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TiO_2 Printed Aluminum Foil: Single-Use Film for a Laser Desorption/Ionization Target Plate

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Single-use aluminum foil-based laser desorption/ionization (LDI) target plates have been developed for mass spectrometry (MS) analysis and provide detection results comparable to those of commercial stainless steel plates while offering a convenient way to avoid the time-consuming surface cleaning process. Additionally, arrays of TiO_2 nanoparticle spots are coated on the foil either by screen-printing or rotogravure-printing followed by sintering to form a mesoporous layer spot to act as an anchor for sample deposition. These TiO_2 spots offer further functions to the Al foil, such as matrix-free laser desorption/ionization or specific affinity for in situ enrichment of phosphopeptides. The single-use TiO_2−Al foils are cheap to produce, easy to use, and well suited for high-throughput proteomics research. They can also be of interest for protein post-translational modifications study.

During recent years, mass spectrometry (MS) has increasingly developed as a method of choice for proteomics research.[1,2] To date, two ionization techniques are most commonly used to volatize and ionize proteins or peptides for mass spectrometric analysis, namely, electrospray ionization (ESI) and laser desorption/ionization (LDI).[1] The former ionizes the analytes directly from a solution and is therefore readily coupled to liquid-based separation tools, while the latter ablates and ionizes the samples out of a solid substrate under laser pulses.[1] Although ESI-MS systems are usually preferred for the analysis of complex samples, matrix-assisted LDI (MALDI),[3] where the samples are deposited together with a matrix, is widely used in proteomics because of its simplicity, excellent mass accuracy, high resolution, and sensitivity.[1]

Sample preparation and matrix/substrate employed are key factors affecting LDI performances.[4] Different substrates and modification methods have been reported to endow LDI target plates with additional functions. Metallic LDI plates totally covered with a hydrophobic coating, but with an array of micrometer sized hydrophilic regions acting as anchors, have been proposed to confine matrix/analyte mixtures upon evaporation within a small area or to desalt biological samples prior to analysis.[5−8] Alternatively, plates functionalized with specific solid-phases have been developed to provide special affinities for targeted biomolecules.[9−13] In that context, while the modification of a steel target plate might be rather straightforward, its regeneration is normally difficult. Therefore, single-use or disposable layers, such as silicon wafer,[14,15] have been proposed as attachments to the LDI plate to form a functionalized substrate for MS analysis.

Herein, a commercial aluminum foil, which maintains a surface condition as efficient as a commercial stainless steel target plate, is used as an economical disposable layer for LDI-MS analysis. While offering a convenient way to avoid the time-consuming polishing and/or washing steps, it is here also demonstrated that an array of TiO_2 based spots can be printed by screen-printing[16,17] or rotogravure-printing[18] technique and then sintered to provide the disposable layer with different additional functions. Indeed, because of its photosensitivity, TiO_2 can act as a substrate for assisting matrix-free laser desorption/ionization[19] or as a redox center for inducing LDI in-source reactions, such as online

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peptide tagging[20] or disulfide bond cleavage.[21] In addition, because of its special affinity toward phosphopeptides,[22,23] the porous TiO_2 spots may also be employed to modify the target plate for in situ enrichment of phosphorylated peptides and further for the analysis of protein phosphorylation,[24] which is involved in a wide range of biological processes.[15,23,25−29]

In comparison with other strategies, the present single-use TiO_2-coated aluminum foil offers a very efficient and convenient approach for producing functionalized target plates. A functional LDI plate was obtained by simply assembling the film on a modified commercial target plate. Indeed by using high throughput screen-printing[16,17] or rotogravure-printing[18] techniques, large-scale production of the TiO_2−Al foil can be easily achieved. Screen-printing produces a thick TiO_2 layer that is very hydrophobic, therefore allowing the loading of large amounts of samples from aqueous solutions. In contrast, rotogravure-printing generates a much thinner layer of TiO_2, which can be more appropriate for ionization during LDI-MS analysis but less efficient for enrichment purposes as the overall specific surface area is decreased. For both printing techniques, the TiO_2 layers were obtained with a high reproducibility, therefore yielding reproducible MS analysis. While the use of nonmodified Al foils provides sensitive protein and peptide MALDI analysis, the TiO_2−Al foil can be employed to carry out matrix-free laser desorption/ionization[30] of peptides with sample amounts down to several hundred femtomoles. The TiO_2−Al foil was also evaluated for in situ phosphopeptides enrichment with β-casein as a model sample. Because of their mesoporous structure, the TiO_2 spots have a very large specific to geometric area ratio, exhibiting a high-trapping capacity for its target species. Generally, it has been demonstrated that phosphopeptides can be selectively identified from only ~100 fmol sample digests with the TiO_2 functionalized foil. More interestingly, it was found that multiphosphopeptides could be preferentially observed when using the rotogravure-printed TiO_2−Al foil.

