TECHNICAL REFINEMENTS OF THE MICROFLUIDIC INTEGRATED SELECTIVE ENRICHMENT TARGET FOR IMPROVED SOLID-PHASE EXTRACTION

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ABSTRACT

The Integrated Selective Enrichment Target (ISET) is a microfluidic platform for solid-phase extraction (SPE) sample preparation and sample presentation prior to matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS). The original design of the ISET consisted of an array of nanovials manufactured by wet-etching of 360 μm silicon, each having a single inlet and outlet. Here it is shown that by substituting the single hole outlet for a 3 x 3 array of outlets for each nanovial, the usefulness, flexibility and robustness of the ISET sample preparation platform is increased.

KEYWORDS: Solid-phase extraction, Mass spectrometry, Proteomics, MALDI MS, ISET

INTRODUCTION

Previously we have demonstrated the efficiency of the ISET platform for reversed-phase extraction of proteomics samples prior to MALDI MS [1,2]. The ISET sample preparation device has also been used in combination with our acoustic sequential/trapping platforms [3, 4] in order to concentrate dilute samples and for performing on-bead digestion of captured proteins [5]. The previous in-house wet-etched pyramidal ISET had worked well from a fluidics perspective, but in certain applications, e.g. biomarker analysis in plasma, a higher binding capacity was desired. The use of wet-etching puts a limit to the ISET/wafer thickness that can be manufactured with sufficient reproducibility of the outlet holes. This thickness limitation obviously limits the volume that can be filled with beads for the selective enrichment of analytes.

In order to address the need of increased capacity and also provide a single-use device with a low cost/analysis a polymeric ISET device was developed and characterized [6]. Unfortunately, the high cost of prototyping using injection molding prevented further progress of this polymeric ISET device. Additionally, previous work had identified two further areas where the single hole outlet ISET device could be improved; The use of soft material beads (e.g. agarose) resulted in clogging of the outlet, and when using a pre-filled ISET in conjunction with automated robotic liquid handlers the amount of liquid that could be loaded onto each ISET position was limited (5-10 μL) by the slow transport provided the single outlet. By implementing an array based outlet hole, i.e. an outlet consisting of several holes, these prior shortcomings of the ISET platform can be addressed.

Figure 1: The example illustrates a reversed-phase ISET sample preparation using vacuum to facilitate liquid transport through the positions. Right to left first pre-packed beads or beads with analyte are loaded (1), this is followed by a wash (2) step to remove contaminants. The analytes are then eluted (3) using the MALDI matrix in an organic solvent at lower vacuum, in order to get the eluted analytes/matrix to crystallize on the underside of the ISET. Finally the ISET is flipped (4) and subjected to MALDI MS analysis. Top right insert shows a close-up of a square 3x3 array outlet hole.
EXPERIMENTAL

The DRIE etched ISET devices where manufactured by GeSiM (Großerkmannsdorf, Germany). All chemicals and peptides/proteins used in this study where bought for Sigma-Aldrich (St. Louis, MO, USA). MALDI mass spectrometry analysis was made using both a Waters M@ldi MS (Milton, MA, USA) and a MALDI-LTQ Orbitrap XL from Thermo Scientific (Waltham, MA, USA). For the automated robotic sample preparation a Biomek 3000 (Beckman Coulter, Brea, CA, USA) or a custom built 8-channel solenoid system (Seyonic SA, Neuchâtel, Switzerland) was used for liquid handling. The beads used for the geometry: area study was Poros™ R2 50 μm beads from Applied Biosystems (Foster City, CA, USA).

RESULTS AND DISCUSSION

The first effort to manufacture ISET devices with increased capacity was made by a direct adaptation of the original ISET design for fabrication in 760-780 μm silicon, with single 15 μm outlets made by DRIE etching. This resulted in two serious problems. The fluorocarbon layer inherently deposited in the DRIE etching process together with a 15 μm single outlet resulted in too large backpressure for reproducible handling of aqueous solutions. Secondly due to the manufacturing process the bead trapping nanovials had to be flat bottomed with a small outlet hole in the centre (middle figure 2) a design more prone to clogging than a pyramidal hole. Also, the initial wetting when liquid is applied to a bead filled perforated nanovial is driven not only by the vacuum but also by capillary force. When wetting by capillary force were faster than the flow rate out from the outlet, the liquid went under the beads and displaced them from the nanovial. This problem was exaggerated when applying water to a hydrophilic ISET nanovial surface filled with hydrophobic beads.

In order to understand how the outlet geometry affects the ISET sample preparation a chip with 96 perforated nanovials spanning 3 different hole geometries (3 x 1 rectangular, 3 x 3 round, 3 x 3 square) and 4 different total outlet areas. This design provided 8 positions of each area and geometry, grouped as shown in figure 3. It was found that the rectangular 3 x 1 array of rectangular holes was promising, but prone to breakage during sample preparation. The round 3 x 3 outlet array was very prone to clogging and only the two largest outlet areas provided acceptable performance. While the square 3 x 3 outlet array was the overall best geometry for robustness of liquid handling in the ISET sample preparation process.

The presented results represent MALDI MS analysis of > 1000 standard samples using ISET reversed-phase SPE sample preparation. This protocol was used for the evaluation because it is well characterized on previous ISET designs. Sample cross contamination was investigated by processing standard samples, in adjoining ISET positions with different concentrations, and it was found that the array outlet did not result in any problems with cross-contamination (data not shown).

In order to investigate the sample preparation reproducibility samples of the same standard digest (50-100 fmol/pos) was processed and analyzed using ISET nanovials prefilled with beads. A correlation between signal intensity and outlet total area was found, where the smaller area array outlet holes provided more intense signal, this was attributed to the slower more homogenous flow through these positions during binding as compared to the larger area holes, figure 4. This observation could be confirmed in an experiment comparing pre-filled ISET positions with transferring the analytes by incubation of the beads in the sample followed by packing into individual ISET positions, the incubated samples provided much higher signal intensity and more peptides having low hydrophobicity were observed.

The difference compared to the original ISET design that uses a pyramidal single hole outlet and this new array outlet design is that the keystone effect cannot be used for retaining the beads in the nanovial and that much larger volumes can be passed through ISET nanovials considerably faster. Another benefit of the array outlet design is that soft agarose beads can be used without clogging the outlets.
Figure 3: Shows area: geometries (μm²) of the evaluation ISET design and observed clogging events. Ok denotes area: geometry combinations providing a fast flow with high loading capacity (>25 μL). Slow flow denotes area: geometry combinations where the flow was not fast enough to enable direct instantaneous application of 25 μL liquid without risk of cross contamination.

CONCLUSION
The usefulness and ruggedness of the Integrated Selective Enrichment Target (ISET) sample preparation device for biomolecules prior to MALDI MS have been increased considerably. Based on the geometry: area optimization experiments two different chip designs, both with a square hole 3 x 3 array outlet and total areas of 4500 resp. 8500 μm² are currently used for biomarker screening, affinity assays and phosphopeptide ISET-SPE protocols in our laboratory.

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