Multinozzle Emitter Array Chips for Small-Volume Proteomics

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Supporting Information

1. Experimental Section

MEA chip design, fabrication, and on-chip bead packing. The next-generation MEA chips were designed using the L-Edit software (v15, Tanner Research Inc.). The fabrication procedures were similar to what we have described in detail for the first-generation MEA chips [1]. As shown in **Figure 1**, all channels on the chip were created between the two silicon wafers. A 2 μm frit was implemented at the end of the LC channels to retain beads. LC channels were packed with Zorbax SB-C18 5 μm beads (pore size of 80 Å, Agilent Technologies). Briefly, beads were suspended in 2-propanol and sonicated to form a solution of mono-dispersed particles. Then the slurry of particles was forced into the channels on the chip through the sample input holes by a pressurized (> 500 psi) helium gas tank. The pressure gauge was shut off in 20 minutes and the system was slowly depressurized for about one hour before switching to the atmosphere pressure. The quality and reproducibility of bead packing was confirmed by monitoring the backpressure of the LC channel under a constant flow rate (e.g., 1 μL/min), and the efficiency of LC separation was validated by the LC-MS analysis of GFP and BSA tryptic digests.

MEA chip assembly. To establish a robust and high-quality fluidic connection to sustain high pressure for on-chip LC separation, we built a manifold to mechanically assemble the MEA chip with capillary tubings that were connected to the outside nanoflow source (**Figure 1d** and **Suppl. Figure 1**). The chip was sandwiched between a PEEK clamping plate and a steel plate, and tightly clamped by screws with a gasket in-between to prevent fluid leakage. The top PEEK plate had 24 threaded ports for Upchurch fittings to provide connections with capillary tubings. The cylinder face of the steel plate was covered with Kapton tape (polyimide film) for electric insulation. The sandwich was then attached to a plastic (acrylic) adapter plate, which was mounted to a motorized rotation stage (Thorlabs), using the screw in the center hole. For easy and precise assembly, four alignment pins were implemented, each on a quarter of the assembly. No fluid leakage was observed for the chip assembly for flow under a pressure of up to 1000 psi.

Preparation of blood cell lysate and trypsin digestion. A 10 μL aliquot of whole blood obtained from healthy donors (Innovative Research, Inc) was diluted in 1× PBS buffer to make cell suspensions of 1×10^6 cells per mL, as validated by Coulter Counter (Beckman Coulter). 100 μL of cell suspension (1×10^5 cells in total) was spun down in a centrifuge at a speed of 5,000 rpm. Supernatant was removed and precipitated cells at the bottom were lysed by freeze/thaw cycles using dry ice. For direct infusion MS analysis of proteins using unpacked channels on MEA chips, blood cell lysate was suspended in 50/50 acetonitrile/H_2O+0.1% formic acid and spun down in a centrifuge to precipitate cell lysate debris. The supernatant was collected and analyzed. The final concentration corresponded to proteins from a lysate of ~200 cells per μL.

For LC-MS analysis of tryptic peptides using channels packed with C18 beads, blood cell lysate was suspended in 25 mM ammonium bicarbonate and spun down to precipitate cell debris. The supernatant was collected and digested by sequencing grade modified porcine trypsin (Promega) at a trypsin: protein ratio of 1:50 (w/w). The mixture was incubated at 37 °C overnight and afterwards 0.6 μL of 10 % formic acid was added to stop digestion. The final sample concentration corresponded to proteins from lysate of ~2000 cells per μL. The sample was aliquoted and stored at -20°C until further analysis.

Nanoelectrospray mass spectrometry. All MS experiments were performed on a hybrid quadrupole/orthogonal Q-TOF API US mass spectrometer (Waters Corp.) with the same MS and MS/MS settings as we described before [1]. The MEA chip assembly was mounted on a rotation stage with an automatic motorized control, which was further attached to a XYZ translational stage (**Suppl. Figure 1**). The solvent was delivered to the on-chip channels from a nanoflow source through connecting tubings. Individual MEA chip emitter was positioned in front of the MS ion cone for nanoelectrospray in the Z spray geometry. High voltage cable was connected to the center region of the silicon-based MEA chip via a metal wire, and provided up to 5 kV for electrospray. This connection strategy minimized tangling between the cable and capillary tubings, and eliminated the electric arcing, during the rotation of the chip assembly. Electrospray process was visualized and monitored using a Waters nanoflow camera kit equipped with a MLH-10 Zoom lenses (Computar). Protein samples (BSA or hemoglobin from red blood cells) were analyzed using MEA emitters by direct infusion using unpacked channels at a flow rate of 600 nL/min.

