material with methanol as the eluent. Ten successive measurements were evaluated indicating that the microchip packed with fresh silica beads is capable of being reused for four times without any loss in lipid extraction process. The initial screening based on high-resolution tandem mass spectrometry data along with discovery profiling approach revealed the presence of 173 identified phospholipid species from microfluidic cell extracts. This work demonstrates the potential of incorporating microchip-based lipid extraction into cellular lipidomics research.

Keywords

lipid extraction; lipidomics; microchip; solid phase extraction

Introduction

Lipids have various biochemical functions including energy storage (fats and sterols), cell structure (lipid bilayers and micelles), and cell signaling.^{1–3} The quantitative study of lipids, or 'lipidomics', is a burgeoning field in bioanalytical chemistry.^{4–6} Low detection limits are important to lipid studies because some lipids occur in trace (picomolar (10^{-12}) – nanomolar (10^{-9})) and even ultra-trace (femtomolar (10^{-15}) and below) concentrations in samples.^{7–9} Two branches of chemical analysis play an important role in 'omics': separation science and mass spectrometry. In lipidomics, distinct subclasses of lipids, obtained by liquid extraction from a cell or tissue mass, are isolated by solid phase extraction, liquid chromatography, or thin-layer chromatography and then analyzed by a further dimension (or two) of liquid chromatography and two or more dimensions of mass spectrometry.^{10,11} One difficulty in profiling the chemical population of a small sample is that the lipidome exists over a wide range of concentrations. The most critical issue in analyzing such samples is that the less

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abundant species can easily get lost if the sample is wasted during inefficient sample processing steps. In addition, a relatively large amount of sample is required in some commonly used techniques such as TLC.^{12,13} One way to overcome these issues is to reduce sample transfer steps and dead volume by applying a microchip platform for all or part of the sample preparation scheme.

Numerous methods in chemistry are on the micrometer scale. Micro total analysis system (μ TAS) also called “lab-on-a-chip” is one of the techniques that shrink an entire experimental device to the scale of a microscope slide.^{14,15} Chemical analysis and chemical synthesis are the two main fields of μ TAS. Many of these concepts were brought to fruition by the application of basic photolithography to creation of channels in glass microchips.^{16–20} During the past two decades, the field of microfluidics has grown to include not only chips that separate compounds by capillary electrophoresis (CE),²¹ but also chips that create gradients, chips that integrate cell lysis with DNA capture, and even chips that allow for nanoscale reactions.¹⁵ The integration of sample pretreatment within microfluidic devices has recently become an important area of emphasis in the field of μ TAS. However, there are very few reports in the area of microfluidic chips associated with lipid extraction for lipidomics analysis.

In this article, a method for a microchip-based solid phase extraction for purification and enrichment of microscale glycerophospholipids is described. Specifically, intact bacterial cells loaded onto a chip are immediately lysed to release their lipid content. The released lipids are captured on a stationary phase and eluted with a suitable elution solvent. On-chip lipid extraction efficiency and reproducibility are evaluated by comparison to the modified Bligh-Dyer method.²² The purified lipids are reconstituted and injected directly into Q-TOF MS. Data are analyzed using both the METLIN Personal Metabolite database²³ and the LIPID MAPS (www.lipidmaps.org) database by integrating with MassHunter Qualitative Analysis software for lipidomics discovery profiling. With this method, we were able to identify over 170 phospholipid species from bacterial cell extracts.

