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Integrated Microstructured Photonic Fiber as a Bifunctional Robust Frit and Efficient Electrospray Emitter of a Packed Column for Capillary Liquid Chromatography−Tandem Mass Spectrometry Analysis of Complex Biological Samples

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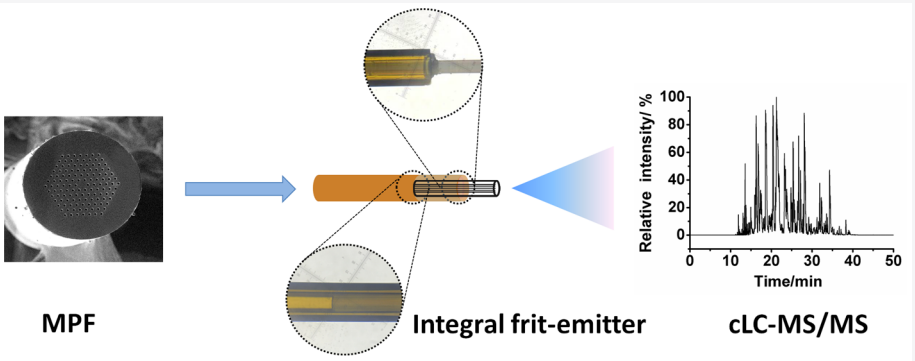
Supporting Information



ABSTRACT: Although capillary liquid chromatography married with tandem mass spectrometry (cLC−MS/MS) has become a powerful technique for proteomics and metabolomics research, it is still a great challenge to fabricate durable capillary-based analytical columns coupling continuous nanoflow (<1 000 nL/min) electrospray ionization (ESI) with MS, owing to the issue of clogging and fragile of emitters. Here, we proposed a simple approach to integrate microstructured photonic fibers (MPFs) into wide bore capillaries with 150 μm i.d., serving as an integral bifunctional frit or/and ESI emitter of packed columns. Two kinds of MPFs containing 126 homogeneous microchannels with different inner diameter, 3.2 μm for MPF-1 and 2.6 μm for MPF-2, were explored for preparation. The octadecylsilicate (ODS) silica-packed column using MPF-1 as a frit exhibited the lowest plate heights of 14.2−19.7 μm for five alkylbenzenes at the velocity of 1.5 mm/s, which were slightly lower than those of packed column with porous polymer monolith (PPM)-based frit by cLC coupling with ultraviolet (UV) detection. Additionally, the packed columns with integral MPF frit-emitters were further applied in analysis of a complex biological sample of digest of Hela cells by cLC−MS. An average of 7109 unique peptides could be identified in a single analysis by using MPF-1 emitter, and 7110 unique peptides were identified by using the MPF-2 emitter, which were superior to the identified result of packed column with an integral tapered tip emitter (6894 peptides). It is obvious that this novel integral MPF-based frit-emitter does not easily suffer from the issues of cracking owing to the silica cladding around independent microchannels (>100), which always encumbers both independent and integral tapered tip emitters for cLC−MS.

Capillary liquid chromatography (cLC) has become a complementary and/or competitive separation technique to conventional LC as a result of many advantages such as shorter analysis time, better separation efficiency, fewer required samples, and greater compatibility with mass spectrometry (MS).[1,2] Up until now, cLC has been widely applied in many fields, such as biology, medicine, pharmacy, the environment, and so on.[3−8] As the "heart" of the cLC separation technique, capillary columns mainly include packed columns, open-tubular columns, and monolithic columns. Among them, the packed capillary column is still popular and widely used in cLC due to higher column efficiency and greater availability of various commercial packing materials. Currently, packed columns are prepared by packing particles into a blank capillary matched with either a tapered tip or a frit for cLC−MS. For making a tapered tip capillary, one end of the capillary is heat-pulled into a narrow aperture by using a butane torch or a pulling instrument. Taking advantage of the "keystone" effect,

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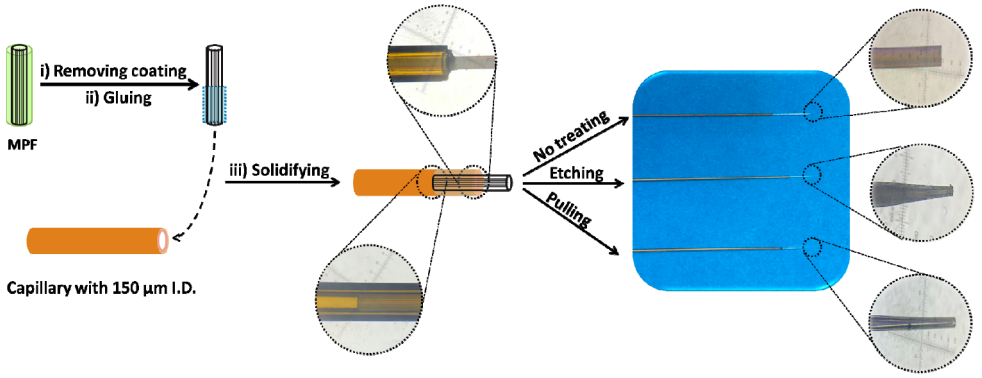
Scheme 1. Design and Fabrication Diagram of Integration of MPF into Capillary Simultaneously Acting as Frit and an ESI Emitter of Packed Column for cLC−MS



a tapered tip could retain the packing particles as a frit and meanwhile transmit electrospray as an emitter.[9] This fabrication approach is simple and minimizes postcolumn dead volume, facilitating to improve separation efficiency. However, a major problem encountered with this kind of column is the issue of clogging and fragility. Once the tip is clogged by small particles or large molecules (such as undigested proteins), the column is no longer usable and must be discarded, thus increasing the cost of analysis. Even worse, if the tip is broken, owing to the extremely thin wall formed by pulling sharply the end of capillary from large diameter (75−200 μm) to narrow (5−20 μm), not only the column could not be used anymore but also the leaking particles may contaminate the MS. Besides these bothering issues, the tapered tip capillary cannot be used to prepare packed column for UV detection.

