NON-COVALENT ANTIBODY IMMOBILIZATION ON POROUS SILICON COMBINED WITH MINIATURIZED SPE FOR ARRAY BASED IMMUNO-MALDI ASSAYS

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ABSTRACT

This contribution presents a new strategy to combine the power of antibody based capturing of target species in complex samples on porous silicon (PSi) with the benefits of microfluidic solid-phase extraction sample preparation on an Integrated Sample Enrichment Target (RP-SPE ISET) and the analysis speed of MALDI MS, figure 1. The method provides high capacity, low background, and simple coupling/immobilization of the antibody as well as being amendable for automation [1].

KEYWORDS: Immuno-MALDI MS, Porous Silicon, Antibody array, solid-phase extraction

INTRODUCTION

Proteomics has spread widely into every field of life science and medicine as an important part of the post-genomic research era. One of the difficulties in proteomics is the high complexity of the samples the that necessitates high-resolution separation technologies interfaced to high sensitivity detection. In this perspective, the combination of immunocapture and Matrix Assisted Laser Desorption/Ionization Mass Spectrometry (iMALDI MS) is a highly desirable combination since the immunocapture reduces the complexity of the sample to be analyzed at the same time as the target species is enriched, thereby increasing the possibility of detecting the analyte of interest at lower abundant levels [2].

EXPERIMENTAL

The immunoaffinity step was performed on an in-house developed 3D-structured high surface area porous silicon (PSi) matrix, which allows efficient antibody immobilization by surface adsorption without any coupling agents in 30-60 minutes [3]. The hydrophilic nature of the porous silicon surface at molecular level provides a low adsorption of background peptides when exposed to complex protein digests or plasma samples, improving the conditions for the antigen specific capture and subsequent readout. At the same time the hydrophobic behavior, due to the nanostructured surface, of the PSi material in combination with the implementation of a silicon dioxide ring around each porous silicon spot effectively confines the samples solution to each antibody covered PSi array position during the assay. The protocol is illustrated in figure 1.

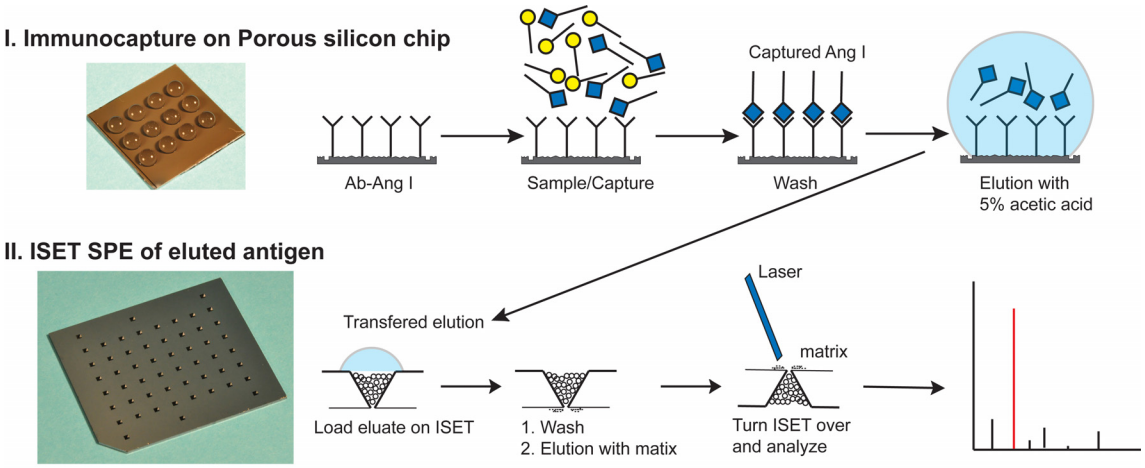

Figure 1: Schematic work-flow of immunocapture, top (I): antibody directly adsorbed on the high-surface area porous silicon is used to capture the antigen Angiotensin I (Ang I). Note confinement of the sample solution within the SiO2 ring. bottom (II) the eluted antigen is transferred and subjected to RP-SPE sample preparation protocol on the ISET chip followed MALDI MS detection.

RESULTS AND DISCUSSION

The use of a silicon dioxide ring around each porous silicon spot effectively confined the samples to each antibody covered PSi array position, circumventing carry-over and enabled loading of up to of 10 µL solution. The hydrophilic nature of the PSi allowed for detergent free assays, which in turn is paramount for the implementation of a SPE step after immunocapture. In earlier work we have reported a chip integrated micro array based solid-phase extraction platform for MALDI MS sample preparation, the Integrated Selective Enrichment Target (ISET) [4-6]. Here the ISET was used to address a key aspect in immunoaffinity MALDI assay, i.e. the SPE sample preparation step after the immunocapture that serves to remove buffer salts and enables re-concentration of the captured antigen after elution from the PSi into well-defined MALDI spots. Also if needed, additional sample preparation steps such as digestion of immunocaptured intact proteins can also be performed onbead in the ISET microarray [7].

A series of experiments were performed using an antibody against angiotensin 1 (Ang I) to verify the performance of the PSi-ISET iMALDI approach. In a first step a sample containing 5 nM Ang I spiked in 50 nM ADH digest before and after assay, was assayed with or without antibody on the PSi, figure 2. After the PSi-ISET iMALDI assay specific capture of the Ang I was unmistakably observed with a very low background (figure 2B).