EXPERIMENTAL SECTION

Chemicals. Titanium dioxide nanoparticles were obtained from Degussa-Evonick (P25, 30 nm average diameter, Germany). Acetonitrile (ACN, 99.9%) and trifluoroacetic acid (TFA, 99.8%) were purchased from Merck (Darmstadt, Germany), while ammonium bicarbonate, citric acid (99%), 2,5-dihydroxybenzoic acid (DHB, 98%), β-casein (from bovine milk, 90%), and trypsin (from bovineancreas) were obtained from Sigma (St. Louis, MO). Angiotensin I (98%) was obtained from Bachem (Switzerland). Ortho-phosphoric acid (>85%), acetic acid (≥99%), ammonium hydroxide solution (20~25%), ethanol, anhydrous terpineol, and ethyl celluloses were produced by Fluka (Germany). α-Cyano-4-hydroxy cinnamic acid (CHCA) is from the Bruker starter kit for MALDI-TOF MS. All these reagents were used as received without further purification. Deionized water (18.2 MΩ cm) was obtained from an ultrapure water system (Milli-Q 185 Plus, Millipore) and used for all experiments.

Fabrication of Screen-Printed or Rotogravure-Printed TiO_2−Al Foil. TiO_2 printed aluminum foil (Weitafresh from WEITAAG, Switzerland) was prepared using a screen-printing[16,17] or rotogravure-printing[18] strategy. The TiO_2 ink was prepared according to a previous report for the fabrication of dye sensitized solar cells.[31] Briefly, in ambient conditions, commercial Degussa-Evonick P25 TiO_2 powder was ground in an alumina mortar at room temperature, then transferred with ethanol to a tall beaker and stirred with a large magnetic bar at 300 rpm for total dispersion. Anhydrous terpineol and a mixture solution of two ethyl celluloses in ethanol were added, followed by stirring and sonication. The dispersion was concentrated by evaporation at 358 °C with 120 mbar at first. The pressure was then decreased to 10 mbar. The ink was finalized with a three-roller-mill grinder (Exakt). Finally, the ink was printed onto an aluminum foil as an array of spots by a screen-printing or a rotogravure-printing procedure. The screen-printing was performed using polyester screens with a mesh opening of 55 μm.[16,17] The rotogravure-printing process was carried out using a cylinder coated with a copper covering. The covering is engraved to define the shape of the spot to be printed, here with a cellular diamond structure (54 cells per cm) with a 62.5 μm center depth. The engraved cylinder begins its cycle by being loaded with the TiO_2 paste while a flexible steel doctor blade scrapes away the excess paste from the nonengraved regions. Upon rotation of the cylinder, the image area is impressed onto the Al foil (Figure 1d). The resulting TiO_2 printed Al foil was subsequently sintered in an oven (Sorvall Heraeus) at 400 °C for 1 h. The oven temperature was ramped from room temperature to 400 °C in 2 h, kept at 400 °C for 1 h, and then cooled down to 60 °C in 9 h. The sintered TiO_2 surfaces on the Al foil were characterized using a KRÜSS DSA 100 Drop Shape Analyzer. The determination of the contact angle was performed with an evaluation method of the Young−Laplace method.

TiO_2 Printed Al Foil for Matrix-Free Laser Desorption/Ionization. Two slits were drilled by electroerosion on a commercial target plate so as to pass a foil through them (Figure 1a). The foil covers the top layer of the LDI plate and is attached underneath by adhesive tapes. If necessary, the film could be mounted on axes to provide a reel-to-reel system. The TiO_2 printed Al foil was placed here manually with the printed part upward. Peptide aqueous solution (1 μL) was deposited on the printed