Frit-containing capillary columns can solve these problems, but an independent electrospray ionization (ESI) emitter must be connected to the outlet of packed column with a zero dead volume union for cLC−MS.[10,11] Although both the emitter and analytical column are easy to be replaced, the separation efficiency dramatically decreases due to the increase of postcolumn dead volume caused by the connection between frit and emitter. How to prepare appropriate and robust in-capillary frits (also called integral frits) is challenging. It is required not only to allow the mobile phase pass-through columns but also to minimize extra-column volume caused by frits.[12] Multifarious strategies were taken to meet these requirements, such as sintering frits,[13,14] porous polymer monolith (PPM) frits,[15−19] magnetically immobilized frits,[20,21] tunnel frits,[22] and so on.[23−25] It is a pity that these strategies generally required tedious procedures or exhibited a short lifetime.

As the key component of nanoESI-MS, spray performance of emitter seriously affects the reproducibility and sensitivity of MS in the analysis of biological samples.[26−30] Great efforts have been made to fabricate nanoESI emitters, such as porous polymer monolith[31] emitters, entrapped microsphere capillary[32] emitters, and array of etched fused-silica capillary emitters,[33] etc. Among them, a tapered capillary tip is still the most widely used emitter due to its simple preparation and commercialization.[34] However, the issue of fragility is always bothering MS operation. In order to improve robustness of the emitter, the technique of electrically conductive materials coating is often used, whereas it still does not solve the issue fundamentally.[35,36]

In 2009, Oleschuk's group proposed a strategy of employing microstructured photonic fibers (MPFs) as independent multichannel electrospray emitters.[37] Although these emitters were not used to couple with packed columns in their case, they offered the possibility of MPFs as nanoESI emitters. Herein, a simple and facile approach was proposed to prepare packed capillary columns by integration of MPF containing 126 channels into capillaries (150 μm i.d.), where MPF acts simultaneously as a frit to retain particles and as an emitter for MS. The resulting packed columns integrated with double MPF frits were evaluated in cLC coupling with ultraviolet (UV) detection. Furthermore, the packed columns matched with integral MPF frit-emitter were successfully applied in the analysis of complex biological samples by cLC−MS/MS.

EXPERIMENTAL SECTION

Chemicals and Materials. Two kinds of MPFs containing 126 microchannels were purchased from NKT Photonics (Denmark), with product series ESM-12 (assigned as MPF-1) and LMA-10 (assigned as MPF-2). Polyimide-coated fused-silica capillaries with 150 μm i.d. × 360 μm o.d. were bought from Polymicro Technologies (Phoenix, AZ). Sylgard 184 PDMS prepolymer base and curing agent were from Dow Corning (Midland, MI). Chlorotrimethylsilane, lauryl methacrylate (LMA), ethylene glycol dimethacrylate (EDMA), 2,2-azobis(isobutyronitrile) (AIBN), γ-methacryloxypropyltrimethoxysilane (γ-MAPS), 1-propanol, 1,4-butanediol, formic acid (FA), dithiothreitol (DTT), iodoacetamide (IAA), and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (St. Louis, MO). Acetonitrile (ACN, HPLC grade) was bought from Merck (Darmstadt, Germany). The water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore Inc., Milford, MA). The octadecylsilane (ODS)-silica particles (100 Å, 3 μm) were bought from Agela Technologies (Tianjin, China). Other chemical reagents were of analytical grade.

Preparation of Packed Capillary Columns Integrated with MPF-Based Frit and Bifunctional Frit-Emitter. Two kinds of MPFs, MPF-1 and MPF-2, were employed for preparation of bifunctional frit-emitters. The preparation procedures were carried out according to the following steps. First, the MPF was cut with an appropriate length (3.0 cm) and then immersed in toluene for 10 min to remove the outer coating. Next, the resulting MPF was painted with a layer of PDMS prepolymer (about 1.0 cm long), which was made by mixing the Sylgard PDMS prepolymer base and curing agent at

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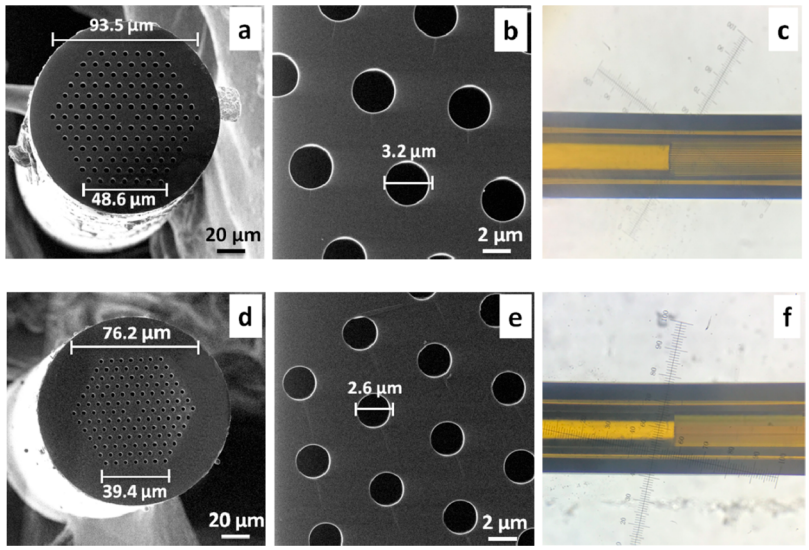

