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Surface assisted laser desorption–ionization mass spectrometry on patterned nanoporous silica thin films

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Abstract

We describe the preparation and use of patterned nanocomposite silica thin films deposited on silicon for laser desorption-ionization mass spectrometry (LDI-MS) without the use of conventional matrices. Ordered nanocomposite silica thin films deposited on silicon substrates were prepared by evaporation induced self-assembly using Brij56 as the surfactant template. The films were then exposed to masked deep-UV light to selectively remove the template to yield isolated regions of ordered nanoporous silica in a field of nanostructured silica. The nanoporous regions act as isolated "wells" that allow for the mass spectral characterization of analytes by laser induced desorption–ionization MS using a commercial MALDI-TOF instrument. We demonstrate the utility of these patterned films for the mass spectral analysis of small organic molecules, such as amino acids, peptides and siderophores. No consistent background signal from the films was observed at laser intensities typically used to desorb/ionize analytes. We also show that the films remain active for over a year when stored at ambient laboratory conditions. Because these patterned nanocomposite films are straightforward to produce, readily modifiable and stable at ambient laboratory conditions, they provide a potentially useful alternative to currently available films and substrates for matrix-free LDI-MS analysis of small molecules.
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Keywords: Nanocomposite; Mesoporous; Patterned; LDI-MS; Matrix-free

1. Introduction

Matrix assisted laser desorption ionization mass spectrometry or MALDI-MS has rapidly gained popularity as a powerful tool for analyses of numerous biomolecules [1–4]. Although the use of a matrix is critical for the effectiveness of this mass characterization technique, especially for proteomic analysis of peptides and proteins (>600 Da), it does present some disadvantages, particularly in the generation of background ions that hinder small molecule analyses. In addition, the use of specific matrices for optimal analysis of different analytes can deter the rapid analysis of unknown samples in mixtures. Thus, researchers have searched to discover substrates that promote ionization, and subsequent desorption, of analytes without generation of a background signal in the low molecular mass range to extend the utility of this characterization tool. LDI-MS techniques that utilize substrates, which generally do not significantly desorb along with analytes, may generally be classified as surface-assisted laser desorption–ionization mass spectrometry (SALDI-MS). This is in contrast to MALDI or surface enhanced laser desorption–ionization mass spectrometry (SELDI-MS) [5,6], which uses matrix molecules or surfaces coated with ionizable organic molecules to promote analyte characterization.

A significant amount of effort has been expended to develop substrates for optimized SALDI-MS [7]. These include a wide range of organic materials, like carbon nanotubes [8–13] and polymers [14–17], inorganic materials, like metal nanoparticles [18], porous silicon [19–21], metal-oxides [22–27] and composite materials [23,28–30], which

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consist of an organic matrix embedded in an inorganic supporting material, as well as gold-supported self-assembled monolayers [28–33]. The most widely used SALDI substrate is porous silicon, which is available under the trade name DIOS (desorption-ionization on silicon). The original DIOS work, which was performed by the Siuzdak group [21], reinvigorated the field of surface assisted mass spectrometry by demonstrating the characterization of molecules (<6000 Da) without the need for a matrix. However, porous silicon can be relatively unstable and requires involved handling procedures, although some of these issues may be overcome through surface modifications to the silicon surface [34]. In fact, one of the significant advantages of DIOS films is the ability to tailor the silicon surface for a variety of SALDI-MS applications [35,36]. In addition to the DIOS chips, nanoscale columns of germanium (Quickmass™ Chips) or silicon [37,38] have been reported to be effective for SALDI-MS. These films are reportedly stable under ambient conditions without observable loss to its LDI-MS activity, which allows for archiving of samples.