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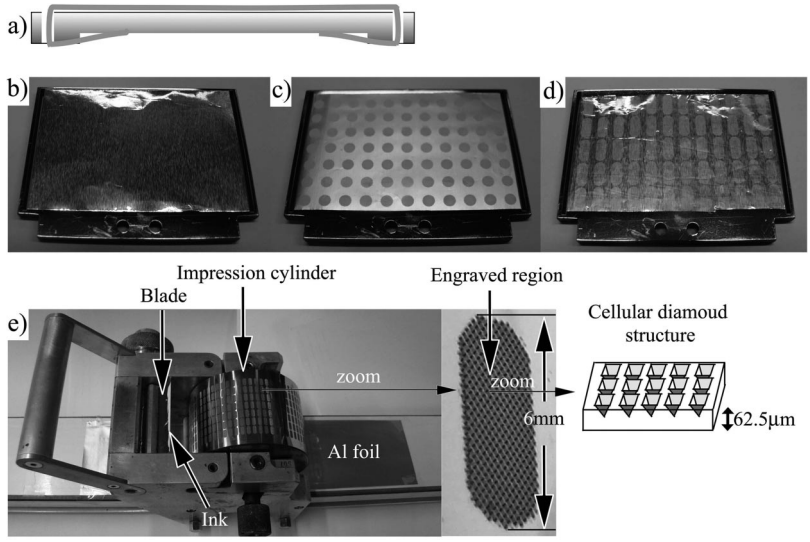

Figure 1. (a) Schematic illustration of assembling the Al foil on a modified commercial target plate. Pictures of the special modified target plate assembled with (b) Al foil, (c) screen-printed TiO_2−Al foil, and (d) rotogravure-printed TiO_2−Al foil. (e) The rotogravure-printing process: picture of the setup, zoomed picture on engraved regions defining the shape of printed pattern, and schematic zoom on the cellular structure of engraved regions.

TiO_2 spots and dried in the ambient condition. Then, a citric buffer (10 mg/mL in deionized water, 1 μL) was deposited as an overlayer and dried for assisting protonation. The LDI-MS analysis was performed on a Bruker Microflex in the positive reflector mode. The instrumental parameters were optimized to obtain satisfactory spectra.

On-Plate Enrichment of Phosphopeptides. The tryptic digestion of proteins was performed according to a reported method.[24] A total of 1 mg of β-casein was dissolved in 1 mL of ammonium bicarbonate solution (25 mM, pH ~8), denatured at 100 °C for 5 min, and digested with trypsin for 12 h at 37 °C (enzyme-to-protein ratio of 1:30 (w/w)). The digests of β-casein were diluted with DHB (2,5-dihydroxybenzoic acid) buffer (20 mg/mL in ACN/water/TFA, 50/49.9/0.1% (v/v)). The TiO_2 coated Al foil was assembled on the modified commercial target plate. Then, the digests (1.5 μL) were spotted on the TiO_2 spots and left for ~15 min before being washed three times with DHB buffer (20 mg/mL in ACN/water/TFA, 50/49.9/0.1% (v/v)). A volume of 1 μL of 400 mM ammoniac aqueous solution was then added to each spot to desorb the phosphorylated peptides bound to the TiO_2. Finally, 1 μL of TFA solution (2% in water (v/v)) was added on the sample spot and dried at room conditions before the deposition of 1 μL of DHB matrix solution (10 mg/mL in ACN/water/H_3PO_4, 50/49/1% (v/v)) for LDI-MS analysis.

LDI-TOF Mass Spectrometry and Data Analysis. LDI-MS analysis was performed on a Bruker Microflex mass spectrometer equipped with a pulsed nitrogen laser emitting at 337.1 nm (Bruker Daltonics, Germany). α-Cyano-4-hydroxy cinnamic acid (CHCA, 5 mg/ml in 50% ACN, 49.9% H_2O, 0.1% TFA) or DHB was employed as the matrix. Samples (1 μL) and matrixes (1 μL) were deposited on the steel target plate, the Al foil, or the TiO_2−Al foil, respectively. All mass spectra were acquired in the positive ion reflector/linear mode and precalibrated by an external calibration in accordance with the manufacturer's recommendations, using a standard peptide mixture (Bruker, Germany). The laser intensity was adjusted to 10% above the threshold to obtain a good resolution and signal-to-noise (S/N) ratio. The data analysis was performed by using the flexAnalysis software from Bruker and FindMod tool available on the ExPASy (Expert Protein Analysis System) proteomics server of Swiss Institute of Bioinformatics (SIB) (http://expasy.org/). The masses and intensities of special peaks were read out using the flexAnalysis, and then initial assignment of each peptide peak was performed using the FindMod tool, where the experimentally measured peptide masses were compared with the theoretical peptides calculated from a specified Swiss-Prot/TrEMBL entry to finish the peak identification.