Figure 1. SEM images of (a, b) MPF-1, (d, e) MPF-2 without coating, and optical microscope images of (c) MPF-1 and (f) MPF-2 as frits in blank capillaries (150 μm i.d. × 360 μm o.d.).

a weight ratio of 10 to 1. Then the painted MPF was carefully inserted into a blank capillary (150 μm i.d. × 360 μm o.d.), and the other part of MPF was exposed. Subsequently, they were put into an oven at 80 °C for 2 h to immobilize the MPF with the inner wall of the capillary. The micrographs were shown in Scheme 1 and Figure 1. Finally, ODS-silica beads were packed into the capillary with the slurry packing technique. In this way, the 3.0 cm-long MPF at outlet of the capillary could act as a frit to retain the particles and meanwhile acted as an emitter to form electrospray for cLC−MS analysis. After cutting off the MPF exposed to the outside of the capillary, the resulting packed capillary column with a 1.0 cm-long MPF frit could be directly connected with UV detector for cLC analysis. After packing with ODS-silica beads, the other 1.0 cm-long MPF frit was similarly prepared at the inlet of the packed column.

Three approaches were employed to improve spray efficiency of MPF-1 emitter for aqueous solutions. First, the silica orifice of emitter was decorated with a toluene solution of 20% (vol %) chlorotrimethylsilane to increase hydrophobicity of the cross-section. Second, the outlet of the emitter was etched with 40% (vol %) HF solution. Third, the outlet of emitter was directly pulled into a tapered tip with a butane torch.

Preparation of Packed Capillary Column Integrated with a Tapered Silica Tip Emitter. First, a 2.0 cm transparent window was made by burning the polyimide coating of the capillary (150 μm i.d. × 360 μm o.d.) with a flame from an alcohol lamp. Second, it was put into the laser puller (Sutter Instrument Co., America). A tapered tip, which can be used as an emitter, was formed at the outlet of capillary though appropriate procedures. Finally, ODS-silica beads were packed into the capillary under a 4 MPa nitrogen pressure.

Preparation of Packed Capillary Column Integrated with PPM-Based Frit. The PPM frit was prepared via *in situ* thermal-initiated polymerization at one end of the capillary with reference to our previous report.[38] First, the inner surface of blank capillary (150 μm i.d. × 360 μm o.d.) was treated with γ-MAPS. Second, a polymerization solution, which contained 100 μL of LMA, 100 μL of EDMA, 170 μL of 1-propanol, 130 μL of 1,4-butanediol, and 2 mg of AIBN, was mixed and sonicated for 5 min. Third, the polymerization solution was introduced into the capillary for 2.0 cm long. Then two ends of the capillary was sealed with silicon rubbers and put them into the water bath at 60 °C for 12 h to synthesize the PPM frit located at the outlet of the capillary, and the PPM frit was then flushed by methanol. Finally, ODS-silica beads were packed into the capillary under a 4 MPa nitrogen pressure. The frit was kept at 1.0 cm long prior to chromatographic analysis, and there was not another PPM frit at the inlet of the packed column.

Helium Ion Microscopy (HIM) Analysis. HIM images of the MPFs were obtained on a Zeiss ORION nanoFab helium ion microscope (Zeiss, Germany), which was equipped with a helium gas field as an ion source.

Evaluation of Packed Capillary Column with cLC-UV. cLC experiments were carried out on a system consisting of an Agilent 1100 micropump (California, America), a 7725i injector, and a K-2501 UV detector (Knauer, Berlin, Germany). The detection wavelength was set at 214 nm. A T-union connector was used as the splitter, which connected the analytical capillary column and a blank capillary (50 μm i.d. × 100 cm). The outlet of the analytical column was connected to an empty polyimide-coated fused-silica capillary (10 cm × 50 μm i.d.) with a Teflontube, where a detection window was made by removing a 2.0 mm-long polyimide coating and located in a position of about 5.0 cm from the outlet of analytical column. The chromatographic data were collected on the HW-2000 software (Qianpu Software, Shanghai, China).

Application of Packed Capillary Column with cLC−MS/MS. A nano-RPLC−MS/MS system, equipped with a Surveyor HPLC system and a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific), was applied in the analysis of peptides according to our group's report.[39] System controlling and data collection were performed with Xcalibur software (version 2.1, Thermo Fisher Scientific). An aqueous solution containing 0.1% FA was used as mobile phase A, and ACN containing 0.1% FA was used as mobile phase B. The digest was first injected into a homemade trap column packed with ODS-silica beads (4.0 cm × 200 μm i.d.) with 100% mobile phase A for 10 min. Then the trapped peptides were

