Chinese Chemical Letters 36 (2025) 110183

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Efficient serum lipids profiling by TiO_2-dopamin-assisted MALDI-TOF MS for breast cancer detection

Xinyue Han[a,1], Yunhan Yang[a,1], Jiayin Lu[a], Yuxiang Lin[b], Dongxue Zhang[a,*], Ling Lin[a,*], Liang Qiao[a,*]

[a] Department of Chemistry, Zhongshan Hospital, and Institutes of Biomedical Sciences, Fudan University, Shanghai 200000, China
[b] Department of Breast Surgery, Affiliated Union Hospital of Fujian Medical University, Fuzhou 350001, China

ARTICLE INFO

Article history:
Received 14 November 2023
Revised 10 June 2024
Accepted 27 June 2024
Available online 28 June 2024

Keywords:
Lipid
MALDI-TOF MS
Breast cancer
Machine learning
TiO_2

ABSTRACT

Lipids serve as fundamental constituents of cell membranes and organelles. Recent studies have highlighted the significance of lipids as biomarkers in the diagnosis of breast cancer. Although liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is widely employed for lipid analysis in complex samples, it suffers from limitations such as complexity and time-consuming procedures. In this study, we have developed dopamine-modified TiO_2 nanoparticles (TiO_2-DA) and applied the materials to assist the analysis of lipids by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The TiO_2-DA can provide large specific surface area and acidic environment, well suited for lipid analysis. The method was initially validated using standard lipid molecules. Good sensitivity, reproducibility and quantification performance was observed. Then, the method was applied to the analysis of 90 serum samples from 30 patients with breast cancer, 30 patients with benign breast disease and 30 healthy controls. Five lipid molecules were identified as potential biomarkers for breast cancer. We constructed a classification model based on the MALDI-TOF MS signal of the 5 lipid molecules, and achieved high sensitivity, specificity and accuracy for the differentiation of breast cancer from benign breast disease and healthy control. We further collected another 60 serum samples from 20 healthy controls, 20 patients with benign breast disease and 20 patients with breast cancer for MALDI-TOF MS analysis to verify the accuracy of the classification model. This advancement holds great promise for the development of diagnostic models for other lipid metabolism-related diseases.

Breast cancer is the most common cancer threatening women's health worldwide. In East Asian, breast cancer accounts for 43.3% of all female cancer cases [1]. Malignant breast cancer is highly invasive and easy to metastasize to lung, liver and other organs, which makes tumor treatment and prognosis difficult, resulting in low overall survival rate of patients [2,3]. Effective clinical treatment strategies for breast cancer include early surgery and chemotherapy, for which accurate early diagnosis is the key to improve survival rate of breast cancer patients [4,5]. To date, the typical approach for diagnosing breast cancer involves a combination of imaging techniques and tissue biopsies. However, this approach might result in delayed tumor detection or even contribute to the potential spread of the tumor. Liquid biopsy based on tumor biomarkers is an emerging technology, which has the advantages of non- or litter invasive, simple operation, low cost and high analytical throughput [6]. Proteins, metabolites and lipids presenting in biofluids have attracted wide interests as potential biomarkers for breast cancer diagnosis in recent years.

Lipids, serving as crucial biomolecules, play dual roles. They serve as primary constituents within cell membranes and organelles, while also creating enduring non-covalent complexes to store energy and engage in protein synthesis [7,8]. Lipidomics was firstly proposed as a branch of metabolomics in 2003, aiming to conduct comprehensive qualitative and quantitative analysis of lipids in organisms, tissues and cells [9]. Through this, the goal is to uncover the connections linking lipid metabolism disruptions and various biological processes [10]. Impairments in lipid metabolism play a pivotal role in the pathogenesis of several conditions, including cancer, cardiovascular disease, and neurodegenerative disorders [11-13]. Recent studies underscore the significance of lipids as potential biomarkers for the precise clini-

* Corresponding authors.
E-mail addresses: dongxuezhang@fudan.edu.cn (D. Zhang), linglin@fudan.edu.cn (L. Lin), liang_qiao@fudan.edu.cn (L. Qiao).
[1] These authors contributed equally to this work.

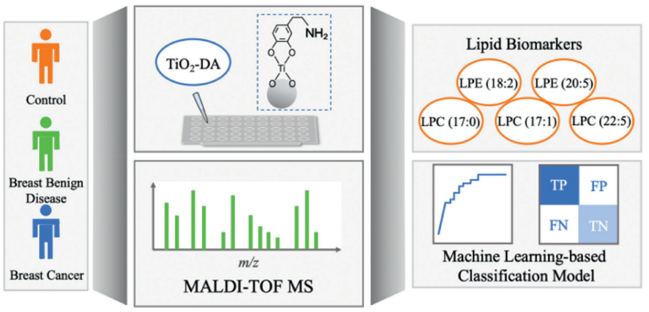
https://doi.org/10.1016/j.cclet.2024.110183
1001-8417/

cal assessment of diseases such as liver cancer, breast cancer, and prostate cancer [14-16]. Analyzing and quantifying lipid profiles in body fluids like blood and urine hold promise for enabling early cancer detection and monitoring the progression and prognosis of tumors. By using lipidomic analysis, Chen *et al.* discovered 15 lipid species, including 4 lyso-phosphatidylcholine (LPC), 6 phosphatidylethanolamine (PC), 2 ether-linked phosphatidylcholine (ePC), and 3 cholesterol ester (CE), that could be used as plasma biomarkers in distinguishing early-stage breast cancer [17]. Eghlimi *et al.* analyzed 166 human plasma samples including 45 healthy controls, 96 non-triple negative breast cancer (TNBC) and 25 TNBC [15]. A panel of 19 lipid biomarkers was capable of distinguishing TNBC from controls. In addition, a panel of 5 lipid biomarkers, including diglyceride (DG) 34:2, PC 40:3, PC 39:8, PC 34:0 and PC 38:9 was capable of distinguishing TNBC from non-TNBC.