Experimental Section

Microchip Fabrication

Schott Borofloat glass plates (1.5 mm thick, Telic Company, Santa Monica, CA) were used to fabricate microchips by standard photolithographic techniques.²⁴ The primary design for the microchips used in this study is a T-shape channel (Figure 1A). Masks were designed in Adobe Illustrator (CS2, Adobe, Inc) and were printed on a high resolution ink-jet printer (Canon Pixma Pro900, 4800 $\times$ 2400 dpi). In order to produce a channel with a width of 300 μ m and a depth of 200 μ m, both top and bottom masks had line features that were 100 μ m wide. A channel with a total depth of 200 μ m was made by etching both the top and bottom of glass pieces 100 μ m deep, and each channel was etched laterally by 100 μ m, giving a channel that was 300 μ m wide at its axis (Figure 1B). The glass was etched using a 14:20:66 (v/v/v) HNO₃: HF: H₂O solution. The etching of the weirs in Figure 1C was observed under a light microscope and a zoom stereomicroscope (Leica Wild, Wetzlar, Germany), and the plate was rinsed with deionized water. The total time of etching was 28 min. The top and bottom chip were subsequently brought into contact while still wet and were held together by capillary action. Finally, the assembly was placed in a furnace (Lindberg/Blue Moldatherm, Thermo Fisher Scientific Inc., Asheville, NC). The furnace temperature was raised at a rate of 10 $^{\circ}$ C/min to 80 $^{\circ}$ C and was held for 30 min, after which it was ramped at the same rate to 610 $^{\circ}$ C and held for 8 hours. The temperature was then decreased to room temperature at a rate of 10 $^{\circ}$ C/min. The chips were examined with the stereoscope to ensure that they were properly aligned and no shift occurred during the bonding process.^{25,26}

The packing material used as the stationary phase was either spherical silica gel (100 Å, 20 µm, Sorbent Technologies, Inc., Norcross, GA) or C18 silica gel (100 Å, Luna 15 µm, Phenomenex, Torrance, CA). A slurry of packing material in methanol was introduced to the reservoir on the side-arm of the chip by applying a mild vacuum through the other 2 reservoirs using pipette tips attached to a vacuum pump (Figure 1D). The packing was done under a zoom stereomicroscope. Once the chips were packed, water was passed through the channel using a syringe pump to test the flow of each chip. The lower bead-hole of the T-shape was sealed with epoxy and left to dry overnight before use (Figure 1E).

Apparatus

The homebuilt microfluidic chip apparatus for solid phase extraction consisted of a Harvard Apparatus Model 11 syringe pump (Harvard Apparatus, Holliston, MA) and a 250-µL Hamilton gas-tight syringe (Hamilton, Las Vegas, NV). Reagents were injected using an HPLC type injection valve (Rheodyne 7725, Rohnert Park, MA) with a 40-µL injection loop. The injection port was connected to the chip with polyether ether ketone (PEEK) tubing (1/16" od× 0.010" id, Upchurch, Oak Harbor, WA) and custom pressure holders or Upchurch Nanoports (Figure 2).

Cell Staining

Sulfurospirillum barnesii strain SES-3 cells were kindly provided by Dr. P. Basu, Duquesne University. *S. barnesii* is a readily available arsenate respiring bacterium with simple lipidome.^{27,28} The cells were treated with a cell-labeling dye that stains intact cells but displays a large decrease in fluorescence when cells are completely lysed.²⁹ The dye DiO (Invitrogen Molecular Probes, Inc., Carlsbad, CA) was prepared according to manufacture's instructions, and the prepared stock solution was stored at -20 °C. To stain the cells, a 0.5 mL portion of live cells was placed in a microcentrifuge tube. One µL of 1:100 diluted DiO stock solution was added to the live cells, and the contents were vortexed for 1 minute. The final concentration of the dye was 0.6 M.

Determination of Cell Lysis

A solution of washed *S. barnesii* strain SES-3 cells was prepared as follows. First, 0.5 mL of cells was placed in a 37 °C water bath for 8 minutes. After heating, the tube was centrifuged for 5 minutes at $6,900 \times g$, during which a pellet was formed and the supernatant was subsequently removed. The cells were then washed in 0.5 mL of 20 mM Tris buffer, and a pipette was used to break up the cell pellet. The tube was vortexed for 1 minute, then centrifuged for another 5 minutes at $6,900 \times g$. The supernatant was removed and discarded after centrifugation. The pellet was finally resuspended in 0.5 mL of 0.15 M Tris buffer.

