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RESEARCH PAPER

Metal/Matrix Enhanced Time-of-flight Secondary Ion Mass Spectrometry for Single Cell Lipids Analysis

LI Hao-Wen, HUA Xin*, LONG Yi-Tao

Key Laboratory for Advanced Materials, School of Chemistry & Molecular Engineering, East China University of Science and Technology, Shanghai, 200237, China

Abstract: The chemical components analysis of single cell is important for the understanding of physiological processes such as cell growth, signal transduction and apoptosis. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a sensitive surface analysis technique with high spatial resolution, which has been used for single cell and micro-area analysis. However, relatively low ionization yield of biomolecules limited its wide applications in single cell analysis. Herein, we used metal substrate and matrix material to enhance the ionization yield of lipids. The signal intensity of phosphatidylcholine (PC 40:0) casted on the matrix/gold-coated silicon substrate was 65 times higher than that on the silicon wafer. The signal enhancement of phosphatidylcholine (PC 34:1) on single cell surface cultured on matrix/gold-coated silicon substrate was observed as well. Owing to the influence of irregular topography and complex chemical environment of cell, the increase of lipids signal was smaller. Delayed extraction mode of ToF-SIMS overcame the effects of cell topography, leading to further enhancement of the signal intensity of lipids. Meanwhile, simultaneous high spatial resolution of chemical imaging and high mass resolution of the mass spectra of single cells were obtained. Our strategies provided new insights into the study of cell metabolism and cell-environment interactions.

Key Words: Single cell analysis; Lipids; Time-of-flight secondary ion mass spectrometry; Noble metal/matrix enhancement; Mass spectrometry imaging

1 Introduction

Single cells show heterogeneities in various aspects such as structure, chemical composition and metabolism. These heterogeneities may affect functions of tissues and organs. Conventional ensemble analysis techniques could not capture these individual differences, leading to difficulties in accurately evaluating and predicting physiological behavior of cells. Thus, single cell analysis is attracting more and more attentions[1]. An important task for single cell analysis is the analysis of lipids, since lipids are largely involved in cell metabolism. Under the stimulation of external environment, the metabolism pathway of cells may change, resulting in composition changes of lipids. For example, after affected by hepatitis C virus, the metabolism of intracellular cholesterol changed, leading to an increase of desmosterol[2,3]. Another report demonstrated that the change of microenvironment induced increase of fatty acids and phosphatidylinositol in human breast tissue[4]. In addition, a significant decrease of cholesterol in rat neuronal tissue was observed after feeding the rats with specially processed cereals[5]. These results showed the close relationship between lipids and cell metabolism. Therefore, single cell lipid analysis is of significant importance for better understanding of cell metabolism processes. Owing to ultra-small size as well as extremely low volume of analytes in a single cell, high sensitivity and high spatial resolution are required for single cell analysis techniques. Thus, single cell analysis is challenging[6]. Single cell imaging is one of the most important aspects in single cell analysis. Current single cell

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*Corresponding author. E-mail: xinhua@ecust.edu.cn
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imaging techniques include optical microscopy[7,8], electron microscopy (EM)[9], scanning probe microscopy (SPM)[10] and mass spectrometry imaging (MSI)[11,12], etc. These techniques provide rich information of single cells regarding the structure, morphology and chemical composition of cells[13,14]. MSI is a label-free chemical mapping technique for simultaneous analysis of multiple components[15,16]. In recent decades, we have witnessed the development of MSI techniques such as matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF-MS)[17], desorption electrospray ionization mass spectrometry (DESI-MS)[18] and secondary ion mass spectrometry (SIMS)[19]. MALDI-ToF-MS and DESI-MS are typically used for the imaging of tissue samples. However, single cell imaging by these techniques was limited due to poor lateral resolution. So far, a best lateral resolution of 5 μm was reported for MALDI-ToF-SIMS[20]. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) with a lateral resolution over 100 nm is suitable for single cell imaging. In addition, high mass resolution was obtained by using a ToF analyzer. Recently, ToF-SIMS has been applied in various applications in single cell analysis[21–23]. However, relatively low ionization yield of biomolecules has limited its further applications in unraveling more complex biological processes. To improve the ionization yield of ToF-SIMS, efforts have been devoted into the development of new ion sources[24], post-ionization techniques[25] and matrix enhancement methods[26]. In this work, 2,4-dihydroxybenzoic acid (DHB) and noble metal substrate were used to enhance the ionization yield of lipids in single cell ToF-SIMS analysis. By further optimization of instrumental conditions, high quality single cell imaging results were obtained.