RESULTS AND DISCUSSION

Aluminum Foil As a Disposable Layer for MALDI-MS Detection. Figure 1 shows the aluminum foil mounted as a disposable layer on a commercial target plate. Samples and matrixes are directly deposited on the Al foil, where MS analysis is then carried out. β-Casein digest and myoglobin (16 951.49 Da[32]) were employed to test the feasibility of using the Al foil as a MS target substrate for the LDI process. Both the MS linear mode and the reflector mode could be run satisfactorily with the Al foil. Parts a and b of Figure 2 show the mass spectra of myoglobin obtained in the positive linear mode on a commercial LDI steel plate and on the Al foil substrate, respectively. In both situations, detection limits of 29 fmol were achieved, where a double charged ion with an m/z value of 8 500 was observed. In the case of peptide mixtures in the positive reflector mode, the disposable Al foil layer provided a detection limit of 4.2 fmol, which is indeed comparable with what obtained on the commercial steel plate, as shown in Figure 2c,d. Therefore, it is clear that the Al foil can be a suitable substrate for LDI-MS detection when compared with a commercial steel plate. Of course, the single-use Al foil disposable layer eliminates the need of time-consuming target plate surface cleaning processes and easily avoids any carry-over pollution.

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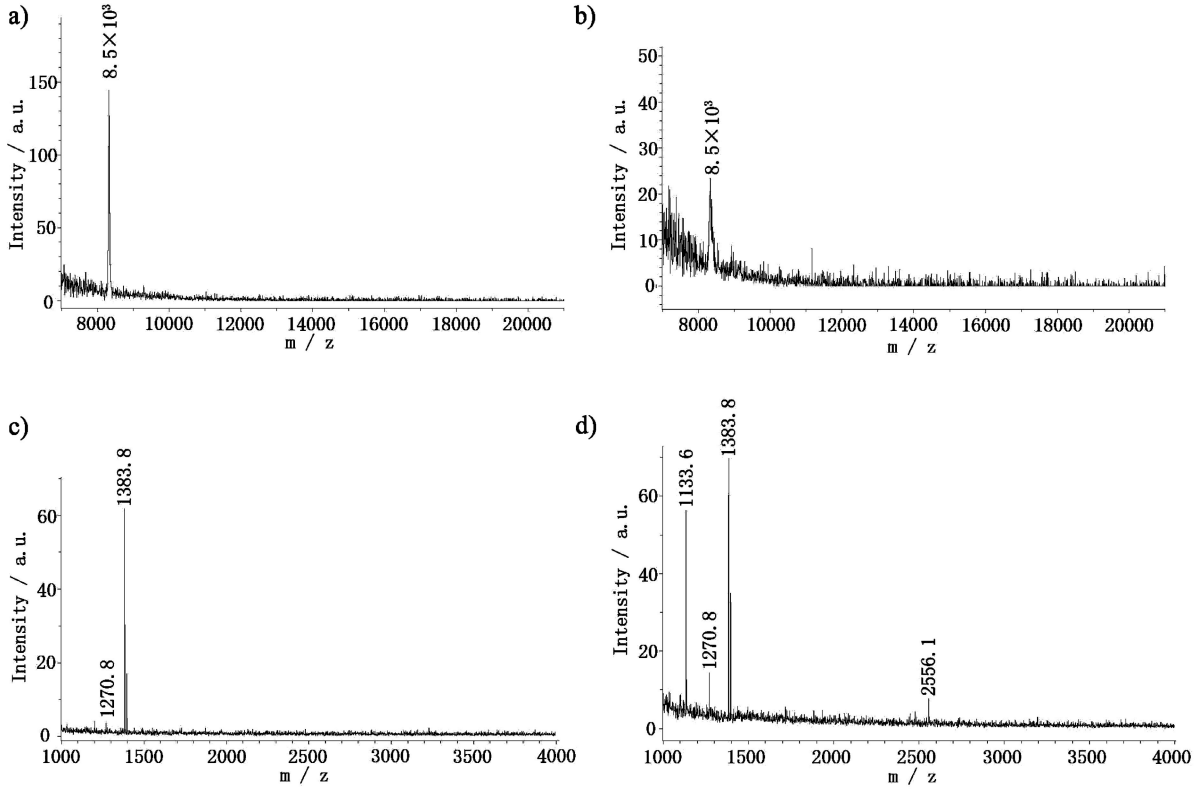

Figure 2. Mass spectra of myoglobin (29 fmol) obtained (a) on a normal target plate and (b) on an aluminum foil modified plate using the CHCA matrix in positive linear mode and mass spectra of β-casein digest (4.2 fmol) obtained (c) on a normal target plate and (d) on an aluminum foil modified plate using the DHB matrix in the positive reflector mode. Identified peaks are highlighted using the *m*/*z*.