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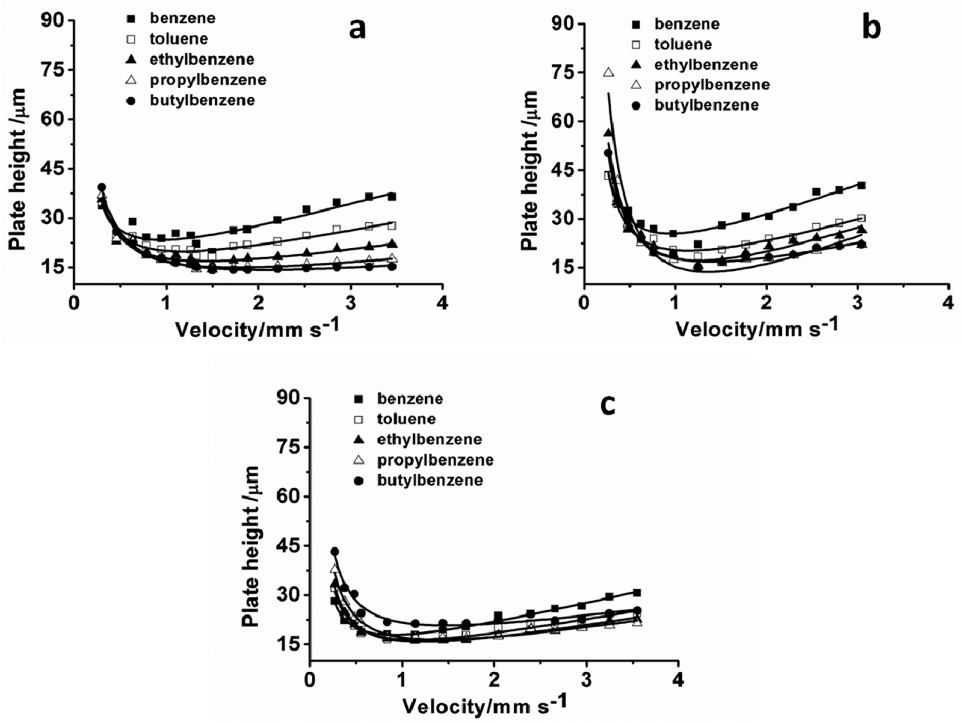

Figure 2. Dependence of the plate height (H) of alkylbenzenes on linear velocity (u) of the mobile phase by cLC on packed capillary columns with (a) MPF-1, (b) MPF-2, and (c) PPM frits, respectively. Experimental conditions: mobile phase, ACN/H_2O (60/40, v/v); column dimension, total 22 cm × 150 μm i.d. (including two 1.0 cm-long frits); UV detection at 214 nm. The curves were fitted according to the van Deemter equation, $H = A + B/u + Cu$.

separated on the packed capillary columns integrated different emitters. The gradient elution was 0−5% (vol %) mobile phase B in 2 min, 5−35% B in 30 min for digest of BSA (or 90 min for digest of Hela cells), 35−80% B in 3 min and retained 80% B for 10 min. The flow rate was set at 80 μL/min before splitting. The spray voltage between spray tip and MS interface was 2.0 kV. The full MS and MS/MS spectra scans ranged from m/z 400 to m/z 2000 in the data dependent mode. The 20 most intense ions were selected for fragmentation by collision induced dissociation (CID) for MS/MS.

The obtained MS spectra were searched on Mascot Daemon (version 2.5.1) protein identification platform (Matrix Science, London, U.K.). The BSA/Human database was downloaded from the Website http://www.uniprot.org/. Mass tolerance was set at 20 ppm for the parent ions, and mass deviation of fragments was set at 0.8 Da for fragment ions. A maximum of two missed cleavages was allowed. The false positive rates (FDRs) were controlled to <1% for identification of peptides.

Cell Culture and Lysis. Human Hela cell line was seeded in RPMI 1640 medium at a 15 cm dish, supplemented with 1% penicillin/streptomycin and 10% bovine serum at 37 °C in 5% CO_2 atmosphere.[40] The cells were harvested when they grew to about 80% density and were rinsed twice with cold phosphate buffered saline (PBS, 0.01 M phosphate, 0.15 M sodium chloride, pH 7.4. Then the dish was scraped in ice-cold lysis buffer, which contained 65 mM DTT, 50 mM Tris-HCl (pH 7.4), 8 M urea, 1 mM EDTA, 0.5 mM EGTA, phosphatase inhibitor, 1% Triton X-100, and 2% protease inhibitor cocktail. Then they were sonicated for 25 min and centrifuged at 25 000g at 4 °C for 30 min. Five volumes of ice-cold precipitation solution (acetone/ethanol/acetic acid = 50/50/0.1, v/v/v) were added into the collected supernatant and placed at −20 °C overnight for protein precipitation. The solution was centrifuged at 25 000g at 4 °C for 30 min for obtaining precipitated proteins. They were washed by ice-cold acetone and ethanol, successively. The protein concentration was determined by BCA assay.

Tryptic Digestion of Proteins. The process referred to our group's report before.[41] A total of 2 mg of BSA/Hela cell proteins, 20 μL of DTT (20 mmol/L in water) solution, and 1.0 mL of denaturing solution, which contains 8.0 mol/L urea and 0.1 mol/L ammonium bicarbonate, were mixed into a centrifuge tube. The mixture was incubated at 60 °C for 1 h. After adding 7.4 mg of IAA, the mixture was incubated in the dark at room temperature for 40 min again. The resulting mixture was diluted 8-fold by 100 mmol/L ammonium bicarbonate solution and followed by digestion in the presence of trypsin (trypsin/protein = 1/25, w/w) at 37 °C overnight. The pH of mixture was then adjusted to 2−3 using 10% TFA aqueous solution. Desalting was performed on a homemade ODS solid-phase extraction (SPE) cartridge with aqueous solution containing 0.1% TFA (vol %) as both loading and washing solution and ACN solution (80%, vol %) containing 0.1% TFA (vol %) as eluting solution. The eluted fraction was dried under vacuum and dissolved in 0.1% FA (vol %) aqueous solution prior to use.