Lysing agent was prepared by adding 20 mg Ompipur egg white lysozyme (EMD Chemicals, Gibbstown, NJ) to 6 mL of 20 mM Tris HCl buffer with a pH of 7.5. To lyse the cells, the lysing agent was loaded onto the microchip by syringe flow using a Harvard PHD 2000 syringe pump at a flow rate of 10 µL/min for 24 s followed by loading of the stained cells for 12 s. The lysing agent was then flowed over the bed at the same flow rate for another 24 s. The loading time was calculated to take into account any dead volume of the column and tubing.

In the loading and lysis steps, cells were imaged fluorescently at the inlet reservoir of the microchip using a Nikon Eclipse E-600 microscope with a 100 W mercury lamp. A FITC-HYQ filter set (excitation bandpass filter 480 ± 20 nm, dichroic reflector 505 nm cut-on, and emission bandpass filter 535 ± 25 nm) was applied for the imaging. The images were recorded using a RT-slider Camera and a QED Camera standalone program. The contrast of the images was enhanced using Adobe Photoshop CS4.

Chip-based Solid Phase Extraction

To flush out any unreacted species and prepare the solid phase for extraction, freshly filled microchips were conditioned with 20 mM Tris buffer (pH 7.5) for 30 min at 10 μ L/min. The solid phase was reconditioned under the same conditions for 10 min before each extraction step. The primary extraction protocol consisted of three pressure-driven wash steps, each performed at a flow rate of 10 μ L/min. First, the injection valve was switched to “load” and 20 μ L of the lysing agent was loaded onto the chip. A sample of 10 μ L cells was injected immediately thereafter, followed by loading of another 20 μ L of lysing agent onto the bed. The valve was then switched back to “inject” and 100 μ L of the wash solution (20 mM Tris buffer) was flowed through the chip to eliminate proteins and other potential non-lipid materials. Finally, the purified lipids were eluted in selected eluent (methanol, isopropanol or acetonitrile on silica bed; chloroform/methanol, 2/1 (v/v) or tetrahydrofuran/methanol, 1/1 (v/v) on C18 bed) and were collected in sample vials for subsequent mass spectrometry analysis. To eliminate carry-over, the solid phase was flushed with methanol for 30 min at a rate of 10 μ L/min between each run.

Conditions of Mass Spectrometry Analysis

An Agilent 6520 Quadrupole Time-of-Flight (nanoESI-Q-TOF) mass spectrometer was used for lipid extracts profiling. Aliquots (20 μ L) from loading, washing, and elution steps were collected during the extraction. The lipid extracts were dried by N_2 and resuspended in HPLC-grade methanol with 10 mM NH_4OH . Samples were introduced by direct infusion using a Harvard PHD 2000 syringe pump at a flow rate of 70 μ L/hr. MS only data was acquired in both positive and negative ion modes. The following settings were maintained throughout the analysis: drying gas flow of 10 L/min, drying gas temperature of 350 $^{\circ}C$, nebulizer pressure of 45 psi, data range of m/z 200–1000, acquisition rate of 1000 ms/spectrum, fragmentor voltage of 150 V, skimmer voltage of 60 V, octopole RF voltage of 250 V and capillary voltage of 3.5 kV. Typically, a 1-min period of signal averaging in the profile mode was employed for each spectrum.

Modified Bligh-Dyer Method

In our protocol, 0.2 mL cultured cells were mixed with 0.75 mL chloroform/methanol, 1/2 (v/v) and vortexed for 1 minute. 0.25 mL chloroform and 0.25 mL deionized water containing 1% acetic acid were added into the mixture and vortexed for another 1 minute. The solution was subsequently centrifuged at 1300 rpm for 5 minutes. The lower phase was collected with a Pasteur pipette and then evaporated followed by reconstitution in 0.5 mL methanol with 10 mM NH_4OH . Samples were introduced into Q-TOF MS for lipid profiling, and the profile was compared to that obtained using the microchip method. In this article, extraction efficiency is defined as the number of identified phospholipid species obtained using the microchip method as compared to the modified Bligh-Dyer method.

