NanoMEA Chip Platform for Proteomics

Booth #507

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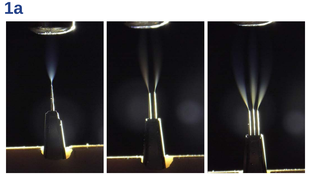
INTRODUCTION

Proteomics of small volumes of biological samples down to single cells has progressed rapidly. However, the sensitivity and reproducibility of ultralow flow LC-MS for proteomics has remained a challenge. We developed a silicon-based, plug and play, nanoflow Multinozzle Emitter Array (nanoMEA) chip to directly address this challenge. NanoMEA chip monolithically integrates our M3 multinozzle emitter and an on-chip LC column, thereby reducing the dead volume and simplifying the plumbing and connection for ultra low flow LC-MS. Herein, we benchmark the performance of nanoMEA chip for small-volume bottom-up proteomics

1a



1b

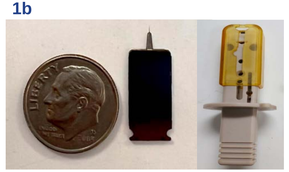


Fig 1a. Newomics nanoMEA chip with a single, double, and triple-nozzle emitter. **Fig 1b.** Newomics nanoMEA chip, bare chip and inside the cartridge as illustrated with a dime.

METHODS

NanoMEA chips were designed using the L-Edit software (v15, Tanner Research Inc.) and fabricated as described [Anal.Chem.2011,83,6082–6089]. The fabricated devices were examined by optical microscopy using a Reichert-Jung Polylite 88 microscope (Reichert Microscope Services) and by scanning electron microscopy using a JEOL 6340F FEG-SEM (JEOL Ltd.). On-chip columns were packed with C18 beads (Dr. Maisch) for bottom-up proteomic applications. Nanoflow LC-MS and proteomic analysis were performed using a Newomics MnESI ion source interfaced to a Thermo Fisher Orbitrap QE+ with a Dionex Ultimate 3000 UPLC. Standard BSA protein tryptic digests as well as HeLa and *E. coli* digests were used for testing the performance of two nanoMEA chips.

Methods	nanoMEA CHIP A	nanoMEA CHIP B
Emitter/Sprayer	MEA emitter (10 µm ID, 1-nozzle)	MEA emitter (10 µm ID, 1-nozzle)
Spray Voltage (V)	2500	2500
Capillary Temperature	250 °C	250 °C
LC Column	75 µm X 15 cm X 1.5 µm C18	75 µm X 20 cm X 1.5 µm C18
Flow rate (nl/min)	300	300
Back Pressure	~225-275 bar	~325-400 bar
LC system	Dionex Ultimate 3000	Dionex Ultimate 3000

2a

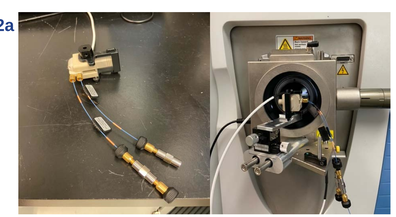


Fig 2a. Plug-and-play nanoMEA chip housing (left Fig.). The nanoMEA chip stage was integrated into the Newomics MnESI ion source (right, Fig. Thermo Fisher legacy version).

2b

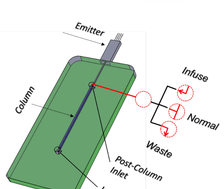
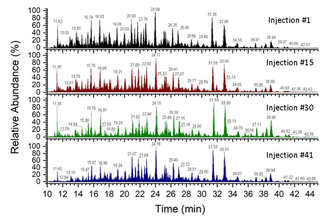


Fig 2b. Functional illustration of the nanoMEA chip. The post column inlet (PCI) affords several novel operating modes. Infusion mode allows for post-column addition of solutions directly into the flow. It is suitable for procedures such as doping and post-column reactions. In the waste mode the PCI also serve as a way to divert column flow from the emitter and MS to the waste during sample loading and column washing/reconditioning.

RESULTS

3a



3b

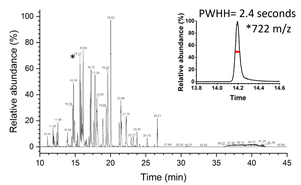
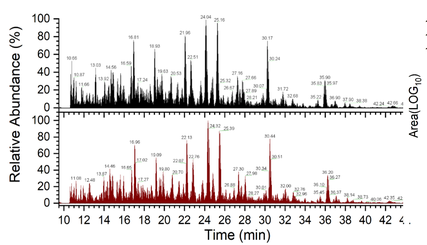


Fig 3a. Consecutive 41 LC-MS runs with 200 ng *E. Coli* digest using nanoMEA CHIP A. The average RSD for the peak retention time for 15 *E.coli* peptides was determined to be 0.30%. **Fig 3b.** Peak width of 2.4 seconds was observed for the peptide with m/z=722 from the LCMS run of BSA digest using nanoMEA CHIP B.

4a



4b

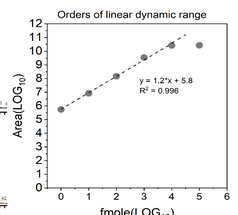
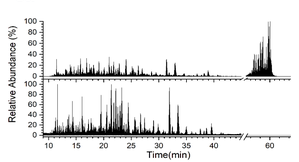


Fig 4a. Reproducibility between two identical nanoMEA CHIP B was assessed by comparing Hela cell digest LCMS runs. Protein counts for each LCMS run did not vary more then 3% between CHIPS. **Fig 4b.** Four orders of linear dynamic range were achieved with the nanoMEA CHIP A using BSA peptide 722 m/z and a Thermo QE+ mass spectrometer.

5a



5b

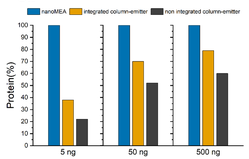


Fig 5a. Two *E. coli* LCMS2 runs were done to illustrate the wash mode for the nanoMEA CHIP A. The first was run in normal mode whereas the second was run in wash mode (open and divert waste using the post column inlet during sample loading and cleaning). **Fig 5b.** Hela digest LCMS2 runs were performed at three different concentrations using the nanoMEA CHIP B, the commercially available integrated column-emitter and non-integrated column emitter.

CONCLUSIONS

1. We have developed the nanoMEA chip that is suitable for proteomic analysis by nanoflow LC-MS, and more sensitive than the commercially available solutions across three sample concentrations.
2. We introduce a novel operational mode for the nanoMEA chip that utilizes the unique post column inlet. The infusion mode allows for the addition of post column solutions. Using the infusion mode, we doped ACN into the LCMS stream and increased the sensitivity by ~25% (data not shown).
3. We introduce another novel operational mode for the nanoMEA chip that utilizes the chip's unique post column inlet to greatly enhances robustness. During column sample loading and column washing, the stream can be diverted through the post column inlet and to the waste, thereby prolonging the lifetime of the emitter tip while reducing the MS contamination.