Supporting Information

Segmented microfluidics-based packing technology for chromatographic columns

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Table of contents

EXPERIMENTAL S1

According to the confocal laser scanning microscopy (CLSM) method reported by Jorgenson and Tallarek *et. al.*[1], two columns were prepared with a hydrophilic particulate stationary phase, Hypersil NH_2, using conventional slurry packing method at a slurry concentration of 2 mg/mL and the current segmented microfluidic method at a slurry concentration of 180 mg/mL, respectively.

To pack the hydrophilic NH_2 phase, the slurry solvent and continuous phase were changed to methanol and hexadecane with 5wt.% EM90, respectively. After packing, 1 mg/mL fluorescein in methanol was used for bed staining, and a mixture of glycerol/DMSO/water, 70/19/11 (v/v/v), was used as immersion liquid and embedding liquid for refractive index matching. The column was then cut into 3 cm segments and the coating of the middle part was carefully removed with a scalpel. The column segments were sealed at both ends and fixed on a slide, then the refractive index matching liquid was applied to the middle part. CLSM imaging was performed with 20x and 40x objective lens under the excitation wavelength of 488 nm.

Reference:

(1) Reising, A. E.; Godinho, J. M.; Jorgenson, J. W.; Tallarek, U. *J. Chromatogr. A* **2017**, *1504*, 71-82.

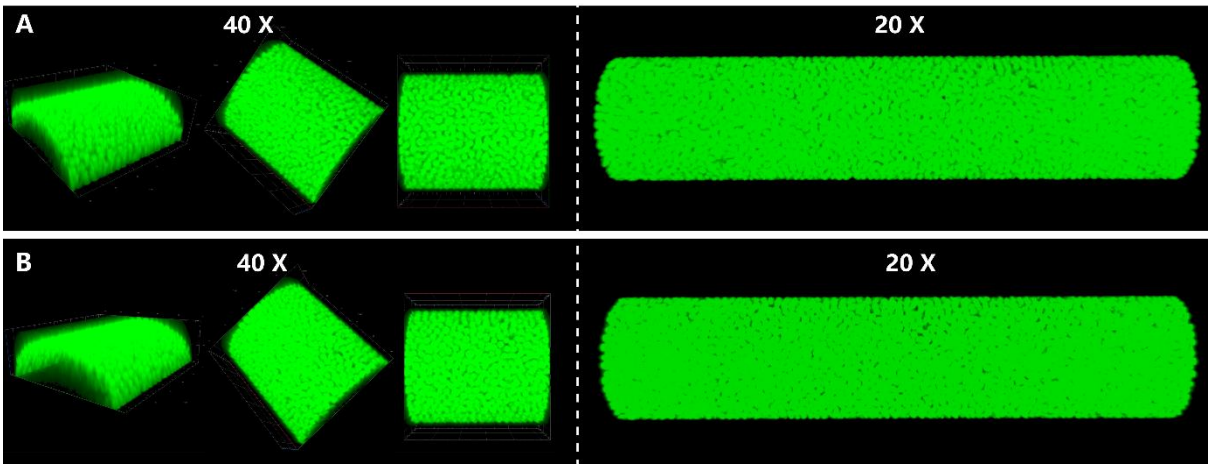

Figure S1. 3D rendering of reconstruction morphology of column bed scanned by CLSM. (A) Bed morphology of the column prepared by segmented microfluidic method with 180 mg/mL particle slurry. (B) Bed morphology of the column prepared by conventional slurry packing method with 2 mg/mL particle slurry. In 40x objective lens, the range of observation is 108 μm long, 100 μm wide and 20 μm high. In 20x objective lens, the observation length extends to 425 μm.

EXPERIMENTAL S2

For nanoLC-MS analysis of HeLa protein digest standard, a 500 cm x 100 μm capillary column packed with Acquity Peptide BEH C18 (5 μm, 130 Å), and a 10 cm x 100 μm capillary column packed with Acquity Peptide BEH C18 (1.7 μm, 130 Å) were prepared using the segmented microfluidic method. A commercial capillary column, Acquity Peptide BEH C18 (10 cm x 100 μm, 1.7 μm, 130 Å) was used for comparison study. Gradient elution was adopted for the nanoLC separation, mobile phase A: water/ACN (98/2, v/v) solution containing 0.1% formic acid; mobile phase B: ACN/water (98/2, v/v) solution containing 0.1% formic acid. For 10 cm short nanoLC columns, the gradient elution program was set as: 0 min, 2% B; 5 min, 10% B; 105 min, 35% B; 125 min, 65% B; 145 min, 95% B. For the microfluidic packed 500 cm long nanoLC column, the gradient elution program was set as: 0 min, 2% B; 80 min, 20% B; 1680 min, 55% B; 2000 min, 75% B; 2160 min, 95% B; 2320 min, 95%B. Flow rate: 300 nL/min; injection amount: 1 μg HeLa protein digest. The nanoLC-MS/MS analysis was conducted on Waters ACQUITY nano-UPLC/Q-Exactive mass spectrometer, online electrospray of the eluting peptides into the Q-Exactive mass spectrometer was achieved with the Easy Spray ion source (Thermo Fisher Scientific, USA). Full MS spectra were recorded at a resolution of 70,000 over a mass range of 350 to 1800 m/z. The automatic gain control target was set to 1×10^6 and a maximum injection time of 50 ms was allowed. The 15 most intense peptide ions were chosen for fragmentation. The MS/MS spectra were recorded at a resolution of 17500 with the automatic gain control target set to 1×10^5 and a maximum injection time of 100 ms. A mass window of 2.0 m/z was applied to precursor selection. Normalized collisional energy for the higher-energy collision-induced dissociation fragmentation was set to 28%. A dynamic exclusion with a time window of 45 s was applied. The acquired nanoLC-MS/MS data were analyzed using Proteome Discoverer (version 1.4) for peptide and protein identification, and the database for HeLa cell lysate was downloaded from Uniprot-SwissProt human database (Reviewed UP000005640 (20397)), parameters were set as follows: Min Peptide length: 4; Max Peptide length: 30; Precursor Mass Tolerance: 10 ppm; Fragment Mass Tolerance: 0.8 Da.