様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月　 ４ 日現在

機関番号：１７１０２
研究種目：研究活動スタート支援
研究期間：2022～2023
課題番号：２２Ｋ２０５６０

研究課題名（和文）Functionalized capillary device for mass spectrometry-based subcellular lipid profiling

研究課題名（英文）Functionalized capillary device for mass spectrometry-based subcellular lipid profiling

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：サブセルラーリピッド分析のためのガラス針デバイスを開発した。サブセルラーリピッド プロファイリングは価値の高い研究分野であるが、サンプル量の制限や構造/分子多様性の高さから大きな課題を伴う。質量分析法(MS)はサブセルラー組成分析の最先端手法の1つであるが、信頼性の高い分析を行うには繊細なサンプル前処理が必要とされる。本研究で開発したガラス針デバイスは、固相マイクロ抽出(SPME)機能により不純物を除去でき、電導性を利用したエレクトロスプレーイオン化MSを実現できる。SPMEは標準リピッドサンプルを用いて実証し、ガラス針デバイスとMSによるサブセルラー分析はNeuro2a細胞を用いて実証した。

研究成果の学術的意義や社会的意義
サブセルラーリピッド プロファイリングは細胞の生理機能や病態解明において極めて有益な研究分野である。しかし、サンプル量の制限と高い構造/分子多様性が大きな課題である。質量分析法(MS)はサブセルラー組成分析に有力だが、複雑なサンプル中の不純物によるイオン抑制が重要な検出問題となっている。本研究で開発したガラス針デバイスは、不純物を除去し、エレクトロスプレーイオン化MSを実現できる。この簡便製作のデバイスは高性能なMS分析を可能にするため、実現可能性の高いサブセルラーリピッド プロファイリング手法として貢献できると期待される。

研究成果の概要（英文）：A simple glass needle device was developed and demonstrated for subcellular lipid analysis. Subcellular lipid profiling is a highly valuable research area, but it is challenging due to the limited sample volume and high structural/molecular diversity. Mass spectrometry (MS) is one of the most advanced techniques for subcellular composition analysis, but it requires delicate sample pretreatment methods to ensure reliable analysis. A major issue in crucial sample detection by MS is ion suppression from impurities present in the complex sample matrix. With our glass needle device, impurities could be removed by its solid phase micro extraction (SPME) function, and electro-spray ionization-based MS would be realized by its electrically conductive character. The SPME was demonstrated using a standard lipid sample, and subcellular analysis via the glass needle device and MS was demonstrated using Neuro2a cells.

研究分野：分離化学

キーワード：mass spectrometry　solid phase extraction　subcellular analysis　lipidomics

１．研究開始当初の背景 (Background of the project)

Subcellular lipid profiling is a highly featured research area but challenging due to the limited sample volume and high structural/molecular diversity. Mass spectroscopy (MS) is one of the most advanced candidates for subcellular composition analysis but calls for delicate sample pretreatment methods to guarantee a reliable analysis. A major issue in crucial sample detection by MS is the ion suppression from impurities presenting in the complex sample matrix. With the purpose to enhance the detection sensitivity, we propose a functionalized needle-based device for sample purification before MS detection to remove ionic impurities. In general, the needle is able to realize solid phase microextraction (SPME) for lipid species, which facilitates the sensitive MS detection of trace sample. This project will contribute to feasible and high-performance MS-based subcellular lipid profiling.

２．研究の目的 (Goal of the research)

We aim to develop a needle-based device for the elimination of interfering molecules from the complex cell membrane compositions based on SPME. This device is adaptable with MS interface for lipid detection. One stage of purpose is to realize the SPME of standard lipid samples for MS detection. Another stage of purpose is to modify the lipid detection method for real subcellular membrane analysis.

３．研究の方法 (Method of the research)

Hydrophobic material is coated on the inner surface of needle for the specific adsorption of lipid species. Electric conductive Pt layer is coated on the outer surface of the needle to equip the needle with conductive function. At the first stage of research, dioleoyl phosphatidylcholine (DOPC) is selected as a standard lipid sample for SPME demonstration. Here the protocol for SPME purification of DOPC was developed. At the second stage of research, we develop protocol for real Neuro2a subcellular membrane analysis.