2 Experimental

2.1 Instruments and reagents

Electron beam evaporation system (Denton Vacuum, LLC, Moorestown, USA) was used to coat metal layers on silicon wafers. Cell density was counted using a Petroff-Hausser cell counter (Hausser Scientific Co., Ltd, Horsham, USA). Cell samples were frozen-dried using a vacuum type freeze drier (Four-Ring science instrument plant Beijing Co., Ltd, Beijing, China). ToF-SIMS measurements were conducted on a ToF-SIMS V spectrometer (IONTOF GmbH, Münster, Germany).

Ammonium acetate (CH_3COONH_4), acetone (CH_3COCH_3), ethanol (CH_3CH_2OH), hydrogen peroxide (H_2O_2) and sulfuric acid (H_2SO_4) were purchased from Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). MCF-7 cells were purchased from KeyGen Biotech. Co., Ltd. (Nanjing, China). 2,4-dihydroxybenzoic acid ($C_7H_6O_4$, DHB) and 10% fetal bovine serum (FBS) were purchased from Sigma-Aldrich. Co., Ltd. (Shanghai, China). All other reagents were of analytical grade. All aqueous solutions were prepared using ultrapure water (≥ 18 MΩ cm, Milli-Q, Millipore). Silicon wafers (1 cm × 1 cm) were purchased from Beijing Zhongjingkeyi Technology Co., Ltd (Beijing, China).

2.2 Experiment methods

2.2.1 Preparation of substrates

The silicon wafers were successively sonicated in acetone, ethanol and pure water for at least 30 min. These wafers were then dried under a flow of nitrogen. A 3-nm thick titanium layer and a 30-nm gold layer were successively coated onto the silicon wafers using a Denton electron beam evaporation system. These wafers were subsequently washed with fresh piranha solution (sulfuric acid-30% hydrogen peroxide, 3:1, *V/V*) for 30 s to remove the organic residues on the surface. The substrates were then washed with pure water and dried under a flow of nitrogen. Phosphatidylcholine (PC 40:0) was dissolved in decane to obtain a 2 mM solution and was further diluted to 50 μM by water.

2.2.2 Cell Culture

MCF-7 cells were seeded on the sterilized gold-coated silicon wafers or uncoated silicon wafers. The cells were cultured in RPMI-1640 medium (GIBCO) supplemented with 10% fetal bovine serum (FBS, Sigma), streptomycin (100 μg mL^{-1}), and penicillin (100 μg mL^{-1}) in a humid atmosphere with 5% CO_2 at 37 °C for 24 h. Cell density was calculated by a Petroff-Hausser cell counter.

2.2.3 Sample preparation

The wafers with cultured cells were removed from the medium, followed by washing with 150 mM ammonium acetate (pH 7.4) for 30 s to remove residual salts on surface. A Kimwipe paper was used to absorb most of the remaining liquid by touching the edge of wafers. 10 μL DHB solution (1 mg mL^{-1}) was added on the cell surface before subsequent fast frozen with –196°C liquid nitrogen. The frozen samples were then dried in a vacuum-type freeze drier.

Approximately 2 μL PC (40:0) solution was cast on the surface of silicon wafers and gold-coated silicon wafers respectively. 0.5 μL DHB solution (1 mg mL^{-1}) was added in the PC (40:0) solution before dropped on the gold-coated silicon wafers to prepare the metal/matrix enhanced samples. All samples were dried under a nitrogen flow.

2.2.4 ToF-SIMS instrument parameters

Positive ion mass spectra and images were recorded using a ToF-SIMS V spectrometer equipped with a 30 keV Bi_3^+

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