On-chip LC-MS/MS analysis. A capillary liquid chromatography system (CapLC) (Waters Corp.) was used to deliver nanoflow for LC separation on the MEA chip. Peptide separation was performed at a flow rate of 300 nL/min using the same LC gradients as we described before [1]. Sample was injected through an autosampler and LC-MS/MS was performed using on-chip LC channels. The most abundant peptide ions for each target protein were used for quantification. Extracted ion chromatograms were acquired to obtain peak areas for selected peptide ions. Peak areas were plotted against the concentrations of protein digests. BSA tryptic digests MS standard (Michrom Bioresources, Inc.) was used to validate the performance of on-chip LC-MS/MS analysis. The peptide ion YICDNQDTISSK (*m/z* 722.8) was selected for calibration and quantitation. The tryptic digests of hemoglobin (both α and β subunits) from RBCs in whole blood were analyzed by LC-MS/MS. The most abundant tryptic peptide ion VNNDEVGGEALGR (*m/z* 657.8) was from the hemoglobin β subunit, and selected for quantifying the number of RBCs. The limit-of-detection (LOD) was defined as the lowest concentration point of target proteins at which the signal-to-noise ratio (S/N) of surrogate peptides was at least 3. S/N was calculated by the peak apex intensity over the average background noise in a retention time region of ±5 min for target peptides.

2. Supplemental Figures

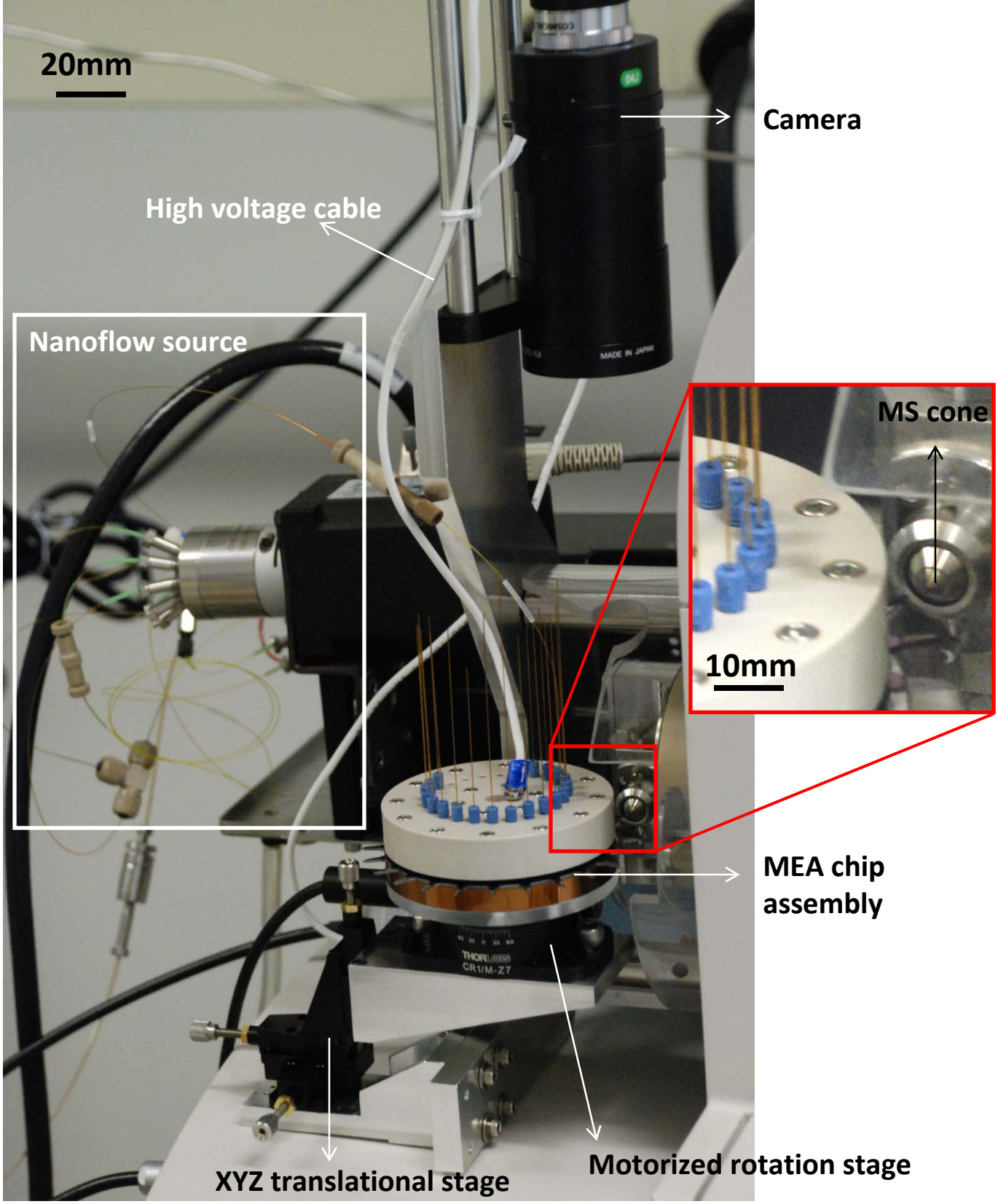
Supplemental Figure 1. The experimental setup for operating the 24-plex MEA chip. It includes a nanoflow source to provide the solvent gradient flow into the MEA chip through tubing connection, a MEA chip assembly, a motorized rotation stage, and a XYZ translation stage to finely tune the position of a particular MEA emitter towards the MS cone, a cable for high voltage supply, and a camera to visualize the position and electrospray process of MEA emitters. The insert shows the Z spray geometry of the emitters on the MEA chip assembly relative to the MS cone of the Waters Q-TOF API US mass spectrometer.

