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Nanoporous Organosilicates Thin Films for Selective Enrichment of Metabolites

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Abstract
In the present study, we propose a platform based on nanoporous SiOCH thin films to improve both selectivity and sensitivity of metabolites recovery, as well as metabolites stability, for Matrix Assisted Laser Desorption / Ionization (MALDI) mass spectrometry analysis. Results suggest that this platform could provide a significant enhancement for low molecular weight biomarker discovery, diagnostic and prognostic.

1. Introduction

The accurate annotation of human pathological fluids for early disease detection, therapeutic response prediction, and disease progression evaluation is a clinico-biological priority. However, the intrinsic complexity of the samples limits the detection of Low Molecular Weight (LMW) disease markers present in trace amounts within a background of abundant non-relevant molecules. Additionally, the detection of these LMW species remains a challenge due to experimental variability, stability during long sample processing, and generation of artifacts as a consequence of unreliable experimental procedures. Adequate sample preparation strategies remain an extraordinary technical challenge [1-2]. To address these bottlenecks that have impeded the use of mass spectrometry in biomarker discovery, we have developed a fast, efficient, and reliable system based on nanoporous organosilicates to improve the detection of specific metabolites.

2. Experimental procedure

Chip fabrication and characterization

Nanoporous SiOCH thin films with tunable features at the nanoscale were fabricated on Si substrates by plasma enhanced chemical vapor deposition [3]. Porosity was introduced via the co-deposition of an organo-silicon matrix and thermally labile organic species (porogens) followed by a UV assisted thermal annealing to remove the porogen part. Porosity was characterized by ellipsometry-porosimetry. Nanoporous SiOCH films with 0 - 30% porosity were obtained, the porosity being open and interconnected with mean pores radius close to 1.3 nm. Film thickness was varied between 180 and 1000 nm. Due to their chemical structure based on a cross-linked Si-O-Si backbone with methyl groups bonded to silicon, these materials are highly hydrophobic whatever the porosity, with a water contact angle of 100°. He plasma post-treatments were used to treat the film surface, leading to a carbon depletion resulting in a strong decrease of the contact angle to 20° with a limited impact on the bulk material.

Fluid fractionation

Nanoporous SiOCH films on Si substrate were automatically cut in 5x5 mm chips. 7.5 µl of urine or serum were spotted in duplicate onto the chip (Fig1) and incubated for 15 minutes at room temperature. The objective is to trap LMW species in the pores while removing larger species from the surface thanks to a washing with sterile deionized water. 8 µl of a matrix solution of 10 mg/ml 9-aminoacridine and 0.1% TFA were deposited on the sample before MALDI acquisition. Mass Spectra were analyzed by a statistical method based on Principal Component Analysis (PCA).

3. Results and discussion

Selective enrichment of metabolites

Mass spectra of serum sample fractionated on nanoporous SiOCH film, as shown Fig. 1, resulted in a significant improvement of species detection in the low mass range. As controls, the same serum sample was fractionated on a nonporous SiOCH film, and on a Si substrate. It can be concluded that it is the porosity and not the SiOCH affinity that constitutes the predominate factor in the enrichment of metabolites. In addition, the same serum sample was applied without washing on a nanoporous SiOCH film, to illustrate the signal suppression in the LMW region due to the presence of highly abundant high molecular weight species [4-5]. These results demonstrate that this physico-chemical-exclusion method enables a specific and significant enrichment of metabolites from complex biological fluids.

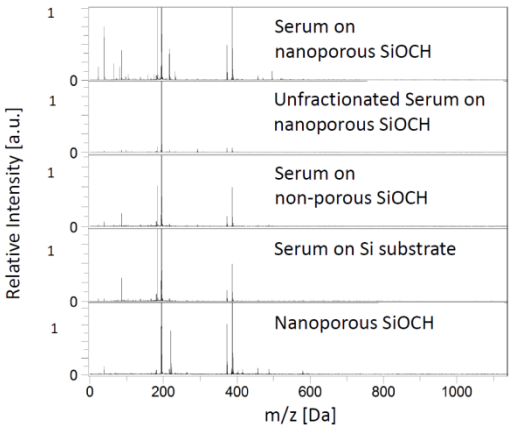

Fig. 1 MALDI Mass Spectra in the range 0-1000Da of serum sample fractionated on nanoporous SiOCH films and controls.

Physico-chemical film properties vs. metabolites recovery

Correlation between the harvesting capacity and the physico-chemical properties of films was studied. Results suggest that the more hydrophobic, the more porous and the

thicker SiOCH films are, the better harvesting capacity is in term of number of species detected and intensity of detection, as shown Fig. 2.

