Multinozzle Emitter Array Chips for Small-Volume Proteomics

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ABSTRACT: High-throughput multiplexed proteomics of small-volume biospecimens will generate new opportunities in theranostics. Achieving parallel top-down and bottom-up mass spectrometry analyses of target proteins using a unified apparatus will improve proteome characterization. We have developed a novel silicon-based microfluidic device, multinozzle emitter array chip (MEA chip), as a new platform for small-volume proteomics using liquid chromatography-nanoelectrospray ionization mass spectrometry (LC-nanoESI-MS). We demonstrate parallel, on-chip, and online LC-MS analysis of hemoglobin and its tryptic digests directly from microliters of blood, achieving a detection limit of less than 5 red blood cells. Our MEA chip will enable clinical proteomics of small-volume samples.

Given that proteome reflects the physiological and pathological states of a patient, proteomics is a powerful tool for early diagnostics of diseases and monitoring of therapeutic responses. The majority of current protein assays in clinical settings are based on enzyme-linked immunosorbent assays (ELISA), which require high-quality antibodies and are hard to achieve high multiplexing (>10) due to the cross-reactivity of antibodies. Mass spectrometry (MS) measures the mass-to-charge ratio of charged species and has become an enabling technology for proteomics.1,2 Aside from de novo identification of target proteins, MS has advantages over ELISA for detecting protein modifications, mutation, truncations, adductations, etc. Once hyphenated with the LC, LC-MS enables separation, identification, characterization, and quantitation of complex mixtures of proteins and peptides. However, the penetration of MS into the in vitro diagnostics market particularly for clinical proteomics has remained low.3−5 Key challenges remain for the MS-based platform to achieve robustness, sensitivity, and throughput comparable to those of ELISA for analysis of small-volume biospecimens such as blood and urine.6−10

In this Letter, we report the design, fabrication, and proof-of-principle applications of 24-plex multinozzle emitter array (MEA) chips for on-chip and online LC-nanoESI-MS analysis of low-volume whole blood samples. The chip is built on our recent breakthroughs in developing the silicon-based microfabricated monolithic multinozzle emitters (M³ emitters) and the first-generation MEA chips for nanoelectrospray MS.11,12 These devices collectively offer a straightforward yet novel solution to the longstanding problem of the efficient coupling between silicon microfluidic chips and ESI-MS and pave the way for large-scale integration on microfluidic chips for MS-based proteomics. Our previous MEA chips achieved both high-sensitivity (via multinozzle emitters) and high-throughput (via multichannel) MS on a single silicon microfluidic chip. However, proteins and peptides were separated by off-chip cartridge-based LC columns, and the fluidic interface was not robust enough for automatic control of high-throughput multiplexed proteomics. Our next-generation MEA chips directly addressed these challenges.

As shown in Figure 1, our MEA chip consists of 24 identical units uniformly distributed in a circular array format on a 4-in. silicon substrate (Figure 1a,c). Each unit (Figure 1b) contains three key functional modules: an input hole for sample injection, a LC channel (with a frit) packed with beads of desired coatings for analyte separation (or unpacked for direct infusion), and a multinozzle emitter for nanoESI-MS identification and quantitation of proteins and peptides. The LC channel has a cross section of 200 μm × 200 μm and a total length of 5 cm (with turns). The emitter has protruding nozzles with a cross-sectional area of 10 μm × 10 μm, a length of 150 μm, and a wall thickness of 0.5 μm. The emitters were constructed between the two silicon layers. The end of the emitter stem was sharpened with an angle of ~20° to enhance the electric fields at the nozzle tips.12 In addition, the chip contains through-holes designed for screws and alignment pins for connection to a fluidic manifold assembly (Figure 1d). The full assembly contains four layers including a polyetheretherketone (PEEK) plate, MEA chip, steel plate, and plastic plate. The top PEEK plate has 24 threaded ports for Upchurch fittings to provide leak-free connections with capillary tubings for delivering solvent gradients from a nanoflow source for LC separation (Suppl. Figure 1, Supporting Information). High voltage for electrospray ionization is provided through a metal wire connected to the center of the MEA chip.

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We first validated the performance of MEA emitters for MS analysis of standard proteins and peptides (Figure 2a). We demonstrated electrospays from emitters with varying numbers of nozzles (Figure 2a-i) and confirmed that the MS sensitivity using MEA emitters was approximately proportional to the square root of the number of spraying nozzles (Figure 2a-ii).13 Importantly, multinozzle emitters achieved sensitivity significantly higher than that of the commercial Picotip capillary emitters. However, the 10-nozzle emitter was a clear outlier. This was probably due to two factors. First, the maximum voltage (5 kV) provided by our current mass spectrometer might not generate electric fields high enough to produce cone-jet mode spray for all the 10 nozzles uniformly. Second, ion collection and transmission by the Z-spray sample cone of our current mass spectrometer was much less efficient for 10-nozzle emitters, because electrosprays were spread out significantly, resulting in a plume much larger in size than the MS cone inlet. Future implementation of a funnel-shaped sample cone for more efficient ion collection14 may further increase MS sensitivity for multinozzle emitters.