RESULTS AND DISCUSSION

Design and Fabrication of MPF-Based Packed Capillary Columns. The MPFs are manufactured by stacking silica capillaries to a desired photonic crystal structure, fusing together, and then pulling as the fiber.[42] Two kinds of MPFs used in this study were acquired from NKT Photonics (Denmark). Figure 1a,b,d,e shows their SEM images without

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Table 1. Fitted Values of *A*, *B*, and *C* Terms in van Deemter Equation: $H = A + B/u + Cu$

	MPF-1 frit			MPF-2 frit			PPM frit		
analytes	*A* (μm)	*B* (10^3 μm^2 s^{-1})	*C* (ms)	*A* (μm)	*B* (10^3 μm^2 s^{-1})	*C* (ms)	*A* (μm)	*B* (10^3 μm^2 s^{-1})	*C* (ms)
benzene	8.68	6.94	7.79	7.03	8.81	10.1	6.01	5.34	6.63
toluene	6.50	7.86	5.73	6.32	8.58	6.58	4.44	6.73	5.34
ethylbenzene	4.64	8.74	4.34	4.56	10.2	5.7	3.91	7.30	4.86
propylbenzene	4.33	9.32	3.06	7.65	10.9	2.77	4.38	8.38	4.37
butylbenzene	4.58	9.99	2.37	1.20	11.9	5.66	9.12	8.57	3.98

an outer layer coating. They contain 126 homogeneous microchannels with 3.2 μm i.d. for MPF-1 and 2.6 μm i.d. for MPF-2, both of which allow continuous flow of liquid but prevent passage of ODS-silica beads larger than 3 μm in diameter due to the "keystone effect" or "size exclusion effect".[43,44] Since the outside diameter of MPFs is only 125 μm after removal of the polyacrylate coating layer, they can be easily inserted into the blank fused-silica capillaries with an inner diameter of 150 μm. The preparation is very simple as the following. First, a 3 cm-long MPF was carefully cut off, making the cross section of MPF as flat as possible with inspecting under an optical microscope and immersed into toluene to remove the outer coating. As Sylgard 184 PDMS glue is compatible with some solvents such as methanol, ACN, and water, etc.,[45] which are typically employed as mobile phases in reversed-phase cLC, it was carefully chosen to immobilize the MPF in the capillary. Second, after the outer wall of MPF was painted with a layer of PDMS glue, the MPF was manually inserted into the blank capillary. In order to minimize dead volume caused by the frit, the insertion length should be as short as possible. Finally, the MPF-based frit was robustly immobilized into capillary after curing at 80 °C for 2 h, which directly served as a bifunctional integral frit-ESI emitter or as an independent in-capillary frit after cutting off the exposed section. On account of mechanical strength, a 1.0 cm-long MPF was optimally selected as an in-capillary frit, shown in Figure 1c,f. After packed with ODS-silica beads, the other in-capillary MPF frit could be made again at the inlet of capillary column, preventing leakage of packing materials. All packed column integrated MPFs were employed to separate small molecules in cLC coupling with UV detection and complex biological samples in cLC−MS/MS, respectively.

Evaluation of MPF-Based Frit in Packed Capillary Column by cLC-UV. For comparison, a thermal-initiated PPM-based frit was fabricated to prepare a packed capillary column. As shown in Figure S1, a polymethacrylate PPM-based frit with 1.0 cm long was formed in a capillary column with 150 μm i.d. Figure 1c,f shows that both MPF-1 and MPF-2 were entrapped into the capillaries, and the interface cuts were very flat. However, the interface of the PPM frit was blurred under the observation of an optical microscope (Figure S1c). To investigate the effect of the frit on column efficiency, three capillary columns matched with MPF-1, MPF-2, and PPM frits were evaluated in cLC−UV after packed with 3 μm ODS silica beads (20 cm in effective length of packing) using alkylbenzenes as probes. The results showed that 5 alkylbenzenes could be baseline separated on three columns when using ACN/H_2O (60/40, v/v) as mobile phase. Their van Deemter plots were obtained by fitting the data of plate height (H) against linear velocity (u) of mobile phase, as shown in Figure 2. The packed column using MPF-1 as a frit exhibited the lowest plate heights of 14.2−19.7 μm for alkylbenzenes (corresponding to 70 300−50 800 theoretical plates per meter) at the velocity of 1.5 mm/s, which were similar to those of the packed column using MPF-2 as a frit (lowest plate heights ranging from 14.5 to 22.1 μm) and slightly higher than those of packed column with the PPM-based frit (lowest plate heights ranging from 16.2 to 21.3 μm). The values of the *A*, *B*, and *C* terms in the van Deemter equation, $H = A + B/u + Cu$, were summarized in Table 1. It was observed that the values of *A*, *B*, and *C* terms for three packed columns were only slightly varied, which suggested that the type of frit does not have much effect on column performance, possibly due to the small dead volume of frits relative to total dead volume in the cLC−UV system.

