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Superhydrophobic candle soot/PDMS substrate for one-step enrichment and desalting of peptides in MALDI MS analysis

Xueyun Wang, Ning Li, Daren Xu, Xiangchao Yang, Qunyan Zhu, Dongyang Xiao, Nan Lu*

State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, PR China

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ABSTRACT

Superhydrophobic substrate is applied in matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) detection due to its confinement effect. The weak interaction of superhydrophobic surface with water/salts makes it potential in one-step enrichment and desalting of peptide in MALDI MS analysis. We fabricate a superhydrophobic substrate by spin-coating poly(dimethyl siloxane) (PDMS) on a candle soot layer. On this substrate, the peptide analytes can be confined and enriched in a small area due to the confinement effect and its strong hydrophobic interactions with PDMS. Meanwhile, the desalting can be easily realized by removing the residual solution after the absorption of analyst molecules due to the weak interaction between water/salt contaminants and the superhydrophobic surface. Using this substrate, angiotensin III (Ang III) in the presence of salt with high concentration (2 M or saturated) can be analyzed, and the peptide sequence coverage of 10 μg/mL myoglobin (MYO) and bovine serum albumin (BSA) digests is enhanced to 51% and 26%, which is 37% and 21% analyzed with the commercial ZipTipC_{18} pipette tips. The LOD of bacitracin A (Bac A) in milk with this substrate is 100 pM and nearly 360 times lower than the LOD of standard testing method. This substrate has potential practical applications in proteomics research and actual sample analysis.

1. Introduction

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) has been playing more and more important role in biological detection because of its great advantage in trace and accurate analysis [1–3], particularly for the analysis of peptides [4–6]. However, the low abundance of peptides, and the salt contaminants in sample for maintaining the structure and activity of peptide [7,8], could result in quite weak MALDI MS detection signals [9–11].

To solve the problem of low abundance, people usually take nanoparticles with large specific surface area to adsorb analyte [12–16], or apply superhydrophobic coating (with water contact angle (CA) > 150°) to condense analyte in a quite small area to improve the sensitivity of MS detection [17–19]. As for desalting, some approaches have been developed in recent years. A common way is to extract and separate analyte from sample solution based on its hydrophobic interaction with hydrophobic materials, such as carbon materials [14,15], poly (tetrafluoroethylene) [20,21], paraffin [22], nylon [23], poly(methyl methacrylate) (PMMA) [11,13,24], and hydrophobin [25], then wash the materials for desalting. Superhydrophobic surface exhibits stronger confinement effect for aqueous solution and weaker interactions with water/contaminants than the hydrophobic surface [26–28]. Herein, we fabricate a superhydrophobic candle soot/poly(dimethyl siloxane) (C/PDMS) substrates by spin-coating PDMS on a candle soot layer for one-step enrichment and desalting of peptide in MALDI MS detection. PDMS is popular for preparing superhydrophobic substrates and exhibits excellent ability in absorbing peptides and proteins because of the strong hydrophobic interactions [29,30]. Candle soot is an ideal material for preparing superhydrophobic substrate due to its suitable nanostructure and low cost [31–33]. On the superhydrophobic C/PDMS substrate, peptide can be enriched in a small area based on the confinement effect of superhydrophobicity and selective capture effect of PDMS. Meanwhile, desalting is achieved by removing the residual solution containing salt contaminants after the absorption of analytes. The limit of detection (LOD) of angiotensin III (Ang III) on this substrate is 3 orders of magnitude lower than that on the traditional MALDI steel plate. Strong MS signals can be obtained when detecting the analytes with salt contaminants of saturated concentration. The created substrates allow for direct analysis of myoglobin (MYO) and bovine serum albumin (BSA) digests, which shows better performance than the commercial ZipTipC_{18} pipette tip. The LOD of bacitracin A (Bac A) in milk with this substrate is 100 pM, which is nearly 360 times lower than the LOD of standard testing method [34]. The superhydrophobic substrate could be applied in one-step enrichment and desalting of peptides for direct

* Corresponding author.
E-mail address: luenan@jlu.edu.cn (N. Lu).

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MALDI MS detection and has great potential in practical used in proteomics research and analysis of actual sample.

2. Experimental section

2.1. Instrumentation

SEM images were obtained from a Hitachi SU8020 Field Emission Scanning Electron Microscope (Hitachi, Japan). CAs and sliding angles (SAs) were measured with 5 μL of water droplet on a Krüss DSA10 MK2 contact angle analyser (Krüss, Germany). The spin-coating procedure was conducted with KW-4A spin coater (Beijing Microelectronics Technology Institute, Beijing, China). Optical images were taken on a Leica EZ4 optical microscope (Leica, Shanghai, China).

2.2. Materials and reagents

Silicon wafers (n type [100]) were obtained from GRINM Advanced Materials (Beijing, China). Candle and milk were bought from a local market. The base/curing agent PDMS was purchased from Dow Corning Corp (Sylgard 184, Dow Corning, USA). Ang III (M_w = 931.10), MYO (M_w = 17000), BSA (M_w = 66430), trypsin TPCK treated from bovine pancreas, trifluoracetic acid (TFA), α-cyano-4-hydroxycinnamic acid (CHCA), and ZipTipC_{18} pipette tips were purchased from Sigma-Aldrich (Shanghai, China). Bac A (M_w = 1422.69) was purchased from Aladdin (Shanghai, China). Other chemical reagents were of analytical grade and purchased from Sinopharm (Beijing, China). The water in this experiment was Milli-Q water from a Millipore System (Marlborough, France).