Screen-Printed or Rotogravure-Printed TiO_2 on Al Foil. As described above, TiO_2 nanoparticles were prepared as an ink-paste and printed on the Al foil as an array of circular spots with 2.8 mm diameter using screen-printing or rounded rectangular spots (2.5 mm × 6 mm) using rotogravure-printing. Screen-printing is a well-established method to print amperometric sensors such as glucose sensors or dye sensitized solar cells. Rotogravure-printing is referred to as an intaglio printing process.[18] The image to be printed is etched into the surface of a cylinder by electron beam and laser etching methods. As shown by Figure 1e, the engraved regions have a cellular diamond structure into which the TiO_2 ink is pushed by a blade. Then, upon the rotation of engraved gravure cylinders, through the long impression-cylinderlike stage, the TiO_2 is applied onto the Al foil. This procedure allows the printing of a series of TiO_2 spot arrays on long continuous tapes, which has significant advantages to meet the demand of mass production. The viscosity of the TiO_2 ink is adjusted to fill the diamond cells, and the cell depth used provided an efficient transfer from the engraved cylinder onto the Al foil to form a homogeneous TiO_2 coating without discontinuities. The printed Al foil was sintered for 1 h at 400 °C to form a stable functional mesoporous TiO_2 layer. Upon inspection using the video camera of the mass spectrometer, it can be found that rotogravure-printing produces sintering thin layers of TiO_2 with many microgrooves, because the full area is not completely covered by the TiO_2 nanoparticles as shown in Figure 3b. On the other hand, screen-printing generates thicker spots, where the whole surface is covered by TiO_2 nanoparticles as shown in Figure 3a. As a result,

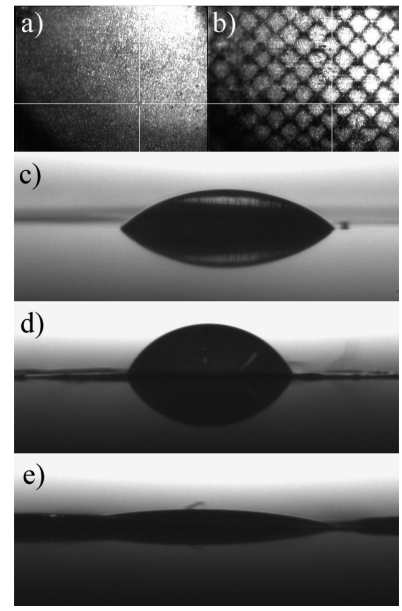

Figure 3. Pictures of the (a) screen-printed and (b) rotogravure-printed TiO_2 layer on the Al foil obtained using the camera inside the mass spectrometer. The contact angle of water on the (c) pure Al foil surface, (d) screen-printed TiO_2 surface, and (e) rotogravure-printed TiO_2 surface.

the screen-printed TiO_2 spots are very hydrophobic (Figure 3d), with a contact angle of 64.2° for water, compared to 44.7° for the nonprinted Al foil surface (Figure 3c). Therefore, as long as the sample remains on the TiO_2 spot, a rather structured droplet is obtained and the TiO_2 can be considered as a sponge allowing

the concentration of a large amount of analytes on a controlled surface area. Indeed, we have found that as much as 10 μL of samples can be trapped on a single screen-printed spot, thus showing great advantage for analyzing peptides/proteins at low concentrations. After the structural properties of lotus leaves were carefully studied, it was proposed that several parameters were essential to provide a surface with self-cleaning and superhydrophibic characteristics, one of the most important parameters being the roughness of the considered surface.[33−35] As a consequence, in the case of the screen-printed TiO_2, it can be supposed that the hydrophobicity of the surface is partly due to its mesoporous structure, which is a result of a surface covered with nanometer sized particles. In contrast, the rotogravure-printed TiO_2 surface is very hydrophilic with a contact angle of only 12.0°, Figure 3e. The rotograved spot can then be used as an anchor spot, as any liquid places on the more hydrophobic aluminum surface will migrate by wetting to the more hydrophilic rotograved spot, the unique surface structure of the rotogravure-printed TiO_2 spots playing here an important role. Indeed, with the abundant microgrooves of the Al surface, the solution can easily spread and flow through a large area. Still, the rotogravure-printed spot holds the advantage of being a very thin layer, which is more appropriate for ionization during LDI-MS analysis.

