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Porous Silicon as a Versatile Platform for Laser Desorption/Ionization Mass Spectrometry

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Desorption/ionization on porous silicon mass spectrometry (DIOS-MS) is a novel method for generating and analyzing gas-phase ions that employs direct laser vaporization. The structure and physicochemical properties of the porous silicon surfaces are crucial to DIOS-MS performance and are controlled by the selection of silicon and the electrochemical etching conditions. Porous silicon generation and DIOS signals were examined as a function of silicon crystal orientation, resistivity, etching solution, etching current density, etching time, and irradiation. Pre- and postetching conditions were also examined for their effect on DIOS signal as were chemical modifications to examine stability with respect to surface oxidation. Pore size and other physical characteristics were examined by scanning electron microscopy and Fourier transform infrared spectroscopy, and correlated with DIOS-MS signal. Porous silicon surfaces optimized for DIOS response were examined for their applicability to quantitative analysis, organic reaction monitoring, post-source decay mass spectrometry, and chromatography.

Desorption/ionization mass spectrometry techniques experienced a renaissance in the early 1980s with the introduction of matrix-assisted secondary ion mass spectrometry (SIMS)[1] and fast atom bombardment (FAB)[2] and their novel use of energy-absorbing matrixes. SIMS and FAB use energetic atoms or ions (e.g., Ar or Cs^+) in conjunction with a matrix to desorb analyte ions through a sputtering process. A useful aspect of the FAB approach is that it uses a liquid matrix to provide a continuously renewed surface, which allows for an intense primary ion beam to impinge upon the sample surface, generating a continuous stream of ions for an extended period of time. By enabling soft desorption/ionization of moderately sized molecules (typically 200−6000 Da), FAB was a widely used technique to determine the mass of labile biomolecules. Matrix-assisted laser desorption/ionization (MALDI), developed in the mid-1980s,[3] has been even more successful due to its ability to efficiently generate intact molecular ions in the gas phase. Using MALDI, large biomolecules (up to and greater than 200 000 Da) can be desorbed and detected as intact species.[4] The matrix material acts as an energy receptor for the pulsed laser beam, using that energy to desorb cocrystallized analytes. A disadvantage of MALDI is that it produces a large amount of matrix background ions, which can obscure or suppress small mass ions, thereby limiting their use to analytes with masses greater than 700 Da and making quantitation of small molecules difficult. Additionally, the choice of matrix is often critical for optimal desorption/ionization.

Recently, we described a matrix-free method using pulsed laser desorption/ionization on porous silicon (DIOS).[5] Porous silicon is a UV-absorbing semiconductor with a large surface area (hundreds of m^2/cm^3) and is produced through electrochemical anodization or chemical etching of crystalline silicon.[6,7] Much of the interest in porous silicon and its morphology derives from its photoluminescent properties, which make it a useful platform for electronic and optoelectronic devices as well as chemical microsensors.[6−10] For its application to laser desorption/ionization mass spectrometry, we believe that the structure of porous silicon provides a scaffold for retaining solvent and analyte molecules, and the UV absorptivity affords a mechanism for the transfer of the laser energy to the analyte. This fortuitous combination of characteristics allows DIOS to be useful for a large variety of biomolecules including peptides, carbohydrates, and small organic compounds of various types. Unlike other direct, matrix-free desorption techniques, DIOS enables desorption/ionization with

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little or no analyte degradation.[5] In this report, we describe the conditions for preparation of porous silicon surfaces, their characterization, and an introduction to various applications of DIOS-MS.

EXPERIMENTAL METHODS

Laser Desorption/Ionization Mass Spectrometry. The laser desorption/ionization measurements were performed in a PerSeptive Biosystems (Framingham, MA) Voyager DE time-of-flight and Voyager STR time-of-flight reflectron mass spectrometers with delayed extraction. The DIOS samples consisting of etched porous silicon wafers (diced to an approximate size of 20 mm × 20 mm) were simply attached to the standard target plates using one-sided tape. Samples were irradiated with a nitrogen laser (Laser Science Inc.), operated at 337 nm and attenuated with a neutral density filter. Ions produced by laser desorption were energetically stabilized during a delayed extraction period of 150 ns and then accelerated through the linear time-of-flight mass analyzer by a 20-kV potential pulse. Spectra shown were typically an average of between 32 and 128 laser pulses. DIOS postsource decay (PSD) measurements were collected with the samples analyzed in this study as well as a standard peptide MRFA (m/z 523.3) using standard MALDI PSD-MS experimental procedures. DIOS and MALDI-MS measurements were performed in positive and negative ionization mode.