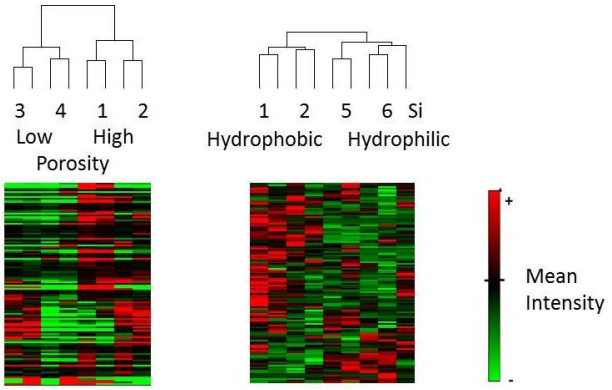

Fig. 2 Effect of porosity density and wettability on serum metabolites harvesting. Hierarchical clustering and specific recovery pattern for each set of films (duplicate): 1 – 2 – 3 – 4 are hydrophobic films with respective porosity of 30 – 20 – 13 – 0%, 5 – 6 are hydrophilic films with porosity of 30 – 20%. Pattern of serum on Si substrate is shown. The relative intensity is gradually indicated with red squares (high intensity), black squares (median) and green squares (low intensity, absence of peak). (SpecAlign and Cluster Treeview Toolbox)

Reproducibility of metabolites recovery

To assess the consistency of our fractionation strategy we screened 4 replicates with the same serum sample. The regression curve comparing the peak intensities recovered from the replicates demonstrated a high reproducibility with a coefficient of regression of 0.9.

Stabilization of metabolites

To assess the protection of trapped metabolites against degradation, *e.g.* enzymatic degradation, 3 nanoporous SiOCH films were incubated with serum, dried after washing, and stored for 3 weeks at room temperature. Metabolites patterns obtained were comparable with those of freshly fractionated serum, as shown on Fig. 3.

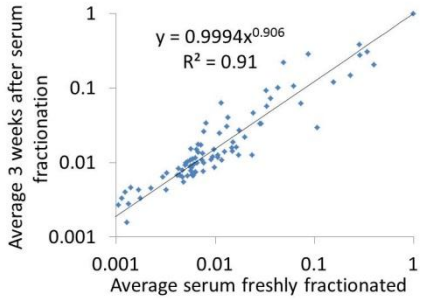

Fig. 3 Linear regression analysis of average intensities of detected mass spectra peaks in freshly fractionated serum triplicate set compared to peaks in another triplicate set measured 3 weeks after serum fractionation.

Diagnostic efficiency

To evaluate the feasibility of our technique to discriminate different stages of cardiovascular diseases from human serum samples, we tested in duplicate 7 pathological serums, from patients with a cardiovascular disease and tissue modification, against 10 normal serums from 10 wealthy patients. A blind statistical analysis (PCA) of the mass spectra obtained enabled to clearly separate 2 major clusters representing specific metabolomics patterns for normal and pathological samples, as shown on the score plot Fig. 4. Moreover PCA enabled identification of metabolites characteristic of the pathological *vs.* normal samples, as shown on the loading plot Fig. 4.

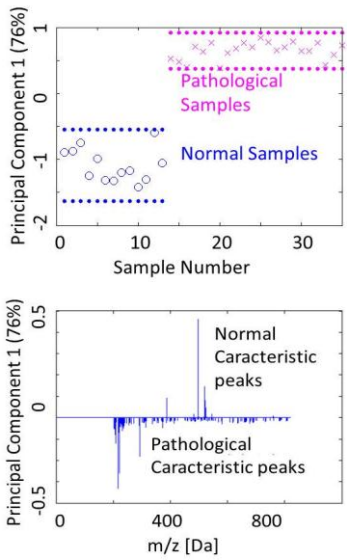

Fig. 4 PCA analysis of 10 normal serum samples *vs.* 7 pathological samples tested in duplicate on nanoporous SiOCH film. Above: score plot. Below: loading plot. (D. Graham NESAC Toolbox)

4. Conclusions

We propose a platform, based on nanoporous SiOCH thin films, that offers a significant enhancement in selectivity and sensitivity of metabolites recovery from complex biological fluids for MALDI mass spectrometry analysis. This low-cost and manufacturable platform offers simple sample acquisition and simple sample long-term storage protocol, and could provide enormous enhancement for LMW biomarker discovery, diagnostic and prognostic.

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