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) stands as a primary tool for analyzing lipids within complex samples. The technique facilitates the differentiation of lipids possessing similar molecular weights and provides insights into their characteristics *via* accurate molecular weight determination and fragmentation pattern profiling [18,19]. Nevertheless, it is important to note that this approach is limited by its complexity, low throughput, and time-consuming nature [20]. Over the recent years, there has been a growing fascination with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) within the realm of lipid analysis [21]. Compared to LC-MS/MS, MALDI-TOF MS offers distinct advantages, including high throughput, robustness, and user-friendly operation [22]. In its initial stages, MALDI-TOF MS primarily found utility in the study of proteins and other macromolecules. However, when employing organic matrices such as α-cyano-4-hydroxycinnamic acid (CHCA), 2,5-dihydroxybenzoic acid (DHB) and sinapic acid, the conventional MALDI-TOF MS often exhibited prominent matrix-derived peaks in the low mass range (*i.e.*, $m/z < 1000$). This phenomenon considerably hindered the accurate detection of small molecules, notably lipids. Furthermore, the effectiveness of ionizing lipids using organic matrices was suboptimal.

Recent studies have introduced a diverse array of inorganic nanomaterials to enhance the MALDI-TOF MS analysis of small molecules [23-26]. Titanium dioxide nanoparticles have garnered considerable interest in lipid detection due to their unique properties of high UV absorbance, photocatalytic properties, chemical stability, non-toxicity, and enhanced ionization efficiency, *etc*. TiO_2 nanoparticles have been widely used as the matrix of MALDI-TOF MS for lipid determination. A noteworthy example is the utilization of dopamine-modified TiO_2 monolith-assisted LDI MS, which has demonstrated successful application in visualizing small metabolites and lipids within mouse brain tissue through mass spectrometry imaging [27]. Similarly, Chen *et al.* applied plasmonic polydopamine-modified TiO_2 nanotube coated with plasmonic gold nanoparticle to assist MALDI-TOF MS to map spatial distribution of lipids within mouse brain tissue sections [28]. However, a pressing exigency persists for pioneering techniques characterized by stable performance and superior efficiency in lipid ionization. These advancements are pivotal in facilitating the quantitative profiling of lipids *via* MALDI-TOF MS and extending their potential application within the realm of clinical diagnostics.

Herein, we introduce a novel approach involving the modification of titanium dioxide (TiO_2) nanoparticles with dopamine (DA) to enhance lipid analysis using MALDI-TOF MS. The efficacy of the TiO_2-DA-MALDI-TOF MS methodology in lipid analysis was initially established through the evaluation of a mixture comprising seven distinct lipid molecules. Subsequently, this technique was extended to scrutinize serum samples sourced from 30 patients with breast cancer, 30 individuals with benign breast disease, and 30 healthy subjects (Table S1 in Supporting information). Based on



Scheme 1. Schematic illustration of the profiling of serum lipids by TiO_2-DA-MALDI-TOF MS for the identification of lipid biomarkers for breast cancer diagnosis.

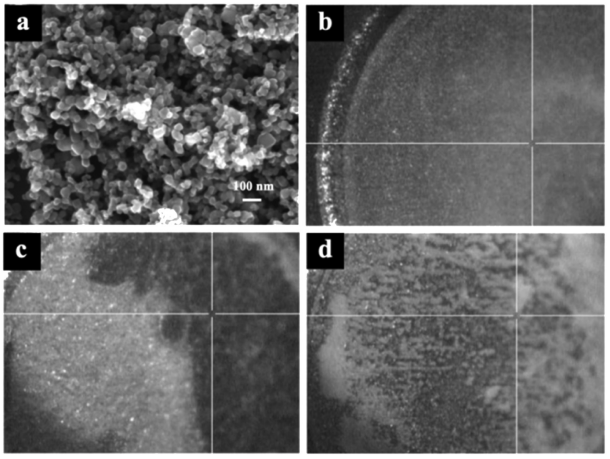


Fig. 1. (a) SEM image of TiO_2-DA with the scale bar of 100 nm. Comparison of crystallization on target plates of (b) TiO_2-DA, (c) 9-AA and (d) CHCA.

the MALDI-TOF MS profiling of lipids extracted from the serum samples, the three groups can be clearly distinguished. To bolster these findings, a parallel application of LC-MS/MS was employed, leading to the identification of five lipid molecules - namely lyso-phosphatidylethanolamine (LPE) (18:2), LPC (17:1), LPC (17:0), LPE (20:5), and LPC (22:4) - that exhibit strong potential as diagnostic biomarkers for breast cancer (Scheme 1).

TiO_2 nanoparticles exhibit exceptional stability in both physical and chemical attributes under laser irradiation, making them a popular choice as inorganic matrices in the MALDI-TOF MS analysis of compounds with modest molecular weights [29,30]. Additionally, TiO_2 nanoparticles have demonstrated efficacy in aiding the detection of diverse molecules, including steroid hormones, peptides, and even proteins [31,32]. However, the potent catalytic hydrolysis tendencies of TiO_2 toward endogenous lipids have been noted, leading to lipid degradation during MALDI-TOF MS analysis [30,33]. The enhancement of TiO_2's attributes for precise lipid characterization within biological systems remains a pressing requirement.

To address this, we optimized the properties of TiO_2 through surface modification. DA has the characteristics of easy oxidative polymerization and robust ultraviolet absorbance. Incorporating DA onto the surface of TiO_2 has been shown to amplify the material's ultraviolet laser absorption and heighten ionization efficiency during MALDI-TOF MS analysis [27]. In light of this, we introduced a small quantity of DA into an ethanol solution of TiO_2 under acidic conditions. This strategy facilitated the surface modification of TiO_2 by fostering covalent interactions between DA and the abundant hydroxyl groups on TiO_2. The electronic microscopy image of the synthesized TiO_2-DA is shown in Fig. 1a. Notably, the polymerization of DA engendered interparticle grafting, endowing the matrix