TiO_2 Printed Al Foil for Matrix-Free Laser Desorption/Ionization. As an efficient photosensitizer, TiO_2 can absorb UV energy under laser irradiation during the LDI process, where electrons can be excited from the valence band to the conduction band, leaving oxidative holes and reductive electrons.[36−38] The generated electrons and holes may be quenched from on-surface or in-volume recombination,[38] therefore releasing heat energy to help the desorption of analytes into gas phase ions for MS analysis. Also, it has been reported that the remaining oxidative holes or reductive electrons can be redox centers for inducing in-source reactions.[20,21] Herein, to prove the use of screen-printed or rotogravure-printed TiO_2 as a possible photosensitized layer in LDI-MS, the modified Al foil was employed for performing matrix-free laser desorption/ionization of peptides.

Angiotensin I (DRVYIHPFHL, 1295.7) was employed as a model sample for matrix-free laser desorption/ionization. Citric acid was added as an overlayer for assisting the protonation. As shown in Figure 4a,b, efficient ionization was achieved on either screen-printed TiO_2 spots or rotogravure-printed ones with a sample amount of 7 pmol, where a strong peak for the singly charged peptide ion with an m/z value of 1295.9 was obtained. However, when the sample amount is decreased to 700 fmol, only the screen-printed one provided satisfactory results. Because the rotogravure-printed porous TiO_2 layer is very hydrophilic, samples were found to cover the whole area of the printed spots. Contrarily, the screen-printed one is rather

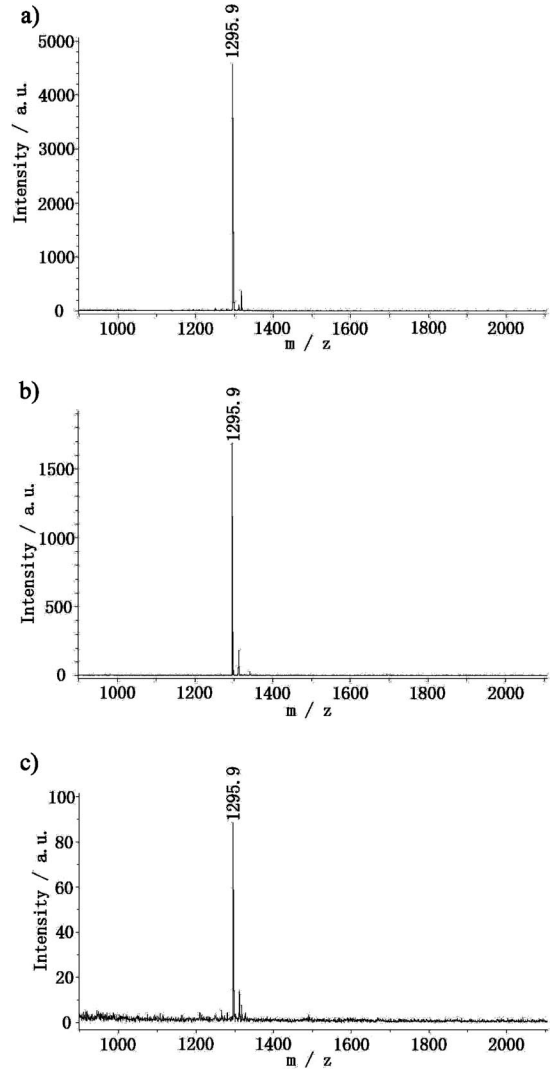

Figure 4. Matrix-free LDI mass spectra of 7 pmol angiotensin I obtained on (a) a screen-printed TiO_2−Al foil modified target plate and (b) a rotogravure-printed TiO_2−Al foil modified target plate with the presence of 10 ng of citric acid in the positive reflector mode. (c) Matrix-free LDI mass spectra of 700 fmol of angiotensin I obtained on a screen-printed TiO_2−Al foil modified target plate in the presence of 10 ng of citric acid in the positive reflector mode.

hydrophobic and would concentrate the sample solution on a reduced area. Considering that the rotogravure-printed spot is ~2.5 times larger than the screen-printed one using the current pattern, the surface concentration of crystallized samples presented on the rotogravure-printed TiO_2 layer is much lower than that on the screen-printed TiO_2 layer, thereby resulting in a poorer MS result. Still, it is clear that the TiO_2 printed Al foil can be employed for carrying out LDI-MS analysis without matrix, being a promising criterion in the scope of a further development.