Although the semiconducting silicon and germanium materials are effective for SALDI-MS characterizations, they require specialized equipment and procedures for their preparation. In contrast, metal-oxides of, for example, silicon or titanium, prepared by sol–gel techniques can be synthesized using basic laboratory equipment and at ambient conditions. As such, several researchers have used metal oxide or modified metal-oxide films for SALDI-MS in search of a stable platform that is competitive with semi-conducting films [22–24]. Recently, ordered nanoporous materials, including aluminum-doped silica [25] and tungsten oxide-titanium oxide [26], which were prepared as bulk powders using an organic surfactant template and deposited on stainless steel MALDI plates, have been demonstrated to be useful for SALDI-MS analysis of small analytes. These materials have the nanoporous structure of the DIOS chips, which appears to promote desorption/ionization processes, but unlike porous silicon these materials posses a narrow pore size distribution that may be useful for creating films with reproducible SALDI characteristics. However, the long-term stability of the sol–gel derived materials, as well as their use for high throughput sample analyses, for SALDI-MS remain issues to be addressed.

Here, we report the use of patterned ordered nanoporous/nanostructured silica thin films deposited on silicon substrates for SALDI-MS characterization of small molecules (<3000 amu). These films are prepared by an evaporation induced self-assembly process, which is a sol–gel process that involves the condensation of silica precursor molecules around an organized surfactant phase [39]. Deep-UV light, in combination with a physical mask, is then used to selectively remove the surfactant phase to create isolated nanoporous regions that are used to promote the ionization/desorption of analytes upon laser excitation. The patterning process allows multiple samples to be spotted and analyzed on a given substrate. A variety of analytes, including amino acids, peptides and siderophores, were characterized at sub-nanomole limits of detection with good resolution. In general, mass spectra of analytes were observed without the appearance of background ions resulting from the nanoporous films. Further, the thin films described here were used to characterize and re-characterize analytes over a period of at least 18 months. The straightforward sample preparation, ability to run multiple samples rapidly on a single active film, and long term stability makes patterned nanoporous silica films deposited on silicon an attractive platform for SALDI-MS analyses.

2. Experimental methods

2.1. Thin film preparation

Ordered nanocomposite silica thin films were prepared by an evaporation induced self-assembly process described previously [39]. Initially a silica sol was prepared by sonicating a mixture of tetraethylorthosilicate (TEOS, 60 mL, >99% from Aldrich), anhydrous ethanol (60 mL, Fisher), >18 MΩ cm^{-1} deionized water (4.7 mL), and 0.07 N hydrochloric acid (0.2 mL, Fisher) for ~15 min. A portion of this solution (10 mL) was then diluted with ethanol (20 mL), water (0.33 mL), and hydrochloric acid (0.07 N, 1.0 mL). A non-ionic surfactant, $C_{16}H_{33}(OCH_2CH_2)_nOH$; $n \sim 10$ (technical name: Brij56, Aldrich) was added to the ethanolic solution at ~4.0 %wt. to yield a final reactant mixture with a mole ratio of 1 TEOS:22C_2H_5OH:4H_2O:0.004HCl:0.085-Brij56. This solution was then diluted with 40 mL of ethanol and allowed to age for at least 30 min prior to thin film deposition. Thin films were deposited onto freshly oxidized single crystal silicon (100) 2 in. wafers by withdrawing the substrate from the surfactant/TEOS solution at 25–200 mm/min. All films were aged at ambient conditions for at least 24 h prior to characterization or patterning by deep UV-light (185–254 nm).

Patterning by deep UV-light [40] was accomplished using a metallic aluminum grid of 1 mm holes that was held in contact with the film by binder clips. The masked nanocomposite silica films were then exposed to deep-UV light, which generates ozone and oxygen radicals, for ~180 min to selectively remove the surfactant generating a pattern of nanoporous silica in a field of nanostructured silica. Ellipsometric values, Δ and Ψ, were collected on both the nanoporous and nanocomposite regions using a VASE spectroscopic ellipsometer (J.A. Woollam Co., Inc.). Thicknesses and indices of refractions were then calculated using a model assembled in the WVase software package (J.A. Woollam Co., Inc.). The mesoscale ordering [41,42] of the films was confirmed by X-ray diffraction, which was collected on a Rigaku Ultima III XRD instrument using Cu Kα radiation.

2.2. Mass spectrometry

Mass spectrometry was performed on a Voyager DE-STR (ABI, Framingham, MA) Time-of-Flight mass