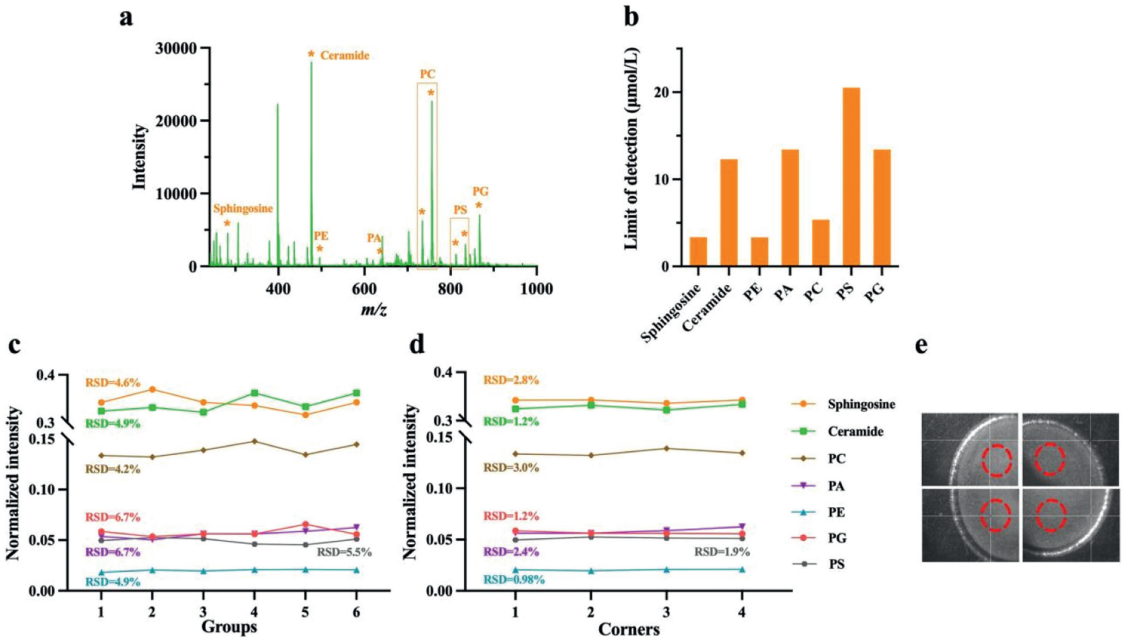


Fig. 2. (a) MALDI-TOF MS spectrum of the seven lipid molecules using TiO_2-DA+CHCA matrix. (b) Limit of detection of the seven lipid molecules by MALDI-TOF MS. (c) Normalized mass spectrum signal intensity of the seven lipid molecules from six sample spots. (d) Normalized mass spectrum signal intensity of the seven lipid molecules at different detection points shown in (e).

with enhanced roughness that bolsters the adsorption capacity of TiO_2-DA for lipids. Simultaneously, the acidic environment inhibits lipid degradation. Upon drying and deposition onto a MALDI target plate, TiO_2-DA exhibited uniform and stable distribution (Fig. 1b). This attribute exhibits the potential to enhance the reproducibility and precision of quantification in MALDI-TOF MS analysis. To contrast, we selected 9-AA and CHCA, both common organic matrices, for matrix crystallization, revealing nonuniformity in organic matrix crystallization (Figs. 1c and d). This nonuniform crystallization can pose challenges to accurate sample quantification.

To ascertain the capability of TiO_2-DA as a matrix for aiding the analysis of lipids through MALDI-TOF MS, a set of seven lipid molecules was selected for evaluation: 18:0–18:1 phosphatidylserine (PS), 18:0–22:6 phosphatidylglycerol (PG), 16:0–18:1 PC, 14:0 phosphatidic acid (PA), 14:0 phosphatidylethanolamine (PE), C10 ceramide (d18:1/10:0), and sphingosine (d18:1) (Table S2 in Supporting information). Among these, PS, PG, PC, PA, and PE are glycerophospholipids, the metabolism of which has been closely linked to the development of various cancers [34,35]. Ceramide and sphingosine fall under the category of sphingolipids, imbalances in the metabolism of which are often associated with inflammation and cancer [36,37]. Notably, sphingolipid-based delivery system has garnered significant attention as potential therapeutic strategy for cancer and inflammatory diseases [38]. In our study, the selection of these seven lipid molecules as the test samples aimed to underscore the potential of MALDI-TOF MS analysis of lipids in the context of breast cancer diagnosis. With a focus on the glycerophospholipids and sphingolipids, the MALDI-TOF MS mass range was set up to *m/z* 1000.

We compared CHCA and two TiO_2-based inorganic nanomaterials, unmodified P25 TiO_2 and TiO_2-DA, for their efficacy in analyzing the seven selected lipid molecules (Table S3 in Supporting information). In the positive ion mode, unmodified P25 TiO_2 exhibited the capacity to detect only three lipid molecules, while both TiO_2-DA and CHCA demonstrated the capability to detect six out of the seven lipid molecules. Combining TiO_2-DA and CHCA facilitated the effective detection of all seven lipid molecules. Given the varied performance of these matrices in terms of lipid desorption and ionization, the ultimate choice of the optimal matrix for MALDI-TOF MS analysis of lipids was TiO_2-DA ethanol solution doped with CHCA.

As depicted in Fig. 2a, distinct signal peaks were observed for all seven lipid molecules. Notably, sphingosine (d18:1) exhibited a hydroxyl group deficiency, 14:0 PE exhibited a loss of the head group, and 16:0–18:1 PC and 18:0–18:1 PS presented peaks with both H^+ and Na^+ ions. It is important to highlight that the sensitivity of MALDI-TOF MS analysis varied among different lipid species. While PC has been recognized as among the most easily detectable lipids using MALDI-TOF MS [39], phospholipids containing quaternary ammonium groups could potentially inhibit the detection of other lipids [7]. Consequently, an optimization of the relative concentrations of the seven lipid molecules in the mixture was performed, ensuring their successful detection *via* MALDI-TOF MS analysis. Specifically, the mixture contained concentrations of 200 μmol/L of 18:0–18:1 PS, 200 μmol/L of 18:0–22:6 PG, 200 μmol/L of 16:0–18:1 PC, 200 μmol/L of 14:0 PA, 200 μmol/L of 14:0 PE, 100 μmol/L of C10 ceramide (d18:1/10:0), and 100 μmol/L of sphingosine (d18:1).