2.3. Preparation of C/PDMS substrate

Silicon wafers were sonicated in acetone, chloroform, ethanol and water for 10 min, respectively, and soaked in a mixture solution of 25% $NH_3{\cdot}H_2O$, 30% H_2O_2 and H_2O (1: 1: 5, v/v/v) at 80 °C for 30 min, then washed with water and dried with nitrogen. The candle soot was collected by placing a clean aluminium foil over the candle flame, and dispersed in 1, 2-dichloroethane (DCE) with a concentration of 0.75% (w/w). PDMS (base/curing agent, 10: 1, w/w) was dissolved in toluene with concentration in the range of 1–5% (w/w). The candle soot films were produced by spin-coating candle soot suspension on clean silicon wafers, whose thickness was controlled by varying the spin-coating times. These prepared films were immersed in different PDMS solutions, then removed the excess PDMS solution by spinning, and cured at 68 °C for 3 h to produce the C/PDMS substrates.

2.4. Desalting with ZipTipC_{18} pipette tips

Desalting with ZipTipC_{18} pipette tips was carried out according to the standard procedures from user guide. In brief, ZipTipC_{18} pipette tip was rewetted with acetonitrile (ACN) for three times and 0.1% TFA for twice. 20 μL of sample solution was aspirated/dispensed for 10 cycles to bind peptides to the pre-equilibrated tip, then washing the tip with 0.1% TFA for three times to desalt. 2 μL of 10 mg/mL CHCA solution (in ACN/0.1% TFA, 1: 1, v/v) was used to directly elute sample onto the MALDI steel plate (MTP 384 polished steel target, Bruker, Karlsruhe, Germany) for MALDI MS analysis.

2.5. Protein digestion

MYO and BSA was dissolved in the ammonium bicarbonate (NH_4HCO_3) solution (100 mM, pH 8.0) with a concentration of 1 mg/mL, and incubated with trypsin (20: 1, w/w) for 12 h at 37 °C. The digests were diluted with 100 mM NH_4HCO_3 solution for MALDI MS analysis.

2.6. Preparation of Bac A sample

Bac A was dissolved in Milli-Q water to reach the concentration of 100 μM, then the stock solution was diluted with milk and store at 4 °C until used.

2.7. Mass spectrometry analysis

Analyte solution (20 μL) was added on different substrates, then kept for 15 min in a closed container and removed the residual droplets using a rubber suction bulb. After spotted 2 μL of CHCA solution on analyte point and dried in air, the substrates were attached to the MALDI steel plate by conductive double-side carbon tape for MALDI MS detection. When detected sample with a standard MALDI steel plate, 20 μL of analyte solution was added on MALDI steel plate and dried in air, then 2 μL of CHCA solution was spotted and dried in air. Each MALDI MS spectrum was obtained in the positive ion mode by 500 laser shots on the Autoflex speed TOF/TOF spectrometer (Brucker, Germany) with a pulsed Nd: YAG laser of 355 nm and acceleration voltage of 20 kV.

2.8. Search parameters and database

The search of peptides from protein digestions was performed with the MASCOT database online (http://matrixscience.com) (Matrix Science Ltd., London, UK). The search parameters were as follows, database: SwissProt; digest used: Trypsin; maximum of missed cleavages: 1; peptide tolerance: ± 2 Da.

3. Results and discussion

3.1. Fabrication of superhydrophobic C/PDMS substrate

The hydrophobicity of a surface depends on its chemical composition and roughness, which can be enhanced by increasing the hydrophobicity of chemical composition and the surface roughness [35]. We fabricated the superhydrophobic substrate with PDMS and candle soot to provide hydrophobic composition and roughness to the surface, respectively. As revealed in Fig. 1a, the candle soot consists of carbon nanoparticles (CNPs) with an approximate diameter of 50 nm. Fig. 1b-d present that the thickness of candle soot film increases from 0.75 to 1.40 and 1.90 μm with increasing spin-coating times from once (F1) to twice (F2) and three times (F3). After coated with PDMS, the gaps among CNPs were gradually filled with increasing the concentration of PDMS (Fig. 2b, Fig. S1a-d, Supplementary material). As plotted in

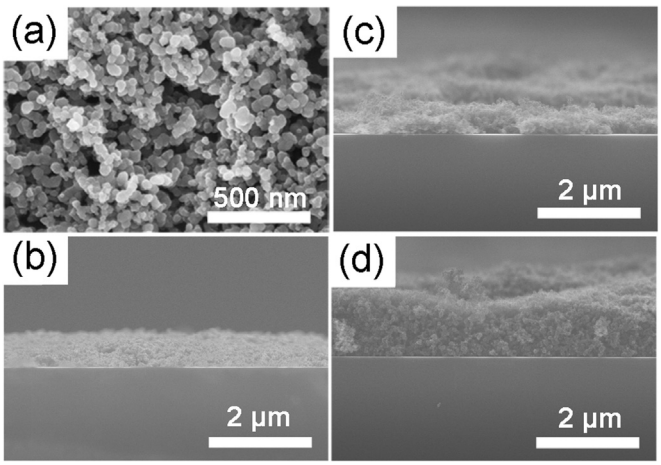

Fig. 1. (a) SEM image of the candle soot. (b) (c) (d) Side images of candle soot film prepared by spin-coating for once, twice, and three times.