Results and Discussion

Overview

The goal of this work was to create a simple microdevice for effective cell lysis followed by lipid capture, cleanup, and recovery. A few preconditions must be met in order to ensure a seamless coupling of solid phase extraction and a microfluidic platform. Firstly, the lysing agent must effectively lyse intact cells in the volume present in the microchannel. Secondly, the released lipids must bind to the extraction bed successfully. Finally, the retained lipids must be eluted from the solid phase with high extraction efficiency and consistent reproducibility (recovery rate).

Evaluation of Lysing Agent

In order to consistently extract lipids from bacteria cells with high efficiency, it is necessary to lyse the cells completely. Figure 3A depicts the bacteria cells after the initiation of flow of the lysing agent into the inlet port of a complete microfluidic device. The fluorescence signal decreased after 1 minute of flow, and was undetectable after 3 minutes (Figure 3B), which confirms the sufficient lysis of the cells. Thus, these lysing conditions were applied in the subsequent experiments.

Adsorption of Lipids onto the Solid Phase

As the lysing agent for cell lysis had been chosen, it was necessary to make sure that released lipids adsorbed to silica surfaces successfully. Several purification goals must be achieved to recover phospholipids from cells and adsorb them to the silica phase in a single step. To make the purification protocol as simple and effective as possible, cell lysis and lipid binding to silica surfaces were accomplished using one single loading buffer. Several factors are taken into account when choosing the loading buffer. Solutions of high ionic strength facilitate the adsorption of phospholipids on the stationary phase as a result of a variety of interactions. At higher ionic strength, the negative potential of silica surfaces is reduced, thereby decreasing the electrostatic repulsion between the negatively charged phospholipid molecules and the bead surface. However, high concentrations of salt reduce the water activity due to the formation of hydrated ions, which facilitates the dehydration of phospholipids and silica surfaces, and drives the lipid to the extraction bed.³⁰ The electrostatic repulsion can be further reduced if the phospholipid is dissolved in a loading buffer below the pKa of the silica (pKa $\approx$ 8), due to the fact that increasing protonation of the surface silanol groups reduces the surface charge.^{31–33} In addition, extremely acidic loading buffer conditions could affect the solubility of lipids and may induce precipitation, which could ultimately lead to a failure of the microfluidic device. Consequently, a buffer of 20 mM Tris HCl with pH of 7.5, was chosen for phospholipid adsorption to silica surfaces.

Microscale Lipid Extraction

According to previous research on the physicochemical properties of silica materials for lipid extraction, surface properties play a critical role in the extraction of hundreds or even fewer cells.^{34,35} Size and shape of the particles, diameter and volume of the pores, and the packing and resin porosity are of major concerns to the overall effectiveness of phospholipid binding. In this work, two types of silica resins, silica beads (100 Å, 20 μ m) and C18 beads (100 Å, Luna 15 μ m), were selected as the packing material to adsorb the phospholipids. The microporous structure prevents the macromolecular proteins from going through the beads, while keeping small analytes including polar lipids from entering and being retained. The performance of lipid extraction on microchip was evaluated by extraction efficiency and reproducibility during the purification process. The recovery of phospholipids from the device was accomplished using an eluent with low ionic strength such as methanol, chloroform, acetonitrile, etc. In contrast to loading buffer, the eluent exhibited a slightly higher back-pressure due to its high viscosity, which caused a drop in flow rate to 8 μ L/min.

In order to evaluate the lipid extraction efficiency, the numbers of identified phospholipid species extracted from the microchip by different eluents were compared with that obtained by the modified Bligh-Dyer method, as illustrated in Figure 4. The overall extraction efficiencies of silica chip with different eluents were 83.3% (methanol), 49.3% (isopropanol), and 29% (acetonitrile), respectively. When the phospholipids were eluted from C18 chip by chloroform/methanol (2/1, v/v) or tetrahydrofuran/methanol (1/1, v/v), the on-chip extraction efficiencies were 71.7% and 61.8% compared to that obtained using the modified Bligh-Dyer method. Thus, silica beads were selected as the packing material with methanol as the eluent for lipid extraction in subsequent experiments. Before each