The mechanical stability of three packed columns was investigated by plotting back-pressure against flow rate, as shown in Figure 3. The result displayed that all of them could

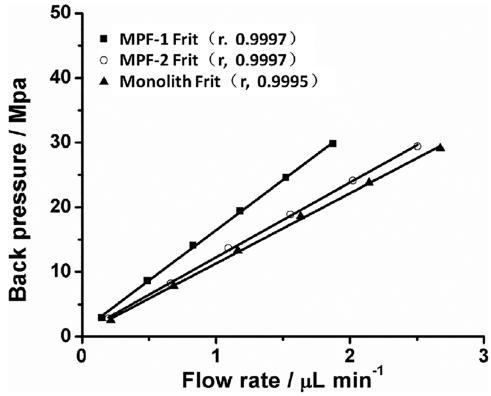

Figure 3. Relationships between flow rate and back pressure on packed capillary columns with different frits. Experimental conditions: mobile phase, ACN/H_2O (40/60, v/v); column dimensions, 22 cm in total length (including two 1.0 cm-long MPF-based frits) × 150 μm i.d. for packed columns with MPF-1 and MPF-2 frits, and 21 cm in total length (including a 1.0 cm-long PPM-based frit) × 150 μm i.d. for a packed column with a PPM frit.

undergo a high pressure of 30 MPa (upper limit of instrument usage) with high linear correlation coefficient (r) above 0.999, which suggested that two columns integrated with MPF frits could provide great mechanical stabilities as much as that of packed column integrated with a PPM frit. The reproducibility was also evaluated in terms of relative standard deviations (RSDs) of retention factor with toluene as a target analyte and thiourea as a void time marker. The RSDs of run-to-run and batch-to-batch (n = 3) were 1.3% and 1.6% for packed column with MPF-1 as frit, and 1.4% and 2.6% for another packed column with MPF-2 frit. Obviously, they were lower than that of a packed column with a PPM frit (1.5% and 6.0%), demonstrating that either MPF-1 or MPF-2 frits could be well

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reproduced compared to the PPM frit. It should be pointed out that the second frit could also be facilely formed by inserting MPF into the inlet of capillary after packing silica beads for improving service life of the packed column, which is impossible for PPM frit. As a result, the design of MPF-based frit offers an excellent approach to fabricate packed capillary columns with double frits owing to a simple preparation procedure.

Evaluation of MPF-Based nanoESI Emitter in Packed Capillary Column by cLC−MS. The packed capillary column, integrated with a 3.0 cm-long MPF-1 frit-emitter at the outlet and a 1.0 cm-long MPF-1 frit at the inlet, was employed to optimize cLC−MS conditions, optical microscope images of which are partly displayed in Scheme 1. On account of the mechanical strength and maneuverability, MPF with 1.0 cm length was embedded into the capillary as a frit, and the other section of 2.0 cm length was exposed as an ESI emitter. Electrospray voltage plays a vital effect on the ESI signals and therefore requires it to be carefully investigated. The BSA digest was selected as a relatively simple biological sample to optimize spray voltage, and the result was shown in Figure S2a and Table 2. A phenomenon of "spitting" emerged during the cLC−MS analysis process when the spray voltage was set at 1.0 kV, and mass spectrum signals were intermittent and discontinuous. When the spray voltage was increased higher than 1.5 kV, continuous signals of MS could be collected although "spitting" still occurred at the beginning of gradient elution (shown in Figure 4a−c), which was caused by both high surface tension and low volatility of highly aqueous mobile phase. However, this phenomenon of "spitting" disappeared when using high organic solvent phase (shown in Figure 4d). When the spray voltage was set in the range of 1.5−2.0 kV, both protein sequence coverage (higher than 80%) and number of identified unique peptides (more than 69) were satisfied, which could be comparable to those of the packed column integrated with a tapered tip frit-emitter (average 80% protein sequence coverage, 64 unique peptides, Figure S2b), which was tapered with the aid of a laser instrument. The spray voltage of 2.0 kV was chosen for following experiments.

Table 2. Influence of Spray Voltage on the Identification Results of BSA Digest on Packed Column with Integral MPF-1 Frit-Emitter[a]

applied voltage (kV)	protein sequence coverage (%)	unique peptides
1.5	80.7	72
2.0	82.7	69
2.5	70.0	55
3.0	68.4	50

[a]The data listed in the table were the average values of three parallel analyses.

The stability of MPF-1 as a frit-emitter was measured by continuous injecting BSA digest in cLC−MS. The number of identified BSA digest remained above 66 using MPF-1 as an emitter after 1 week at a spray voltage of 2.0 kV, which displayed good stability. In addition, two kinds of packed columns integrated with MPF-1 and MPF-2 emitters were flushed with 1.0 mol/L NaCl aqueous solution for 72 h. It was found that the emitters were not clogged, indicating that the MPF had good tolerance.

