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Combinatorial Nanoporous Silica Chips for Selective Serum Proteomics

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Abstract

We developed Nanoporous Silica Chips (NSC) as enabling tools for human serum fractionation. We demonstrated that the NSCs can specifically harvest the low abundant, low molecular weight components of human serum for proteomic analysis, biomarker discovery and early diagnosis of cancer. The NSCs were synthesized using surfactant polymers as templates. The selection of the surfactant type and concentration allowed for the precise control over the resultant nanostructure and pore size and, consequently, over the selective recovery of specific proteins and peptides. The MSCs also allowed for the long-term preservation of the sample by assuring the stability of proteins and peptides in the nanoporous matrix. We believe this novel nanotechnology will substantially impact the field of clinical proteomics.

There is an intense interest in serum proteomics to improve the understanding of cancer pathogenesis and to discover new diagnostic biomarkers for the early detection of tumors using mass spectrometry technology. The low-molecular weight (LMW) region of the blood proteome is a source of diagnostic markers for diseases [1], but the presence of highly abundant proteins and the large dynamic range of serum/plasma proteins limits the sensitivity of low abundant species detection. Innovative technologies capable of finding biomarkers indicative of patient clinical status are mandatory [2]. Nanoporous silica, synthesized by surfactant directed self-assembly, is an attractive candidate material for its unique architectural, physical and chemical properties [3] which, in turn, are determined by the processing parameters during the Evaporation-Induced Self-Assembly (EISA) [4]. This material combines the intrinsic physical and chemical properties of inorganic or hybrid matrices with a highly defined nanoporous network having a tunable pore size and connectivity, high surface area and accessibility, and a specific orientation with respect to the substrate. Thanks to these features nanoporous silica has been used in many promising applications in the field of absorption, separation, chemical sensing, catalysis and drug delivery.

We developed a novel size-exclusion strategy for the removal of the high molecular weight proteins and for the specific isolation and enrichment of LMW species (fig. 1) [5]. This approach is based on combinatorial Nanoporous Silica Chips (NSC) able to fractionate and selectively harvest peptides and proteins present in complex human biological fluids and to protect them from enzymatic degradation. The MSCs operate with rapidity, high reproducibility, no sample pre-processing, and substantial independence from sample acquisition and storage temperature. They can be used as selective surfaces for exploratory screenings, enrichment of low abundant biomarkers and the early detection of diseases. A series of NSCs with tunable pore sizes, pore structures and surface properties were developed, thoroughly characterized and used to fractionate specific proteins in the low mass range. Using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, we established the correlation between the harvesting specificity and the physico-chemical properties of nanoporous silica surfaces (fig. 2). Furthermore, we proposed an improved pathway to coat the NSC's surfaces with functional groups to overcome the limitations of previous, conventional conjugation methods. With the pre-treatment on the surface by plasma ashing, we were able to conjugate chemical groups at high density regardless of the template polymer used. The chemically modified NSCs displayed enhanced harvesting capability and differential specificity for the LMW proteome. All together our data suggested that the introduction of this nanoporous material with fine controlled properties will provide a powerful platform for proteomics application in the clinical setting [6].

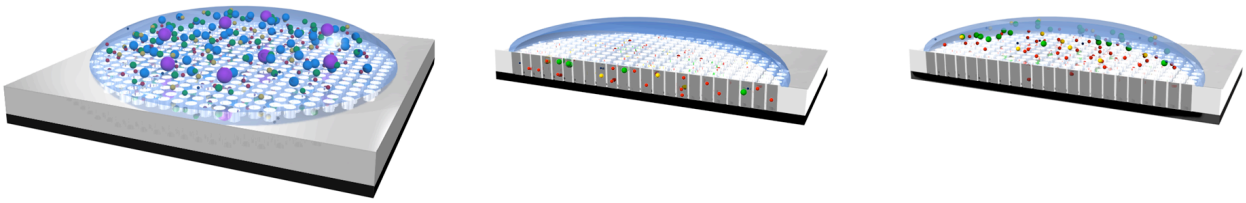

Figure 1. The protocol scheme of serum fractionation on the nanoporous silica substrate; From left to right: the serum is incubated on the surface of the NSCs. The LMW proteins and peptides penetrate in the nanoporous matrix and are retained by the chip during the washing steps. The fractionated LMW species are eluted and analysed on MALDI.

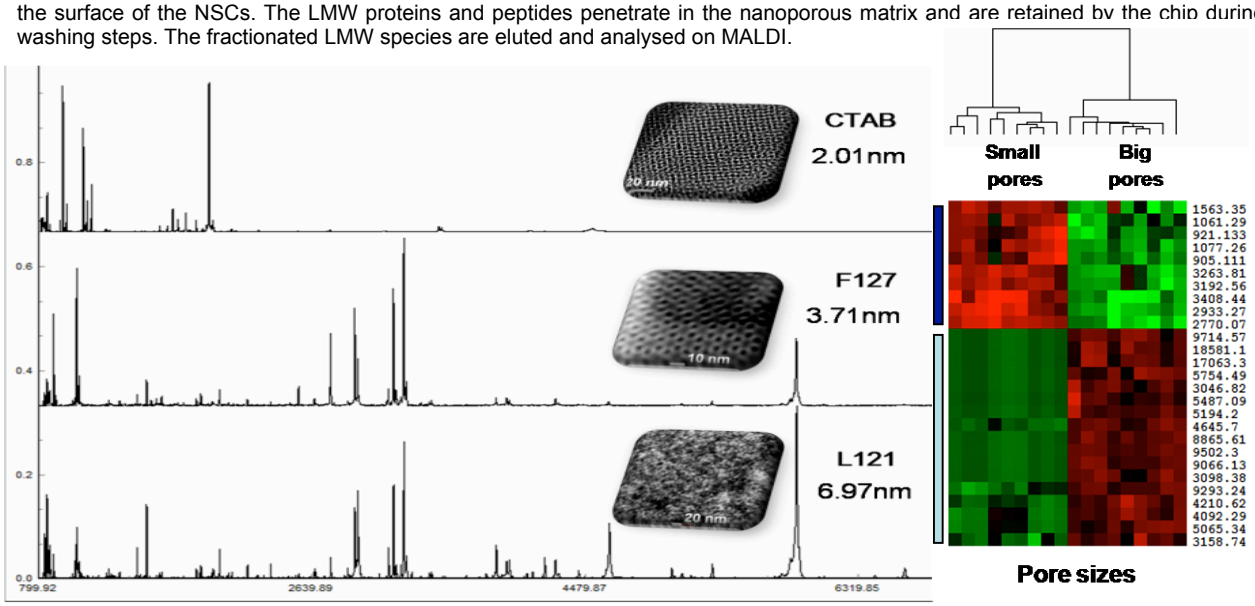

Figure 2. MALDI MS profiles of selective LMW proteins recovered from NSCs with different pore sizes (left), and supervised hierarchical clustering and specific recovery pattern for a set of nanochips differing for pore size (right). The relative intensity is gradually indicated with red squares (high intensity), black squares (median) and green squares (low intensity or absence).

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