Supplemental Figure 2. Performance validation of the 24-plex MEA chip. (**a**) Reproducibility of on-chip LC-MS analysis. Standards of 1 pmole GFP were injected and analyzed by a LC channel packed with C18 beads on the MEA chip. Extracted ion chromatograms of *m/z* 785.8 ion from four consecutive runs are shown. The retention times are indicated for each run. (**b**)

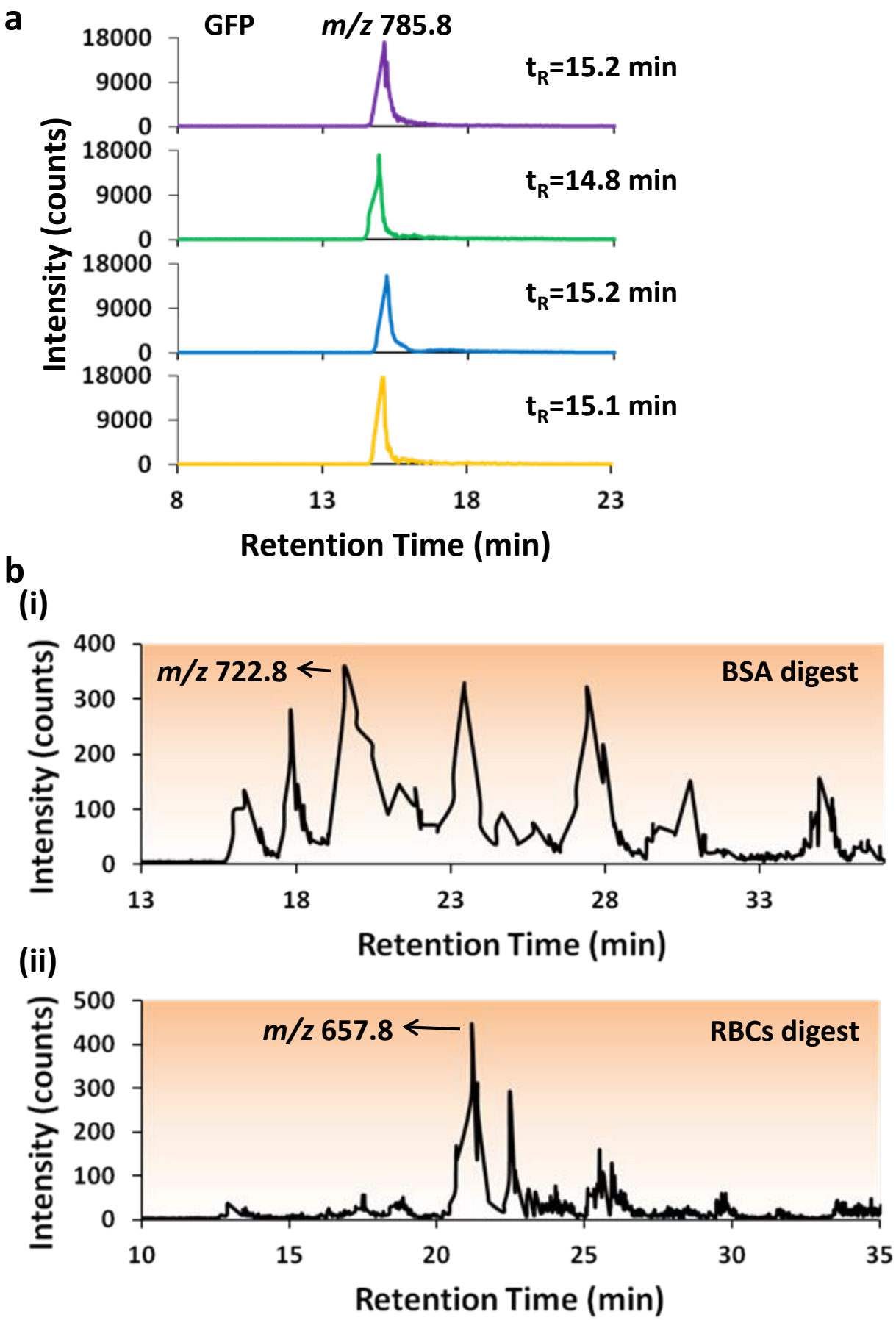
Efficiency of on-chip LC-MS separation. Base peak intensity (BPI) chromatograms for LC-MS analysis of tryptic-digested BSA (**i**); and RBC lysate (**ii**) are shown. 100 fmole BSA digests and lysate of ~6000 RBCs were used for the analysis, respectively. The arrows indicate the retention time of the base peaks for selected tryptic peptide ions (*m/z* 722.8 and 657.8 for BSA and RBC lysate, respectively). The unsmooth lines in the BPI chromatograms were due to the switching between MS and MS/MS mode during data-dependent acquisition.

3. References

(1) Mao, P.; Wang, H. T.; Yang, P.; Wang, D. *Anal Chem.* **2011**, *83*, 6082-6089.



Supplemental Figure 1



Supplemental Figure 2