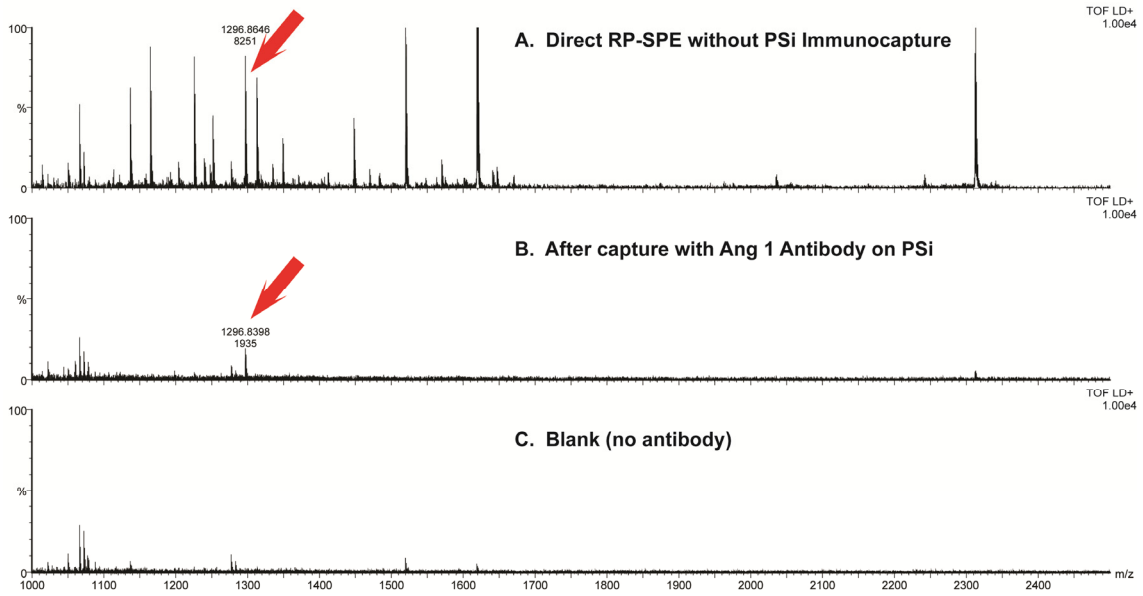

Figure 2: Specific capture of angiotensin 1. The top spectra (A) results from a direct ISET RP-SPE of 10 µl of a sample solution containing 5 nM Ang I and 50 nM ADH digest, without any immunocapture. Middle spectra (B) shows specific capture of Ang I (red arrow) from 10 µl of the sample used above, after immunocapture on porous silicon and ISET RP-SPE. Bottom spectra (C) show the results of an assay without Ang I-Ab on the porous spot. Note the reduction of complexity where very little unspecific binding can be observed on the porous silicon and that no Ang I can be observed.

Plasma represents the most widely available clinical material for large-scale clinical studies. In experiments using undigested plasma diluted 1:10 with PBS, Ang I could not be detected after a direct ISET SPE sample preparation step at low nM Ang I levels. Although, after processing the same samples on the PSi-ISET iMALDI platform Ang I could be clearly observed both at 5 and at 1 nM levels, figure 3.

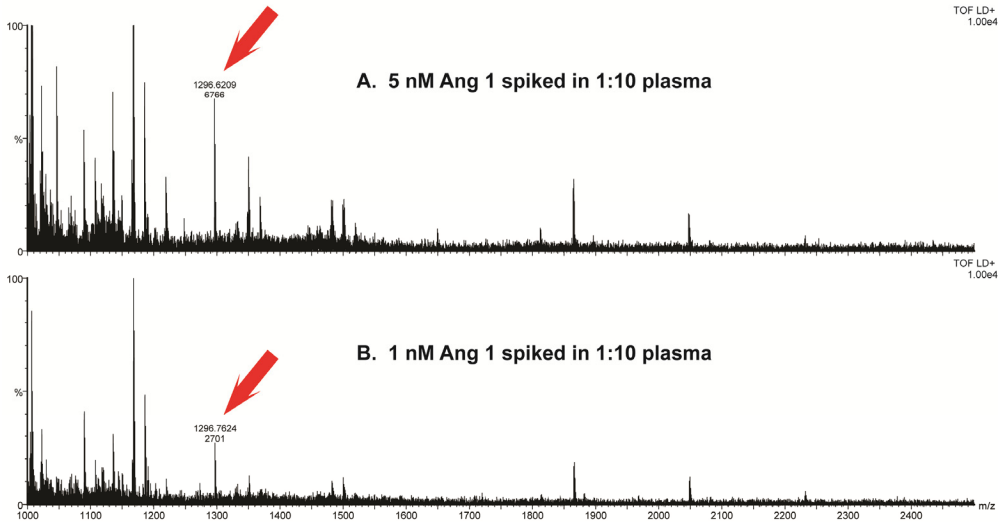

Figure 3: Immunocapture from plasma samples, 10 µl, plasma 1:10 diluted in PBS was spiked with low concentrations of Ang 1. Top spectra (A) plasma at a level of 5 nM and bottom spectra (B) spiked with 1 nM Ang 1. No Ang 1 signal could be observed by a direct ISET RP-SPE.

CONCLUSION

The presented PSi-ISET iMALDI platform combines for the first time the benefits of immunoaffinity capturing on PSi with microfluidic SPE in an array format for high speed readout using MALDI MS. Ongoing work will target multiplex arrays on the PSi capturing platform linked to automated sample handling and RP-ISET and MALDI MS readout in clinical samples.

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