４．研究成果 (Achievement of the research)

1. Hydrophobic modification of glass material

Hexadecyltrimethoxysilane was selected as the hydrophobic coating material (Fig. 1 (a)). A cover glass was coated with hexadecyltrimethoxysilane by evaporation of the coating material in vacuum environment. Water contact angle test resulted with contact angle of 57° for bare glass and 105° for coated glass (Fig. 1 (b)). This indicates that the hydrophobicity increased after the surface modification of glass with hexadecyltrimethoxysilane.

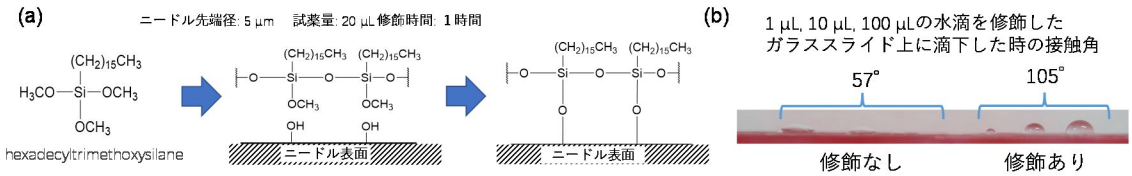

Figure 1. (a) coating process for hydrophobic surface modification. (b) contact angle test with water drop on noncoated glass (left) and coated glass.

2. SPME demonstration

To illustrate the SPME process, we employed DOPC as a standard lipid sample and maltotriose (G3) as a marker of elution solution. The 100 nM DPOC sample solution was prepared by dissolving DPOC into phosphate-buffered saline (PBS) to mimic cell culture environment (Fig. 2). The organic elution solution was prepared by dissolving 200 nM G3 into a solution containing 75% methanol, 10% dimethyl sulfoxide and 10 μM acetic acid.

For SPME, the sample solution was aspirated into the needle from the tip end and incubate for 5 min. Then, the elution solution was injected into the needle from the rear end. The MS detection was conducted with Orbitrap Exploris 120 from Thermo Fisher Scientific.

Fig. 2 illustrates the result of a typical MS analysis of the output from a needle. Three stages are found on the time scale: ① No signal related to DOPC nor G3 is found. This stage is supposed to be the washing process when only aqueous solvent in the sample solution was detected. ② Only G3 were detected. This stage is supposed to be the beginning of the elution process when the organic solvent was at a low concentration. ③ Both DOPC and G3 were detected. This stage is supposed to be the elution of DOPC molecules from the inner surface of the needle. This result indicates that the SPME was successful with our developed needle device.

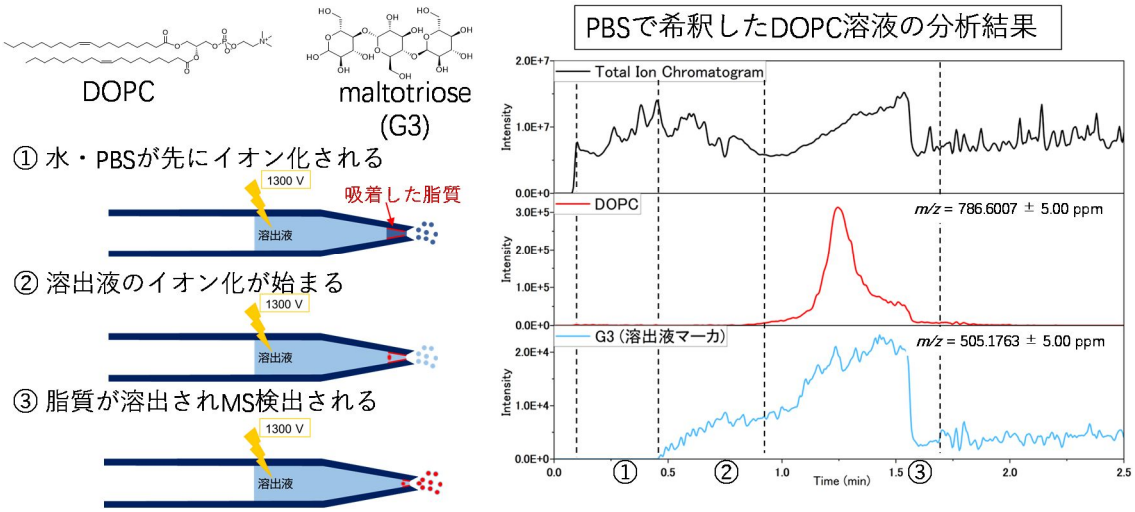

Figure 2. The illustration of SPME scheme and the MS analysis result corresponding to each step of SPME process.