On-Plate Phosphopeptide Enrichment Using TiO_2 Printed Al Foils. With a very large specific to geometric area ratio and strong specific affinity for phosphate groups, the TiO_2 sintered nanoparticle phase on the Al foil can be a very efficient solid extractor for selectively adsorbing phosphorylated peptides from a complex mixture. Figure 5 schematically represents the phosphopeptide enrichment strategy based on the use of a TiO_2−Al foil disposable layer. A functionalized plate for easy

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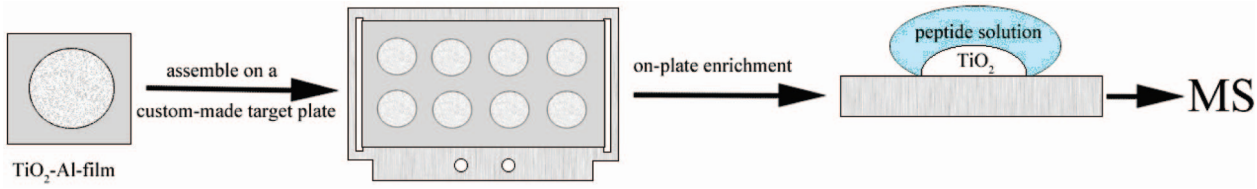

Figure 5. Schematic representation of on-plate enrichment of phosphopeptide using the TiO_2 printed Al foil.

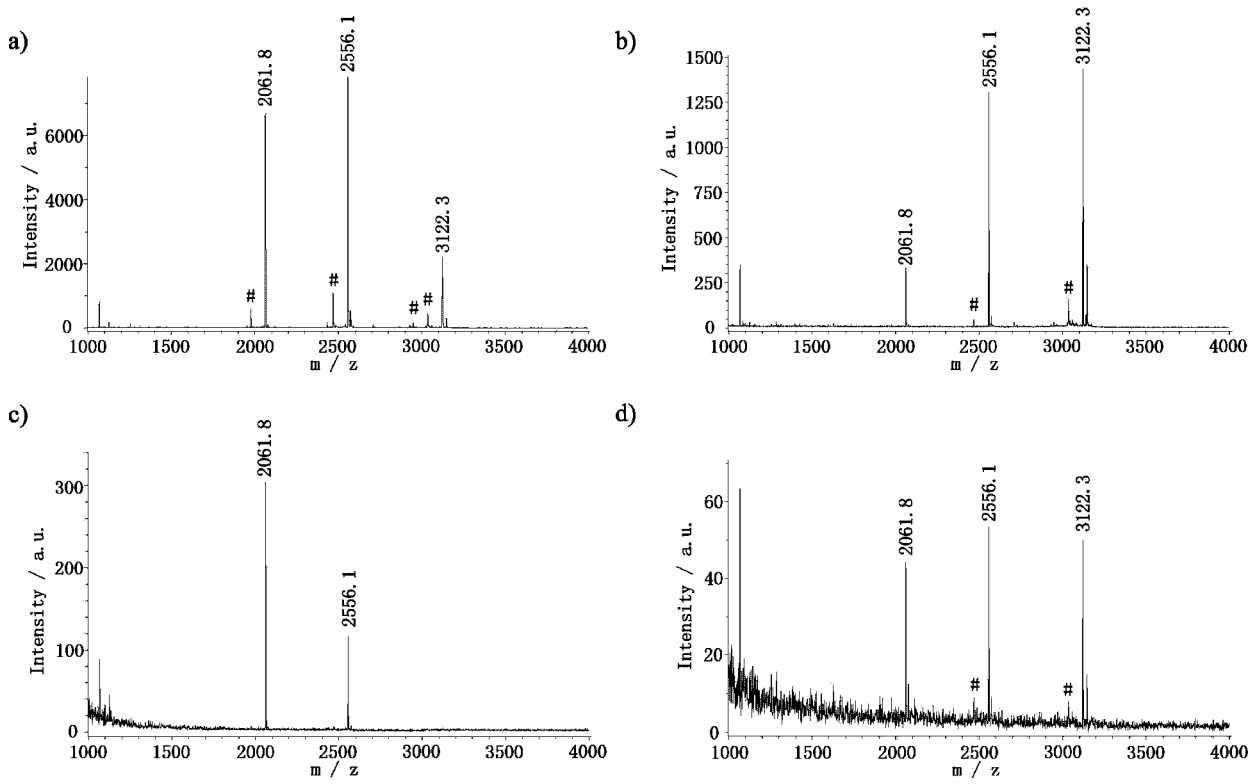

Figure 6. Mass spectra of the tryptic digest of β-casein (1.2 pmol) obtained on a screen-printed TiO_2−Al foil modified target plate and (b) a rotogravure-printed TiO_2−Al foil modified target plate using the on-plate phosphopeptide enrichment method. Mass spectra of the tryptic digest of β-casein (120 fmol) obtained on a screen-printed TiO_2−Al foil modified target plate and (b) a rotogravure-printed TiO_2−Al foil modified target plate using the on-plate phosphopeptide enrichment method. Phosphopeptides are highlighted using the *m*/*z*. # tagged peaks correspond to dephosphorylated fragments generated during the MS detection.