Gradient dilution was employed on the seven lipid molecules to evaluate the detection limits of this strategy. The findings unequivocally established detection limits lower than 20 μmol/L for all seven lipid molecules, with PC, ceramide, and sphingosine showcasing even more impressive detection limits below 5 μmol/L (Fig. 2b). Particularly noteworthy was the heightened sensitivity observed in the detection of sphingosine and phospholipids containing ammonium groups by MALDI-TOF MS. This heightened sensitivity renders the strategy adept at fulfilling the demands of serum lipid analysis, especially considering that total lipid content in human serum can reach 5 mmol/L. Consequently, the MALDI-TOF MS analysis approach underpinned by TiO_2-DA can be feasibly expanded to encompass human serum lipid analysis, thereby offering a theoretical framework for comprehending lipid metabolism in pertinent diseases.

To ascertain the signal's reproducibility in the context of TiO_2-DA-MALDI-TOF MS analysis, six distinct sample spots were chosen from a single MALDI target plate for analyzing the mixture of the

seven lipid molecules. Normalization was performed by summing the total peak intensity of the seven lipid molecules. The results, presented in Fig. 2c, exhibited a high degree of consistency among different sample spots, characterized by a relative standard deviation (RSD) of normalized intensity that remained under 8%. Furthermore, the normalized signal intensity across different regions within a given sample spot also exhibited noteworthy consistency, as evidenced by RSD values under 3% (Figs. 2d and e). Collectively, these results underscore the aptitude of the TiO_2-DA matrix to co-crystallize uniformly with lipids, engendering a high level of analysis reproducibility. This in turn serves as the bedrock for conducting relative quantitative analysis.

Quantification performance of the TiO_2-DA-MALDI-TOF MS in lipid analysis was further explored. Given the distinct performance characteristics of MALDI-TOF MS when analyzing different lipid classes, we conducted relative quantitative analysis using a subset of six molecules representing three lipid classes: PC, PG, and sphingosine. Specific lipid species, including 18:0–22:6 PG, 16:0–18:1 PC, and sphingosine (d18:1) were employed as internal standards at a final concentration of 200 μmol/L. Notably, 16:0–18:1 PG, 14:0–18:0 PC, and sphingosine (d18:0) were chosen as analytes and were tested across a concentration range spanning 40–400 μmol/L. Each set of lipids within the same class was combined and subjected to MALDI-TOF MS analysis. As shown in Fig. S1 (Supporting information), a strong linear correlation emerged between the relative signal intensity and the corresponding relative concentration of the lipids within the same lipid class. This correlation reinforces the feasibility of executing relative quantification *via* internal standard calibration utilizing standard lipids from the identical lipid class.

With the excellent performance in the analysis of standard lipid samples, the TiO_2-DA-MALDI-TOF MS was further applied to analyze lipids extracted from human serum. We introduced a set of seven lipid molecules, 18:0–18:1PS, 18:0–22:6PG, 16:0–18:1 PC, 14:0 PA, 14:0 PE, C10 ceramide (d18:1/10:0), and sphingosine (d18:1), as internal standards (IS) to facilitate relative quantitative calibration. These standards were incorporated into the serum samples. Our study encompassed firstly a collection of 90 serum samples, including 30 from individuals diagnosed with breast cancer, 30 from individuals with benign breast disease, and 30 from healthy control subjects.

In Fig. S2 (Supporting information), the MALDI-TOF mass spectra ($n = 3$) of a serum sample extracted from a healthy individual were observed, exhibiting distinctive signals corresponding to the seven internal standard lipid molecules. A total of 51 features were extracted from the MALDI-TOF mass spectra across all samples. Due to the challenge of identifying the specific molecules corresponding to the peaks, we opted for 16:0–18:1 PC, a stable and consistently detectable lipid, as our reference internal standard. Normalization was firstly performed against the H^+ ion peak of the internal standard (m/z 761), and subsequently against the total peak intensity while eliminating the contributions from matrix-related peaks and the seven added lipid molecules. These matrix-related and added lipid peaks were also excluded from the subsequent machine learning analysis.

The normalized MALDI-TOF MS features underwent rigorous statistical analysis. As illustrated in Fig. S3a (Supporting information), the healthy control group (Ctl, in pink), benign breast disease group (BD, in green), and breast cancer group (BC, in blue) demonstrated discernible separation through PCA, suggesting notable variations in lipid profiles among the three groups. Employing supervised discriminant analysis techniques, including partial least squares-discriminant analysis (PLS-DA), sparse partial least squares-discriminant analysis (sPLS-DA) and orthogonal partial least squares-discriminant analysis (OPLS-DA), we achieved effective separation among the groups, as depicted in Figs. S3b-f (Supporting information). OPLS-DA, which is tailored for binary group comparison, displayed distinctive separation for each pair of groups. Given the relatively larger number of samples compared to features in our study, the risk of overfitting associated with the supervised discriminant analysis methods was mitigated.

Table 1
Five lipid biomarkers of breast cancer identified by MALDI-TOF MS and LC-MS/MS.

LC-MS/MS	MALDI-TOF MS	Lipid
478.288 $[M+H]^+$	478.2 $[M+H]^+$	LPE (18:2)
508.339 $[M+H]^+$	508.2 $[M+H]^+$	LPC (17:1)
510.346 $[M+H]^+$	532.7 $[M+Na]^+$	LPC (17:0)
522.260 $[M+Na]^+$	522.2 $[M+Na]^+$	LPE (20:5)
572.366 $[M+H]^+$	594.0 $[M+Na]^+$	LPC (22:4)

Differential features were pinpointed using volcano plot analysis with t-test P-value < 0.05 and fold change (Fc) > 1.5 or Fc < 0.67 as the criteria, highlighting distinctions between each two groups (Fig. S4 in Supporting information). Between the healthy control group and the breast cancer group, one feature exhibited significant upregulation, while five features showed substantial downregulation. In the comparison between the healthy control group and the benign breast disease group, three features demonstrated significant downregulation. For the benign breast disease group and the breast cancer group, one feature displayed significant upregulation, and two features exhibited noteworthy downregulation (Table S4 in Supporting information). In total, 7 distinctive features were identified after eliminating redundancy. Additionally, when focusing solely on the t-test outcomes, 11 significant features with P-values < 0.05 were observed across any two groups among the three (Table S5 in Supporting information).

