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ThP 099 NanoMEA Chip Platform for Proteomics

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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 challange. We developed a silicon-based, plug and play, nanoflow Multinozzle Emitter Array (nanoMEA) chip to directly address this challenge. NanoMEA chip monothically integrates our M3 multinozzle emitter and an on-chip LC column, thereby reducing the dead volume and simplifying the plumbing and connection for ultraflow LC-MS. Herein, we benchmark the performance of nanoMEA chip for small-volume top-down and bottom-up proteomics.

Methods

NanoMEA chips were designed using the L-Edit software (v15, Tanner Research Inc.) and fabraicated as we published before [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 and C4 beads for bottom-up and top-down proteomcis applications, respectively. Nanoflow LC-MS and proteomic analysis were performed using a Newomics MnESI ion source interfaced to a Thermo Orbitrap Eclipse Tribrid with a Vanquish Neo UPLC, or a TIMS-PASEF TOF equipped with a nanoElute. Standard proteins and their tryptic digests as well as HeLa digests were used for testing the performance of our nanoMEA chips.

Preliminary Data

We developed a prototype MEA chip with 10 um single and double emitters and an on-chip LC column. The columns have 75um and 150 um id with a length ranging from 5 cm to 20 cm. The chips were tested with a variety of chromatography packing beads, including 1.9 um C18 packing for RPLC and 3 um C4 packing for intact protein analysis.We achieved 3 second peak widths using C18 packing for peptide 722 (m/z) from BSA digests. A comparason was made for a nanoMEA chip with a chip based internal column: 75um X 15cm and 1.9 um packing. This prototype chip was developed with one nozzle and with two nozzles. We show that an 50-80% increase in sensivity when we used two nozzles rather then 1 nozzle. As such, two nozzles splits the 400 nl/min flow rate for each 10 um nozzle to 200 nl/min, thereby increasing the sensitivity through increase in ESI efficiency.We compared the nanoMEA chip ptrototype to traditional ESI in details. A fused silica column with the same column demensions and packing was produced in house and connected to a 10 um silica Picotip emitter tip. We found that the single nozzle nanoMEA Chip had a 25% lower peak width and 50% higher sensitivity for BSA digests and a double nozzle MEA chip had nearly 2-fold higher overall sensitivity. Proteomic analysis for the 2 nozzle nanoMEA chip yielded 3 fold higher peptide counts for lower amounts of Hela digest when compared to the traditional ESI.

Novel Aspect

First demonstration of a monolithic silicon microfluidic chip that integrates a LC column with multinozzle emitter for ultralow flow proteomics.