extraction, the silica beads were reconditioned by flushing methanol for 30 min, followed by another flush with 20 mM Tris buffer for 10 min. In a measurement where 10 μ L of 34:2 PC and 34:1 PI (Sigma-Aldrich Chemical Co., St.Louis, MO) standard mixture were loaded at a final concentration of 5 μ M for ten successive extractions, the average phospholipid recovery rate for the microchip was 55.34% and 46.12%, respectively (Figure 5). It is shown that the reproducibility of lipid extraction from silica bed decreases after the fourth run. In order to overcome this issue, new silica beads were packed to the chip and the lipid recovery rate went back to around 65% for 34:2 PC. In summary, the microchip packed with fresh silica beads is capable of being reused for four times without any loss in lipid extraction process.

To ensure that phospholipids were eluted from cells only when the eluent was applied, fractions were collected and analyzed for a phospholipid elution profile during extraction and the results are shown in Figure 6. During this extraction step, a sample of 10 μ L cells sandwiched with 20 μ L lysing agent was loaded onto the silica bed at a flow rate of 10 μ L/min. After a wash with 20 mM Tris HCl buffer (100 μ L, pH 7.5), during which any non-lipid materials were removed, the lipids were eluted in methanol. Eighteen fractions of the eluate (20 μ L each) were collected at the outlet of the device during each step of the extraction (cells loading/lysing, non-lipid materials washing, and lipids elution). The phospholipid 34:1 PI was chosen for lipid quantification during the extraction process by the nanoESI-Q-TOF mass spectrometer. As shown in Figure 6, the major elution of 34:1 PI occurred in the first 80 μ L of the elution phase, with negligible concentrations of lipid detected in other steps. This result indicates that there was no significant loss of lipid during the loading and washing steps, and most of the extracted 34:1 PI was eluted from the first several tens of microliters. These results are consistent with the fact that 34:1 PI was eluted reproducibly in the first 80 μ L of the elution phase, which suggests that the lysing agent/loading buffer did not interfere with binding of the lipid to the silica bed or impact lipid recovery negatively.

Lipid Profiling

In order to identify the molecular species of phospholipids from *S. barnesii* cells, the nanoESI-Q-TOF MS/MS was applied along with “untargeted” (discovery profiling) approaches for lipidomics research. The data analysis workflow consists of interrogation of lipid extracts data files from MS by a METLIN Personal Metabolite database and LIPID MAPS database. By significantly narrowing the list of possible identities, phospholipid species were identified using a custom subset of METLIN Database relevant to lipid metabolites in the mode of accurate-mass information searching. Sample data files were subsequently imported in MassHunter Qualitative Analysis software by loading the lipid database that contains the formula of each lipid species. The data files were queried by each formula using a “Find by Formula” function, which finds and extracts all the co-eluting isotopes, salt adducts, and dimers that belong to each empirical formula.

Six major classes of phospholipid were identified with this method, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidic acid (PA), and their lyso variants. Within these classes, each of the target mass identified in the profiling processes was searched against the METLIN database over a 10 ppm mass window. A mass error window of 5 ppm was set for the empirical formula calculation. Three major lipid classes, PC, PS and PE, are detected in positive ionization mode (+ESI),^{36–39} while four major lipid classes, PI, PS, PG and PA, are detected in negative ionization mode (–ESI).^{40–42} In addition, the lyso variants for all these phospholipids (LPE, LPC, LPA, LPI, LPS, and LPG) can be detected in both modes. Finally, the results from METLIN searches were incorporated into Excel spreadsheets for further interpretation.

Link to the LIPID MAPS database was also provided to predict possible phospholipids species present in the cell extracts. This application allows for input of the m/z value of an unknown lipid ion or a batch of lipids to predict the most possible molecular species, based on internally generated lists of side-chains and head groups of glycerophospholipids and sphingophospholipids. The parameters used were listed as follows: intensity threshold of 1000, mass tolerance of ± 0.01 amu, and ion mode of $[M+H]^+$ or $[M-H]^-$.