To delve deeper into the biological implications of these differential features, LC-MS/MS was employed to characterize lipids within serum samples from a healthy individual (Table S6 in Supporting information). The MALDI-TOF MS spectra of the lipid sample from the healthy subject encompassed all the 11 significant features. Through a comparative analysis of identification results by LC-MS/MS and the m/z values of the 11 significant features obtained from MALDI-TOF MS, a total of 5 prospective lipid biomarkers for breast cancer were discerned with a mass tolerance of 1000 ppm (Table 1). Notably, all the five identified lipids were lyso-subtype of glycerophospholipids. Lyso-phospholipids are generated by removing a fatty acid chain from an intact glycerophospholipid molecule. Therefore, they usually consist of a glycerol backbone, a phosphate group, a polar head group, and a single fatty acid chain. This structure makes lyso-phospholipids more hydrophilic than non-lyso phospholipids. Lyso-phospholipids, as signaling molecules, influence various cellular processes such as cell proliferation, differentiation, and migration [40]. Some lysophospholipids, such as lyso-phosphatidic acid (LPA) and lysophosphatidylcholine (LPC), are known to play a role in the regulation of inflammatory responses during tumor progression [41,42]. In the setting of pancreatic ductal adenocarcinoma (PDAC), pancreatic stellate cells undergo trans-differentiation into activated cancer-associated fibroblasts, which secrete LPCs into the microenvironment. PDAC cells then use these LPCs to facilitate the synthesis of their cell membranes. Moreover, these cancer cells upregulate the expression of autotaxin, an enzyme responsible for converting LPC into LPA. This ensuing LPA, in a positive feedback loop, triggers AKT signaling and substantively bolsters the processes of cancer cell proliferation and migration [43].

We utilized 16:0–18:1 PC and 14:0 PE as internal standards, specifically corresponding to LPC and LPE, to execute the normalization process. Initial normalization of the lipid biomarker peak intensities was accomplished by the corresponding internal standards. Subsequently, normalization by the total peak intensity (excluding matrix-related and the seven added lipid molecules' peaks)

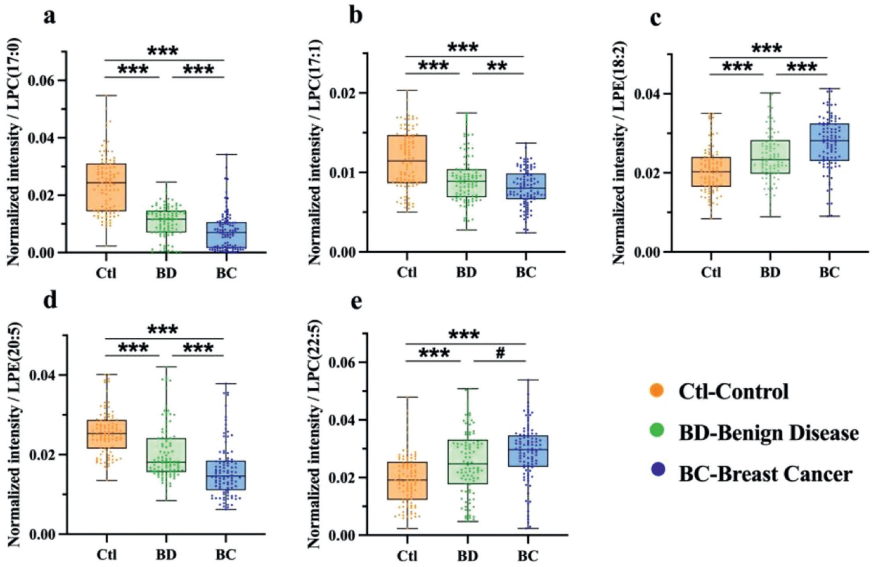


Fig. 3. Box plots of the relative quantities of (a) LPC (17:0), (b) LPC (17:1), (c) LPE (18:2), (d) LPE (20:5), and (e) LPC (22:4) in sera among healthy controls (Ctl), benign breast disease (BD) and breast cancer groups (BC). Error bars represent the standard deviation. "#", "**", and "***" indicate *P*-values less than 0.05, 0.01, and 0.001, respectively. The boxes mark the first and third quantile and the lines inside the boxes mark the median.

was conducted. The relative quantities of each lipid biomarker across the three different groups were depicted in Fig. 3. Notably, all five lysophospholipids exhibited significant variations in relative quantity between any two groups. Among these five biomarkers, LPC (17:0), LPC (17:1), and LPE (20:5) within the breast cancer and benign breast disease groups demonstrated downregulation in comparison to the healthy control group. Conversely, LPE (18:2) and LPC (22:4) in the breast cancer and benign breast disease groups displayed significant upregulation relative to the healthy control group.

While there is currently limited literature discussing these five lipid molecules as breast cancer biomarkers, numerous studies have highlighted the pivotal role of lipid metabolism reprogramming in cancer development [44,45]. For instance, LPE induced calcium signaling by activating phospholipase C and a membrane bound receptor, thereby triggering chemotactic migration and facilitating cellular invasion in ovarian cancer cells [46]. Kühn *et al.* proposed that alterations in blood lipid composition manifested several years prior to the diagnosis of malignancies. Elevated concentrations of LPCs, particularly LPC (18:0), exhibited a consistent association with reduced risks of breast, prostate, and colorectal cancers, regardless of underlying variables. In contrast, heightened levels of PC (30:0) were linked to an escalated cancer risk, demonstrated by regression analyses using data from a prospective case-cohort study involving 835 incident cancer cases [47]. Taware *et al.* conducted a comparative phospholipidomic analysis of invasive ductal carcinoma (IDC) of the breast, as well as benign and control breast tissue samples. Through the application of targeted LC-MS approach, their investigation unveiled a total of 11 phospholipids showcasing significant differential expression. These disparities were observed when assessing 33 IDC samples against an equal number of age-matched benign and control samples. Notably, among these phospholipids, LPI (20:3), PE (22:1/22:2), LPE (20:0), and PC (20:4/22:4) emerged as the most prominently associated with IDC tissue samples [48].