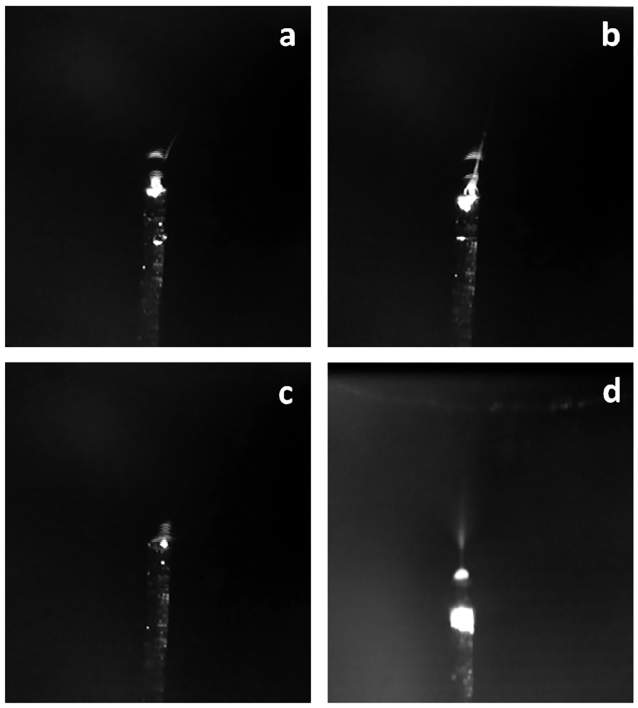

Figure 4. Optical images of MPF-1 emitter at a spray voltage of 2.0 kV. Experimental conditions: column dimension, 24 cm × 150 μm i.d. (including 1.0 cm-long frit at inlet and 3.0 cm-long frit/emitter at outlet of the packed column); packing material, 3 μm ODS beads; mobile phases, (a−c) H_2O/FA (99.9/0.1, v/v) and (d) ACN/H_2O/FA (80/19.9/0.1, v/v/v); flow rate, 900 nL/min.

Inspired by the aforementioned results, MPF-based emitters were further applied in the analysis of a more complex sample of digest of Hela cells by cLC−MS. This cell line is widely used in cancer research, biological experiments, and cell culture and has become a very important tool in medical research owing to the ability of unlimited reproduction.[46,47] In our case, three approaches were tried to improve the spray efficiency of the MPF-1 emitter. First, the orifice of the MPF-1 emitter was decorated with chlorotrimethylsilane to improve the hydrophobicity of the cross-section (assigned as decorated MPF emitter).[37] Second, the outlet of the MPF-1 emitter was etched with 40% HF solution (assigned as etched MPF emitter).[35] Third, the outlet of the MPF-1 emitter was directly tapered with a butane torch (assigned as tapered MPF emitter).[48] The optical microscope images of them are presented in Scheme 1. It could be observed that both etched and tapered MPF emitters were similar to the commercially available ESI emitter (a tapered tip emitter) in appearance. In addition to these four kinds of MPF-1 frit-emitters, both an integral MPF-2 (no treating) and a tapered tip emitter were also employed to integrate with the packed capillary columns and applied in separation of Hela cells digest by cLC−MS/MS under the same conditions.

The scene of electrospray in the analysis process was photographed and partly exhibited in Figures S3 and S4. It was found that the phenomenon of "spitting" electrospray still emerged, but frequency of "spitting" was increased under highly aqueous mobile phase employing the decorated MPF emitter. A donut-like liquid drop appeared at the outlet of etched MPF emitter (Figure S3a−c), but only part of the liquid drop sprayed into the orifice of the MS could provide a

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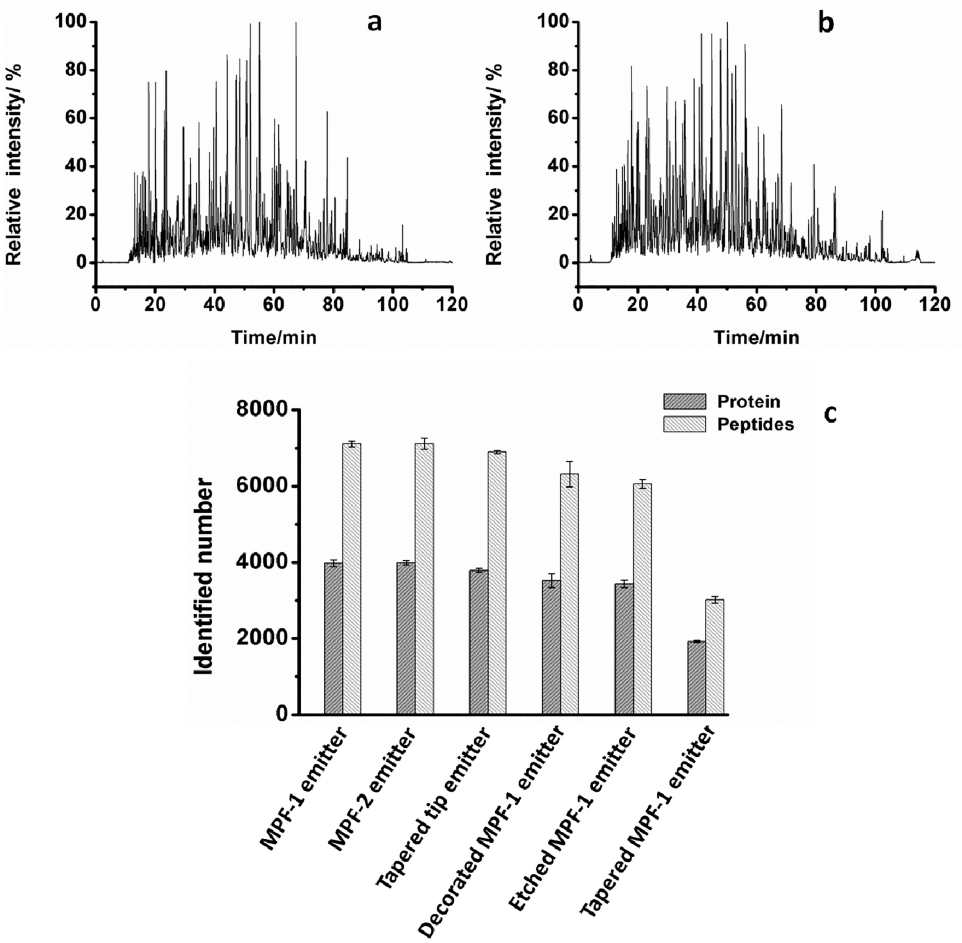