We next validated the performance of 24-plex MEA chips as a unified platform for parallel analysis of standard proteins and their tryptic peptides. We used one unpacked channel for direct infusion MS analysis of full-length proteins and another channel packed with C18 beads for LC-MS analysis of their tryptic digests. We first confirmed the reproducibility of on-chip LC-MS analysis using Glu-fibrinopeptide B (GFP) standards (Suppl. Figure 2a, Supporting Information). We then tested the separation efficiency and detection sensitivity using bovine serum albumin (BSA) and its tryptic digests (Figure 2b and Suppl. Figure 2b(i), Supporting Information). We achieved high-sensitivity detection of full-length BSA (~24 fmole) using direct infusion MS analysis and a limit of detection (LOD, signal-to-noise ratio S/N = 3) of ~400 attomole (i.e., 10^{-18} mole) BSA tryptic digests for the m/z 722.8 ion (YICDNQD- TISSK) using LC-MS/MS. We observed a linear correlation between the LC-MS peak area and the amount of BSA tryptic digests (Figure 2b-ii). The MS sensitivity is comparable to those of conventional cartridge-based LC-nanoESI-MS or HPLC-chip/MS (Agilent), which are typically in the range of ~1 fmole for detecting BSA tryptic peptides.

We then evaluated the performance of our MEA chips for small-volume proteomic analysis of pooled human blood samples. We started with less than 10 μL of whole blood. By direct infusion MS of the blood lysate, we confidently detected the heme group and several multiply charged species of hemoglobin (Hb) α and β subunits from as few as 5 red blood cells (RBCs) (containing ~2.3 fmole Hb) (Figure 2c-i). This sensitivity is still lower than that of the current ELISA assay, which is typically in the range of 0.1−1.0 fmole (assuming 1−10 μL of 100 fmol/mL samples), depending on the characteristics of the antibody–antigen interaction. We achieved a LOD (S/N = 3) of Hb tryptic digests from ~90 RBCs for the m/z 657.8 ion (VNNDEVGGEALGR) using LC-
We also observed a linear correlation between the LC-MS peak area and the number of lysed RBCs (Figure 2c-ii). Therefore, one of the immediate clinical applications of our MEA chips may be low-cost and high-throughput detection of Hb variants using a pinprick of blood in newborns.
screening. With both top-down and bottom-up MS analyses of Hb using extremely small volumes of blood on a single device, our MEA chip platform is expected to have distinct advantages in accuracy, resolution, and throughput for Hb analysis over existing methods, including matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS and direct infusion electrospray MS.

In summary, we have developed a Si-based, scalable, and integrated MEA chip that has the potential to become an enabling platform for MS-based small-volume proteomics. We have demonstrated on-chip and online LC-MS analysis of microliters (e.g., pricks) of human blood. On our MEA chips, multinozzle emitters improve MS sensitivity, and on-chip and online multichannel LCs increase throughput. Detection of full-length proteins and their tryptic peptides on a unified platform facilitates protein identification, characterization, and quantitation. Our 24-plex MEA chip also opens up the possibility of multiplex analysis of multiclass analytes in parallel from a single sample on the same platform. For example, by packing separate LC channels using C4 and C18 beads, we can characterize the plasma proteome at both protein and peptide (endogenous or tryptic digests) levels. We can also use TiO2 beads for on-chip enrichment of phosphopeptides and phosphoproteins for phosphoproteomics. The separation efficiency of on-chip LC columns can be improved by increasing their length (e.g., to 15 cm) using more turns or larger wafers (e.g., 6 in.). In addition, implementation of sub-2 μm particle columns may enable UHPLC capability and hence improves chromatographic separation. To be comparable to immunoassays, the ESI-MS sensitivity of MEA emitters can be further improved by increasing their nozzle numbers and optimizing their geometry, configuration, and sharpening. The detection sensitivity of MEA chips can be further increased by interfacing them with the latest mass spectrometers such as Q-TOF SYNAPT G2MS (Waters Corp.), which has the sensitivity for peptide detection and quantitation in the low attomole range. The throughput of MEA chips can be further increased by integrating more channels (e.g., from 24 to 96) and by optimizing the multiplex LC operation to increase the MS duty cycle using the strategy of staggered parallel separations. If incorporated with functional modules for sample preparation such as cell lysis and on-chip protein digestion, plus automatic fluidic switching among on-chip LCs, and further interfaced with quantitative proteomics strategies such as multiple reaction monitoring (MRM) and “sequential window acquisition of all theoretical fragment-ion spectra” (SWATH), our MEA chip will serve as a low-cost, fully-integrated, high-sensitivity, and high-throughput platform for multiplexed proteomics of small-volume biospecimens.

■ ASSOCIATED CONTENT

1 Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): D.W. is the founder and P.M. is the co-founder of Newomics Inc.

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