The effectiveness of the five potential lipid biomarkers in differentiating patients with breast cancer, patients with benign breast disease and healthy controls was further explored. We randomly selected two thirds of the breast cancer group, the benign breast disease group and the healthy control group as the training data, and set the remaining samples as the test data. Relative quantities of the five lipid molecules were used to construct classification models based on PLS-DA. As shown in Fig. 4a, receiver operating characteristic curve (ROC) analysis and cross-validation were performed on the training data of the 20 healthy controls and 20 breast cancer patients, and the results showed that the AUC value was 1, indicating that the five lipid biomarkers can efficiently distinguish breast cancer patients from healthy individuals. The model was then applied to the test data set composed of 10 healthy controls and 10 breast cancer patients (Fig. 4d). All samples were correctly classified, and the sensitivity, specificity and accuracy of this classification model for differentiating healthy individuals and breast cancer patients reached 100%. Further, we carried out ROC analysis and classification model construction for healthy control and benign breast disease, as well as benign breast disease and breast cancer, respectively. As shown in Figs. 4b and e, the AUC value of ROC analysis for healthy control group and benign breast disease group was 1, and the sensitivity, specificity and accuracy of the classification model in identifying patients with benign breast disease reached 100%. As shown in Figs. 4c and f, the AUC value of ROC analysis for patients with benign breast disease and breast cancer was 0.982, and the sensitivity, specificity and accuracy of the classification model to identify patients with breast cancer from benign breast disease were 90%, 100% and 95%. These results demonstrated that the classification model based on MALDI-TOF MS analysis of the five lipid biomarkers could be used to distinguish patients with breast cancer, patients with benign breast disease and healthy individuals with high accuracy.

We further collected another 20 serum samples from healthy controls, 20 serum samples from patients with benign breast disease and 20 serum samples from patients with breast cancer (Table S7 in Supporting information) for MALDI-TOF MS analysis. The 90 previously collected samples from the healthy control, benign breast disease and breast cancer groups were employed as the training data, and the 60 newly collected samples were employed as an independent test dataset. The relative quantities of the five identified lipid biomarkers, LPC (17:0), LPC (17:1), LPE (20:5), LPE (18:2) and LPC (22:4), were used to construct classification models based on PLS-DA. Cross-validation were performed on the training data, and the trained model was then applied to the independent

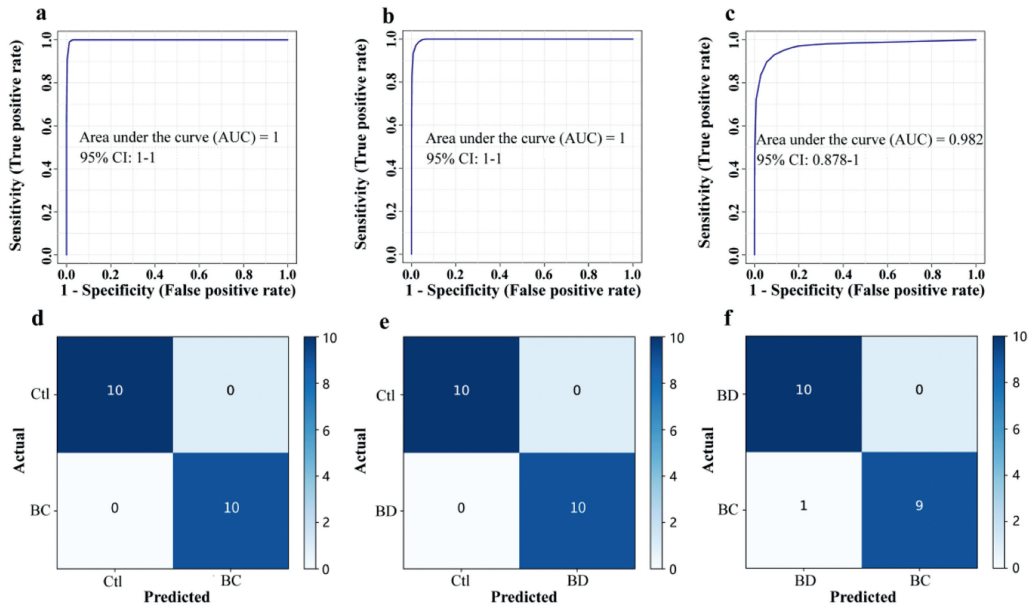


Fig. 4. ROC analysis with 10-fold cross-validation on the training data of (a) 20 healthy control samples (Ctl) and 20 patients with breast cancer (BC), (b) 20 Ctl and 20 patients with benign breast disease (BD), (c) 20 BD and 20 BC, using the classification model built by PLS-DA using only the five lipid biomarker features. Confusion matrix of the classification results on the test data of another (d) 10 Ctl and 10 BC, (e) 10 Ctl and 10 BD, (f) 10 BD and 10 BC.

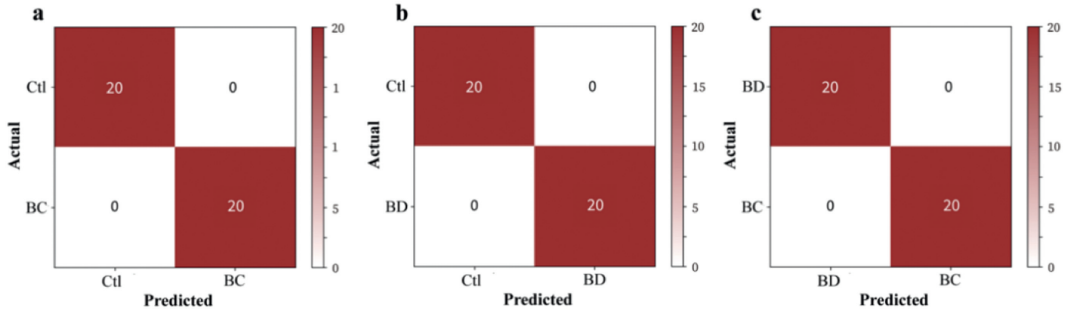


Fig. 5. Confusion matrix of the classification results on an independent test data of another (a) 20 healthy control samples (Ctl) and 20 patients with breast cancer (BC), (b) 20 Ctl and 20 patients with benign breast disease (BD), and (c) 20 BD and 20 BC. The classification model was built based on 90 samples of 30 Ctl, 30 BC and 30 BD with cross validation by PLS-DA using only the five lipid biomarker features.