Figure 5. Separation chromatograms of the analysis of digest of Hela cells on packed columns integrated with a (a) MPF-1 emitter, (b) tapered tip emitter by cLC−MS/MS, and (c) comparison of the number of identified proteins and peptides on packed columns integrated with different emitters. Experimental conditions: (a) 24 cm × 150 μm i.d. (including a 1.0 cm-long frit at the inlet and a 3.0 cm-long frit-emitter at the outlet of the packed column), (b) 22 cm × 150 μm i.d.; packing material, 3 μm ODS beads; mobile phase A, 0.1% aqueous FA; mobile phase B, 0.1% FA in AC;, gradient, 0−5% B in 2 min, 5−35% B in 93 min, 35−80% B in 8 min, and retained 80% B for 10 min; flow rate, 900 μL/min. The error bars represent the standard deviation of three parallel experiments.

continuous and stable electrospray, owing to destroying the outer layer of microchannels after etching the tube wall of MPF (Figure S3d). For the tapered MPF-1 emitter, a stable spray could be observed throughout the LC−MS analysis (Figure S4), which was similar to that of a packed column with a tapered tip emitter (Figure S5). It was surprised that a Taylor cone could be clearly observed during the analysis when directly employing the MPF-2 emitter (Figure S6), indicating that the microchannel distribution of MPFs had an important effect on the electrospray efficiency.

The collected chromatograms are presented in Figure 5a,b and Figures S7 and S8, and the database search results are shown in Figure 5c. Although no any remarkable difference could be observed among these chromatograms, the identification results were quite different. The 7109 unique peptides and 3981 protein could be identified in a single analysis by employing the MPF-1 as an emitter without any modification, which were comparable to those using MPF-2 as an emitter (7110 peptides and 3990 proteins). It was surprising that they were both slightly superior to that of packed column using the tapered tip as an emitter (6894 peptides and 3789 proteins). However, exploring the decorated, etched, and tapered MPF-1 emitters did not improve the identification amount (6316 peptides and 3519 proteins), even though these kinds of emitters facilitated to improve the electrospray performance under a highly aqueous mobile phase. These results might be due to the cumulative effect of the droplet. In this scenario, a part of the peptides was gathered into the droplet on the surface of the emitter, generating an "enrichment effect", and then entered into the MS, which would improve their sensitivity and increase identification quantity. As for both etched and tapered MPF-1 emitters, part of samples were lost and did not enter into the MS instrument, although the latter could offer well spray similar to the tapered tip emitter during the whole process of gradient elution analysis. Interestingly, although the spray effect of the MPF-2 emitter was much better than the MPF-1 emitter, there was no obvious increase of the identified result of Hela digest for the former. As mentioned above, the microchannel distribution of MPFs would affect the electrospray efficiency, and the emitting region of MPF-1 was slightly larger than that of MPF-2 (Figure 1a,d), possibly resulting in a larger plume and fewer samples would be transported into MS. However, there was almost no difference on number of identified peptides using two MPFs, which might be due to the limitations on the instrument sensitivity and resolution. In

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summary, both of the packed columns employing MPFs as emitters without any treatment exhibited the excellent ability in cLC−MS analysis of complex biological samples.

CONCLUSIONS

We have proposed a very simple approach to prepare robust frits or/and emitters by integrating MPF into wide-bore capillary columns within several hours. Stable performance and great reproducibility for both the frits and emitters of capillary columns could be obtained. The resulting ODS silica beads-packed capillary columns exhibited high column efficiency comparable to that of a packed column using a PPM frit, preparation of which requires multiple procedures and longer time, under the same condition for cLC. Furthermore, MPFs could be used to prepare double frits at two ends of a capillary column. Moreover, they also displayed favorable ESI efficiency as integral frit-emitter for analysis of complicated Hela cells digest in cLC−MS, which was superior to that of integral tapered tip frit-emitter. Most importantly, MPF emitters are not easily cracked owing to the silica cladding around microchannels, which always encumber both the independent and integral tapered tip emitters for cLC−MS/MS. It is optimized as expected that the design and fabrication of novel MPF can further improve the ESI efficiency of packed columns in cLC−MS, and this kind of bifunctional MPF-based frit-emitter will be gradually popular and commercialized in the near future.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.9b04997.

Figure S1, images and optical microscope image of PPM as frit in the fused-silica capillary; Figure S2, separation chromatograms of tryptic digest of BSA on packed capillary columns integrated with MPF-1 and tapered tip emitter by cLC−MS/MS; Figure S3, optical images of at a spray voltage of 2.0 kV and SEM images of cross-section of an etched MPF-1 emitter; Figure S4, optical images of tapered MPF-1 emitter at a spray voltage of 2.0 kV; Figure S5, optical images of tapered tip emitter at a spray voltage of 2.0 kV; Figure S6, optical images of MPF-2 emitter at a spray voltage of 2.0 kV; Figure S7, separation chromatograms of tryptic digest of Hela cells on packed capillary columns integrated with different treated MPF-1 emitters by cLC−MS/MS; and Figure S8, separation chromatogram of tryptic digest of Hela cells on packed capillary column integrated with an MPF-2 emitter by cLC−MS/MS (PDF)

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Notes

The authors declare no competing financial interest.

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