Low mass peaks (below m/z 350) were assigned to free fatty acid as a result of in-source collision-induced fragmentation. Peaks of lyso variants for all kinds of phospholipids were in the range of m/z 350–600. Most of the major phospholipid classes (PE, PC, PG, PS, PA, and PI) fell in the range between m/z 600 and 1000, which consisted of about 70% (about 380 peak envelopes) of the MS peaks (Figure 7A,7B). As shown in Figure 7C and 7E, 34:2 PC was detected with $[M+H]^+=758.561$, and a characteristic phosphocholine head group peak at m/z 184.073 was shown in MS/MS spectrum by applying 20 eV on the extracted parent ion. Relatively small peaks of lysophosphatidylcholines ($[LPC+H]^+$ and $[LPC-H_2O+H]^+$) correspond to the loss of one fatty acyl substitute as a ketene $[M-R_2CH=C=O]^+$. In Figure 7D and 7F, the peak of m/z 835.585 was assigned to 34:1 PI and its parent ion was extracted for fragmentation at 45 eV. Lysophosphatidylinositols ($[LPI-H]^-$ and $[LPI-H_2O-H]^-$), lysophosphatidic acids ($[LPA-H_2O-H]^-$), and the characteristic head group fragments including glycerophosphoinositols ($[GPI-H_2O-H]^-$ and $[GPI-2H_2O-H]^-$), fatty acid ($[FA-H]^-$), inositolphosphate ($[IP-H_2O-H]^-$ and $[IP-2H_2O-H]^-$), and glycerophosphate ($[GP-H_2O-H]^-$) were observed for the specific PIs identification. Fragmentation of these phospholipid species in both ionization modes yielded a wealth of structural information. In each case, the lipid identification process was aided by head group fragmentation, lyso-lipid formation, and fatty acid fragments. By using LIPID MAPS mass prediction tool, many possible phospholipids species and their structures (double bonds, carbon numbers of fatty acyl chains, and head groups) were determined. Table S1 in supporting information shows a summary of proposed phospholipids based on METLIN database⁴³ and LIPID MAPS database matches of the measured mass of the parent ions with their theoretical mass over a 10 ppm mass window. The tandem mass spectrometry was a complementary approach for identification of phospholipid isomers. With this “untargeted” lipidomics approach, as many as 173 phospholipid species were identified from on-chip bacterial cell extracts.