test data set. As shown in Fig. 5, the sensitivity, specificity and accuracy of the classification models to identify each group of the mixed samples of healthy individuals, patients with benign breast disease and breast cancer reached 100%. The result demonstrated that the classification model based on the MALDI-TOF MS analysis of the 5 lysophospholipids molecules could achieve the diagnosis of breast cancer with high accuracy, demonstrating the potential of the 5 lipid biomarkers in the prediction of breast cancer disease

As lipidomics advances, it becomes increasingly evident that lipids occupy pivotal roles in regulating processes such as cell proliferation, apoptosis, and development. The orchestration of lipid metabolism profoundly impacts tumor progression, invasion, and metastasis, thus establishing lipids as valuable candidates for cancer diagnosis and prognosis [49]. Earlier investigations focusing on breast cancer tissue have identified overexpression of certain genes involved in lipid metabolism, underscoring the potential utility of phospholipids as diagnostic biomarkers for breast cancer. These insights also raise the prospect of leveraging metabolic regulation as a treatment strategy [50]. The systemic impact of lipid metabolic reprogramming on tumor cell behavior, growth, and communication highlights the complex crosstalk between tumor cells, cancer metabolism, gene expression, and the tumor immune microenvironment [51]. In has been reported that upregulation of lipid metabolism genes was discovered in breast prior to cancer diagnosis [52]. In our study, we identified five lipids closely associated with breast cancer. These lipids belong to the classes of PC and PE. Both PC and PE metabolism are integral to cancer cell biology, impacting oncogenic transformation, tumor progression, and cell survival [53]. These phospholipids are not only fundamental components of cell membranes but also play pivotal roles in signaling pathways that regulate cell proliferation, survival, and migration. Alterations in the levels of PC and PE, as well as in their biosynthetic pathways, have been associated with cancer progression and the development of resistance to therapy.

With a wider scope, the lipid profile present in serum is integral to a variety of physiological and pathological processes, including inflammation, metabolism, and other critical events. Sphingolipids, in particular, have been highlighted for their involvement in inflammatory processes and are recognized as potential signaling molecules that significantly contribute to cellular functions. The biosynthesis and catabolism of sphingolipids are integral to body functions, including their participation in signaling, impacting cell proliferation, death, migration, and invasiveness, and notably, in inflammation and central nervous system development. Their disruption or dysregulation can lead to various pathologies,

including inflammatory diseases, highlighting the importance of sphingolipid homeostasis [54]. In addition, lipids such as cholesterol and triglycerides play crucial roles in metabolic processes, impacting functions from cellular membrane composition to hormone synthesis and energy storage. Cholesterol as a fundamental constituent of cellular membranes, imparts structural integrity and fluidity to membranes, thereby impacting various cellular functions [55]. Dysregulation of cholesterol homeostasis is implicated in a myriad of metabolic disorders, including atherosclerosis, cardiovascular disease, and metabolic syndrome [56]. These highlights illustrate the complexity and diversity of the roles that serum lipids play in signaling pathways across various physiological and pathological contexts.

To date, most existing studies that aim to investigate metabolic pathways associated with breast cancer primarily rely on LC-MS techniques [57,58]. Our study establishes a novel method based on the TiO_2-DA matrix, expanding the application potential of MALDI-TOF MS for lipid quantification. Furthermore, it opens new possibilities for precise diagnoses across diverse cancer diseases by utilizing lipids as biomarkers. Notably, compared to LC-MS-based lipidomics, MALDI-TOF MS offers the distinct advantages of high throughput and cost-effectiveness. This renders the technique suitable for the initial screening of significant lipid features pertaining to a specific disease, with subsequent identification of these features attainable through LC-MS/MS methods.

In summary, we have pioneered the use of dopamine-modified TiO_2 nanoparticles in tandem with MALDI-TOF MS for the analysis of human serum lipids, and identified five lipid biomarkers for the detection of breast cancer. This innovative discovery has the potential of significantly improving the accuracy of identifying and classifying different subtypes of breast cancer, which is a critical factor in customizing patient treatment plans. We intend to further validate these lipid biomarkers through clinical trials and explore their full potential in personalized medicine for breast cancer patients. In addition, the development of this novel inorganic matrix extends the application of MALDI-TOF MS to the identification of complex lipid profiles in biofluids. Tt introduces a realm of new possibilities for diagnosing various diseases linked to lipid metabolism.

Ethical statement

All serum samples utilized in this study were obtained from Fujian Medical University Union Hospital, with the explicit consent of the subjects for the utilization of these biological materials exclusively for research purposes. The study protocol was approved by Ethics Committee of Fujian Medical University Union Hospital (Fujian, China) (No. 20200321), and complied with all relevant laws and regulations of China.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

CRediT authorship contribution statement

Xinyue Han: Investigation, Formal analysis, Data curation. **Yunhan Yang:** Investigation, Formal analysis. **Jiayin Lu:** Investigation. **Yuxiang Lin:** Resources. **Dongxue Zhang:** Writing – review & editing. **Ling Lin:** Writing – review & editing, Resources. **Liang Qiao:** Writing – review & editing, Resources, Supervision.

Acknowledgments

This work was supported by National Natural Science Foundation of China (NSFC, Nos. 22074022, 22374031), and the Ministry of Science and Technology of China, National Key R&D Program of China (No. 2022YFC2704300).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2024.110183.