and rapid in situ phosphopeptides enrichment is obtained by assembling the TiO_2 printed Al foil on a modified target plate, which can be important for developing online methods to concentrate phosphopeptides prior to MS analysis. Furthermore, with the integration of a preliminary separation step, such as capillary electrophoresis or liquid chromatography, it could be anticipated that a high-throughput analysis platform for targeted proteomics could be obtained. Different from prereported on-plate phosphopeptides extraction methods, the employment of the TiO_2 printed Al foil disposable layer can avoid the destructive modification of the steel target plate[13] or the difficult surface regeneration process.[24] In comparison with the reports on the use of a chemically modified silicon wafer[14,15] as a disposable layer for phosphopeptide enrichment, the TiO_2 printed Al foil is produced in a much easier and cheaper way, and the screen- or rotogravure-printing is amenable to large-scale reproduction.

The strategy of on-plate phosphopeptide enrichment was here studied by extracting phosphorylated peptides from the digest of β-casein, which is a major protein component of bovine milk, and widely used as a model protein in the study of protein phosphorylation, containing 5 different phosphorylated sites on serine.[39] The experimental condition and buffer employed were optimized.[24] More precisely, 1.5 μL of a tryptic digest of β-casein was deposited on the TiO_2 printed Al foil layer and subsequently incubated 15 min at room temperature. After washing, the captured phosphorylated peptides were directly analyzed by LDI-MS. As shown in Figure 6a,b, when the amount of tryptic digest on one TiO_2 spot is 1.2 pmol, the obtained mass spectra are quite good by using both the screen-printed and the rotogravure-printed TiO_2−Al foil, where three peaks corresponding to phosphopeptides are clearly observed together with their dephosphorylated ions[24] while nonphosphopeptides were not detected (see Table 1). A detailed analysis of the results suggests that the rotogravure-printed film shows an advantage in detecting multiphosphorylated peptides, where the peak at 3122.3 that corresponds to a peptide with four phosphate groups dominates the mass spectrum. This phenomenon can still be observed with

(39) Cuccurullo, M.; Schlosser, G.; Cacace, G.; Malorni, L.; Pocsfalvi, G. *J. Mass Spectrom.* **2007**, *42*, 1069–1078.

Table 1. Detailed Information of the Observed Phosphorylated Peptides from Tryptic Digest of β-Casein[a]

observed m/z	no. of phosphate groups	aa	amino acid sequence
2061.8	1	β(33−48)	FQSEEQQQTEDELQDK
2556.1	1	β(33−52)	FQSEEQQQTEDELQDKIHPF
3122.3	4	β(1−25)	RELEELNVPGEIVESLSSSEESITR

[a] The phosphorylation sites are underlined.

a lower sample amount. Indeed, when only 120 fmol of sample is loaded, the mass spectrum obtained with a screen-printed TiO_2−Al foil shows only two peaks accounting for singly phosphorylated peptides while the peak at $m/z = 3122.3$ is completely absent. In contrast, the mass spectrum obtained with the rotogravure-printed TiO_2−Al foil showed an intense signal for the multi-phosphopeptide. This affinity for multi-phosphopeptides is still unclear. It has been observed repeatedly that that multiply phosphorylated peptides are retained with high efficiency from peptide/phosphopeptide mixtures but are poorly recovered in the eluate fraction. Therefore it is possible that the thin structure of the rotogravure-printed TiO_2 favors the desorption of phosphorylated peptides, thereby enhancing the detection of multiphosphopeptides. To be mentioned, the screen-printed layer shows mass spectrum with a better signal/noise, which is in accordance with what is previously observed during the matrix-free laser desorption/ionization experiments.

CONCLUSION

We have used a commercial Al foil as a cheap and disposable layer for LDI-MS analysis. For standard MALDI experiments, the attached foil can provide a surface condition as good as the stainless steel target plate while offering great convenience avoiding the cumbersome polishing and/or washing procedures. By modification of the Al foil with TiO_2 using screen-printing or rotogravure-printing technology, a TiO_2-coated Al foil can be easily and reproducibly obtained. This TiO_2−Al layer shows good ability for matrix-free laser desorption/ionization or in situ enrichment of phosphopeptides. With its high versatility and convenience, the proposed Al foil-based MS target plates represent a valuable alternative for high-throughput proteomics research while offering unique capabilities for the study of protein phosphorylations.

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