Conclusions

A microscale solid phase extraction method was developed for the extraction of glycerophospholipids from bacteria cells with integration to analysis by small scale lipidomics. Buffer solution of 20 mM Tris HCl at pH 7.5 containing 20 mg Omnipur egg white lysozyme was demonstrated to work effectively as a lysing agent for *Sulfurospirillum barnesii* strain SES-3 cells on microfluidic devices. Among different types of stationary phase and elution solvent for lipid capture tested, the best extraction was obtained by using a silica phase with methanol as eluent. Lipids purified with the developed method were suitable for subsequent lipidomics analysis and compared favorably with lipids extracted by a commonly utilized Bligh-Dyer method. By incorporating both fast and robust peak finding algorithms and visualization software, many phospholipids were found to have matches to the METLIN and LIPID MAPS databases. In summary, this work presents a highly efficient method for microchip-based solid phase extraction for use with micro scale lipidomics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- (1). van Meer G, Voelker DR, Feigenson GW. Nat. Rev. Mol. Cell Biol. 2008; 9:112–124. [PubMed: 18216768]
- (2). Wymann MP, Schneider R. Nat. Rev. Mol. Cell Biol. 2008; 9:162–176. [PubMed: 18216772]
- (3). Brasaemle DL. Journal of Lipid Research. 2007; 48:2547–2559. [PubMed: 17878492]
- (4). Wenk MR. Nat. Rev. Drug Discov. 2005; 4:594–610. [PubMed: 16052242]
- (5). Han X. Front. Biosci. 2007; 12:14.
- (6). Fahy E, Subramaniam S, Brown HA, Glass CK, Merrill AH, Murphy RC, Raetz CRH, Russell DW, Seyama Y, Shaw W, Shimizu T, Spener F, van Meer G, VanNieuwenhze MS, White SH, Witztum JL, Dennis EA. J. Lipid Res. 2005; 46:839–862. [PubMed: 15722563]
- (7). Yan K-P, Hao J, Dan N, Chen C. Artificial Cells, Blood Substitutes and Biotechnology. 2008; 36:19–33.
- (8). Ivanova PT, Milne SB, Myers DS, Brown HA. Current Opinion in Chemical Biology. 2009; 13:526–531. [PubMed: 19744877]
- (9). Axelsen PH, Murphy RC. Journal of Lipid Research. 2010; 51:660–671. [PubMed: 19767534]
- (10). Han X, Gross RW. Journal of Lipid Research. 2003; 44:1071–1079. [PubMed: 12671038]
- (11). Han X, Gross RW. Mass Spectrometry Reviews. 2005; 24:367–412. [PubMed: 15389848]
- (12). Paglia G, Ifa DR, Wu C, Corso G, Cooks RG. Analytical Chemistry. 2010; 82:1744–1750. [PubMed: 20128616]
- (13). Rohlffing A, Muthing J, Pohlentz G, Distler U, Peter-Katalinić J, Berkenkamp S, Dreisewerd K. Analytical Chemistry. 2007; 79:5793–5808. [PubMed: 17590015]
- (14). Chen X, Cui D, Liu C, Li H, Chen J. Analytica Chimica Acta. 2007; 584:237–243. [PubMed: 17386610]
- (15). Reyes DR, Iossifidis D, Auroux P-A, Manz A. Analytical Chemistry. 2002; 74:2623–2636. [PubMed: 12090653]
- (16). Seiler K, Harrison DJ, Manz A. Analytical Chemistry. 1993; 65:1481–1488.
- (17). Jacobson SC, Hergenroder R, Koutny LB, Ramsey JM. Analytical Chemistry. 1994; 66:1114–1118.
- (18). Jacobson SC, Hergenroder R, Moore AW Jr. Ramsey JM. Analytical Chemistry. 1994; 66:4127–4132.
- (19). Jacobson SC, Hergenroder R, Koutny LB, Ramsey JM. Analytical Chemistry. 1994; 66:2369–2373.
- (20). Jacobson SC, Koutny LB, Hergenroder R, Moore AW, Ramsey JM. Analytical Chemistry. 1994; 66:3472–3476.
- (21). Jemere AB, Oleschuk RD, Harrison DJ. Electrophoresis. 2003; 24:3018–3025. [PubMed: 12973805]
- (22). Bligh EG DW. Can J Biochem Physiol. 1959; 37:7.
- (23). Smith CA, Maille GO, Want EJ, Qin C, Trauger SA, Brandon TR, Custodio DE, Abagyan R, Siuzdak G. Ther. Drug Monit. 2005; 27:747–751. [PubMed: 16404815]
- (24). Ko, WH.; Suminto, JT. Semiconductor Integrated Circuit Technology and Micromachining. Wiley-VCH Verlag GmbH; 2008.
- (25). Jemere AB, Oleschuk RD, Ouchen F, Fajuyigbe F, Harrison DJ. Electrophoresis. 2002; 23:3537–3544. [PubMed: 12412122]