References

[1] H. Sung, J. Ferlay, R.L. Siegel, et al., CA Cancer J. Clin. 71 (2021) 209–249.
[2] N. Harbeck, F. Penault-Llorca, J. Cortes, et al., Nat. Rev. Dis. Primers 5 (2019) 66.
[3] K. Krug, E.J. Jaehnig, S. Satpathy, et al., Cell 183 (2020) 1436–1456.e31.
[4] D. Zardavas, A. Irrthum, C. Swanton, M. Piccart, Nat. Rev. Clin. Oncol. 12 (2015) 381–394.
[5] N. Pashayan, A.C. Antoniou, U. Ivanus, et al., Nat. Rev. Clin. Oncol. 17 (2020) 687–705.
[6] S.M. Batool, A. Yekula, P. Khanna, et al., Cell. Rep. Med. 4 (2023) 101198.
[7] J. Schiller, R. Suss, J. Arnhold, et al., Prog. Lipid Res. 43 (2004) 449–488.
[8] G.J. Ma, B.K. Yoon, T.N. Sut, et al., View 3 (2022) 20200078.
[9] X.L. Han, R.W. Gross, J. Lipid Res. 44 (2003) 1071–1079.
[10] A. Shevchenko, K. Simons, Nat. Rev. Mol. Cell Biol. 11 (2010) 593–598.
[11] X. Gao, L. Lin, A. Hu, et al., Talanta 245 (2022) 123475.
[12] D. Wolrab, R. Jirásko, E. Cífková, et al., Nat. Commun. 13 (2022) 124.
[13] J.H. Yoon, Y. Seo, Y.S. Jo, et al., Sci. Adv. 8 (2022) eadc9317.
[14] L. Lin, Y. Ding, Y. Wang, et al., Hepatology 66 (2017) 432–448.
[15] R. Eghlimi, X. Shi, J. Hrovat, et al., J. Proteome Res. 19 (2020) 2367–2378.
[16] L.M. Butler, C.Y. Mah, J. Machiels, et al., Cancer Res. 81 (2021) 4981–4993.
[17] X. Chen, H. Chen, M. Dai, et al., Oncotarget 7 (2016) 36622–36631.
[18] X. Han, R.W. Gross, Mass Spectrom. Rev. 24 (2005) 367–412.
[19] T. Xia, F. Zhou, D. Zhang, et al., Nat. Commun. 14 (2023) 4263.
[20] J. Wang, X. Han, Trends Analyt. Chem. 121 (2019) 115697.
[21] G. Han, D. Li, Q. Lin, et al., Chin. Chem. Lett. 34 (2023) 107421.
[22] X. Han, D. Li, S. Wang, et al., Mater. Today Bio 17 (2022) 100486.
[23] J. Tan, W.J. Zhao, J.K. Yu, et al., Adv. Healthc. Mater. 1 (2012) 742–750.
[24] Y. Zhao, H. Li, G. Xu, et al., Talanta 243 (2022) 123365.
[25] J. Yang, L. Huang, K. Qian, Exploration 2 (2022) 20210222.
[26] R. Wang, Z. Gu, Y. Wang, et al., Adv. Funct. Mater. 32 (2022) 2206670.
[27] Q. Wu, J.L. Chu, S.S. Rubakhin, et al., Chem. Sci. 8 (2017) 3926–3938.
[28] D. Chen, M. Du, Y. Huang, et al., Nano Res. 16 (2023) 3028–3039.
[29] K. Shrivas, T. Hayasaka, Y. Sugiura, M. Setou, Anal. Chem. 83 (2011) 7283–7289.
[30] R. Zhang, Q. Qin, B. Liu, L. Qiao, Anal. Chem. 90 (2018) 3863–3870.
[31] G. Piret, D. Kim, H. Drobecq, et al., Analyst 137 (2012) 3058–3063.
[32] S. Wang, J. Zhang, W. Cai, X. Shao, Chin. Chem. Lett. 30 (2019) 1024–1026.
[33] L. Morosi, P. Spinelli, M. Zucchetti, et al., PLoS One 8 (2013) e72532.
[34] R.S.E. Young, A.P. Bowman, E.D. Williams, et al., Cell Rep. 34 (2021) 108738.
[35] W. Wang, S. Wu, Z. Cen, et al., Cell Rep. 41 (2022) 111582.
[36] A.H. Janneh, C. Atkinson, S. Tomlinson, B. Ogretmen, Trends Cancer 9 (2023) 782–787.
[37] X. Zheng, Z. Hou, Y. Qian, et al., Nat. Immunol. 24 (2023) 802–813.
[38] C.M. Jogdeo, S. Panja, S. Kanvinde, et al., Adv. Healthc. Mater. 12 (2023) 2202400.
[39] K.A. Al-Saad, W.F. Siems, H.H. Hill, et al., J. Am. Soc. Mass Spectrom. 14 (2003) 373–382.
[40] S.T. Tan, T. Ramesh, X.R. Toh, L.N. Nguyen, Prog. Lipid Res. 80 (2020) 101068.
[41] K. Kano, J. Aoki, T. Hla, Annu. Rev. Pathol. 17 (2022) 459–483.
[42] P. Liu, W. Zhu, C. Chen, et al., Life Sci. 247 (2020) 117443.
[43] F.R. Auciello, V. Bulusu, C. Oon, et al., Cancer Discov. 9 (2019) 617–627.
[44] Z. Gong, Q. Li, J. Shi, et al., Cell Metab. 34 (2022) 1960–1976 e1969.
[45] Y. Xiao, D. Ma, Y.S. Yang, et al., Cell Res. 32 (2022) 477–490.
[46] K.S. Park, H.Y. Lee, S.Y. Lee, et al., FEBS Lett. 581 (2007) 4411–4416.
[47] T. Kühn, A. Floegel, D. Sookthai, et al., BMC Med. 14 (2016) 13.
[48] R. Taware, T.H. More, M. Bagadi, et al., RSC Adv. 11 (2020) 397–407.
[49] R. Guo, Y. Chen, H. Borgard, et al., Molecules 25 (2020) 4864.
[50] M. Hilvo, C. Denkert, L. Lehtinen, et al., Cancer Res. 71 (2011) 3236–3245.
[51] X. Chang, P. Xing, Lipids Health Dis. 21 (2022) 43.
[52] N. Marino, R. German, X. Rao, et al., npj Breast Cancer 6 (2020) 50.
[53] M. Cheng, Z.M. Bhujwalla, K. Glunde, Front. Oncol. 6 (2016) 266.
[54] B.M. Quinville, N.M. Deschenes, A.E. Ryckman, J.S. Walia, Int. J. Mol. Sci. 22 (2021) 5793.
[55] K. Simons, E. Ikonen, Nature 387 (1997) 569–572.
[56] I. Tabas, G. García-Cardeña, G.K. Owens, J. Cell Biol. 209 (2015) 13–22.
[57] M. Huang, H.Y. Li, H.W. Liao, et al., Rapid Commun. Mass Spectrom. 34 (Suppl 1) (2020) e8581.
[58] Y. Wei, P. Jasbi, X. Shi, et al., J. Proteome Res. 20 (2021) 3124–3133.