3. Subcellular analysis

The above mentioned SPME method was adapted to the subcellular analysis of Neuro2a cell species. As is shown in Fig. 3 (a) and (c), a bi-needle system was employed for subcellular membrane collection. Here one needle was employed for fixing the cell and the other one was employed for collection subcellular membrane. We successfully collected the neurite part and main cell body part from single cells. The MS analysis results are shown in Fig. 3 (c) and (d). Typical 4 types of lipids are found in both subcellular sections along with other lipid species. The peak area ratio indicates that a heterogeneity distribution of lipid in these subcellular sections.

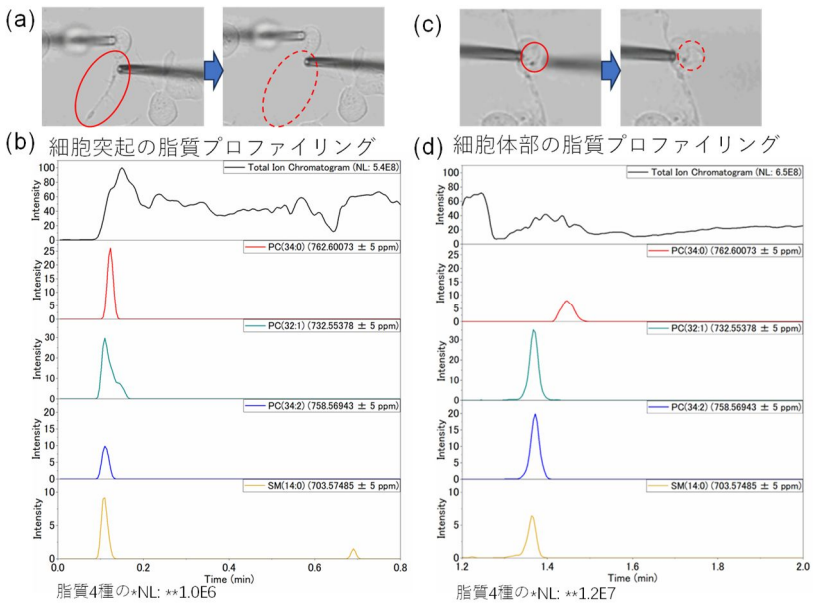

Figure 3. (a) and (b) image of neurite part collection and the MS analysis result. (c) and (d) image of main cell body membrane collection and the MS analysis resutl.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　1件）

１．著者名 Chenchen Liu, Koji Otsuka, Takayuki Kawai	４．巻 0
２．論文標題 Recent advances in microscale separation techniques for glycome analysis	５．発行年 2024年
３．雑誌名 Journal of separation science	６．最初と最後の頁 0
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/jssc.202400170	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　2件）

１．発表者名 Chenchen Liu, Yamaguchi Yoshinori, Kawai Takayuki, Kubo Takuya, Otsuka Koji
２．発表標題 Online Fluorescent Imaging Method for Improving the Accuracy of Quantitative Capillary Electrophoresis
３．学会等名 74th Academic Conference of the Japan Society of Electrophoresis
４．発表年 2023年

１．発表者名 Chenchen Liu, Yoshinori Yamaguchi, Takayuki Kawai, Takuya Kubo, Koji Otsuka
２．発表標題 Online Fluorescent Imaging Method for Accuracy Improvement of Quantitative Capillary Electrophoresis
３．学会等名 51st International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC2023)（国際学会）
４．発表年 2023年

１．発表者名 Chenchen Liu
２．発表標題 New studies on poly(ethylene glycol)-based hydrogels in electrophoresis
３．学会等名 APCE & CECE & ITP & IUPAC 2022（国際学会）
４．発表年 2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関