- (26). Yuen PK, Kricka LJ, Fortina P, Panaro NJ, Sakazume T, Wilding P. *Genome Res.* 2001; 11:405–412. [PubMed: 11230164]
- (27). Luijten MLGC, de Weert J, Smidt H, Boschker HTS, de Vos WM, Schraa G, Stams AJM. *Int J Syst Evol Microbiol.* 2003; 53:787–793. [PubMed: 12807201]
- (28). Finster K, Liesack W, Tindall BJ. *Int J Syst Bacteriol.* 1997; 47:1212–1217. [PubMed: 9336931]
- (29). Loew LM, Simpson LL. *Biophysical journal.* 1981; 34:353–365. [PubMed: 7248466]
- (30). Moura SP, Carmona-Ribeiro AM. *MRS Proceedings.* 2005; 900:98–104.
- (31). Kobayashi M, Juillerat F, Galletto P, Bowen P, Borkovec M. *Langmuir.* 2005; 21:5761–5769. [PubMed: 15952820]
- (32). Goyne KW, Zimmerman AR, Newalkar BL, Komarneni S, Brantley SL, Chorover J. *Journal of Porous Materials.* 2002; 9:243–256.
- (33). Stumm, W.; Morgan, JJ. John Wiley and Sons. New York: 1996.
- (34). Christie, WW. *Lipid Analysis-Third Edition.* The Oily Press; Bridgwater: 2003.
- (35). Van Horne, KC. *Sorbent Extraction Technology.* Analytichem International; Harbor City, CA: 1985.
- (36). Hsu F-F, Turk J. J. Am. Soc. Mass. Spectrom. 2000; 11:437–449. [PubMed: 10790848]
- (37). Hsu F-F, Turk J. J. Am. Soc. Mass. Spectrom. 2003; 14:352–363. [PubMed: 12686482]
- (38). Ekroos K, Ejsing CS, Bahr U, Karas M, Simons K, Shevchenko A. J. *Lipid Res.* 2003; 44:2181–2192. [PubMed: 12923235]
- (39). Hong J, Cho K, Kim YH, Cheong C, Lee K-S, Jung JH. *Rapid Commun. Mass Spectrom.* 2001; 15:1120–1126. [PubMed: 11404849]
- (40). Hsu F-F, Turk J. J. Am. Soc. Mass. Spectrom. 2000; 11:797–803. [PubMed: 10976887]
- (41). Hsu F-F, Turk J. J. Am. Soc. Mass. Spectrom. 2000; 11:892–899. [PubMed: 11014451]
- (42). Hsu F-F, Turk J. J. Am. Soc. Mass. Spectrom. 2001; 12:1036–1043.
- (43). Sana TR, Roark JC, Li X, Waddell K, Fischer SM. *J Biomol. Tech.* 2008; 19:258–266. [PubMed: 19137116]

Figure 1.

Design of the microfluidic device. (A) The schematics of the microchip for cell lysis and lipid extraction. (B) Cross-sectional diagram of the SPE channel. (C, D) Images of the weir structure and the packed column. (E) Silica beads are packed in the microchannel through the bead-hole on the side-arm of the chip using a mild vacuum until the channel is fully packed.

Figure 2. The microdevice setup used for extraction of lipid from bacterial cells. The flow system consists of a syringe pump, which is connected to an HPLC type injection valve for loading the lysing agent and Tris buffer to the microchip.

Figure 3. Fluorescence images of on-chip cell lysis under microscopy at 20 $\times$ magnification. (A) Image taken when DIO labeled cells were flowed into the inlet irradiated with 480–560 nm light. (B) Image taken after flow of the lysing agent over 3 minutes.

Figure 4. Comparison of on-chip extraction efficiencies of phospholipid using different eluents. Silica (black) and C18 (gray) resins were applied as packing materials. The error bars represent the variation of four individual experiments. Values represent mean $\pm$ S.E. of triplicate determinations.

Figure 5. Phospholipid recovery rate on the microchip over ten successive extractions. Standard mixtures of 5 μ M 34:2 PC (black) and 34:1 PI (gray) were used. Values represent mean $\pm$ S.E. of ten successive measurements in triplicate determinations.

Figure 6.

Extraction profile of 34:1 PI from the silica chip eluted by methanol. Fractions (20 μ L in volume) were manually collected during loading, washing and elution steps.

Figure 7.

Mass spectra of phospholipid extracts from *S. barnesii* cells eluted from silica bed of microchip by methanol. Six major lipid classes were detected in positive (A) and negative (B) ionization modes, e.g. 34:2 PC (C) and 34:1 PI (D) were detected and their structures were identified with corresponding tandem mass spectra (E, F).