NanoESI-MS. The steroid analyses were performed on a PE Sciex API III (Alberta, Canada) modified with a nanoESI source from Protana A/S. The orifice was set at −115 V, and ESI voltage was set at −650 V. A curtain gas of ultrapure nitrogen was pumped into the interface at a rate of 0.6 L/min to aid evaporation of solvent droplets and prevent particulate matter from entering the analyzer region. Desolvated ions entered the analyzer via the vacuum interface and were guided by entrance optics. Normal-sized palladium-coated, borosilicate glass capillaries from Protana A/S were used for sample delivery. The collision-induced dissociation (CID) experiments were performed with ultrapure argon as a collision gas. The precursor ion mass spectra were acquired by scanning the first quadrupole, while collisions with argon (target thickness of 3.0×10^{15} atom/cm^2) in the second quadrupole produced ion dissociation. The third quadrupole was used to mass select the fragment ion of interest. Sulfated steriods were detected through a loss of 97 (HSO_4^-) in the negative ion mode. Spectra were the result of averaging from 50 to 200 scans depending on the number of scans necessary to obtain a signal-to-noise ratio greater than 50.

IR Measurements. Fourier transform infrared (FT-IR) spectroscopic measurements were collected on a MIDAC FT-IR spectrometer, M series (Irvine, CA), equipped with a diffuse reflectance accessory from PIKE Technologies (Madison, WI). Spectral resolution was 4 cm^{-1} and typically 128 interferograms were acquired per spectrum.

XPS measurements were acquired on a Physical Electronics Quantum 2000 scanning ESCA mMicroprobe. This system uses a focused monochromatic Al Kα X-ray (1486.7 eV) source for excitation and a spherical section analyzer. The instrument has a 16-element multichannel detection system. The X-ray beam used was a 100-W, 100-m-diameter beam that is rastered over a 1.5 mm by 0.2 mm rectangle on the sample. The X-ray beam is normal to the sample, and the X-ray detector is at 45° from normal. The high energy resolution scans were collected using a pass energy of 23.5 eV. For the Ag 3d 5/2 feature, these conditions produce fwhm of better than 0.75 eV. The collected data were referenced to an energy scale with binding energies for Cu 2p 3/2 at 932.67 ± 0.05 eV and Au 4f at 84.0 ± 0.05 eV. Quantitation of elemental composition was performed by literature methods.[33]

Field emission scanning electron microscope (FESEM) measurements were performed on a scanning electron microscope (LEO 982) with point-to-point resolution of 1 nm at an accelerating voltage of 30 kV and 4 nm at 1.0 kV. The high resolution is achieved using a Schottky field emission source, a beam booster that maintains high beam energy throughout the microscope column, an electromagnetic multihole beam aperture changer, and a magnetic field lens.

Solvents and Materials. Stock solutions of sulfated steroids synthesized in our laboratories were prepared at 5 μg/μL and stored at −20 °C. Steroid sulfates were dissolved in ethanol; desired dilutions were prepared with MeOH/H_2O (70:30, v/v) for working solutions and stored at −20 °C. Methanol, ethanol, dichloromethane, chloroform, hexane, *sec*-butyl alcohol, and acetic acid were purchased from Sigma Chemical Co. (St Louis, MO). Ethyl alcohol was purchased from Quantum Chemical Co. (Tuscola, IL). HF (48−51%) was obtained from Acros. Single polished n-type silicon (100) wafers, resistivity 0.5−2 Ω-cm, thickness 525 ± 50 μm, 100-mm diameter, were obtained from Silicon Sense (Nashua, NH).

Porous silicon surfaces are prepared by electrochemically etching in a Teflon cell. The cell is composed of two sections that are held together by polyethylene screws. Silicon surfaces of n-type low resistivity (0.01−0.02 Ω·cm) and nominal thickness (0.5 mm) are cut to fit over the bottom of the etching cell chamber while resting on the Teflon cell base. A 0.1-mm-thick gold foil (anode) is placed under the silicon wafer to provide electrical contact, and a platinum wire (cathode) is positioned in the cell cavity as a counter electrode. The etching solution (24% w/v HF solution in ethanol) is added to the cell cavity. During the etching procedure, the silicon wafers are illuminated with white light from a model I-150 fiber-optic light source (Coherent Inc.) with a 150-W tungsten filament bulb. The light intensity is ∼50 mW/cm^2. Under illumination, the silicon is exposed to a constant current density (∼4 mA/cm^2) that is passed through the cell chamber from the gold foil anode to the platinum electrode cathode. After the desired etching time (typically 1−2 min), the sample is washed in absolute ethanol and then dried in N_2 flow.

Photopatterning is a convenient visualization feature for depositing and obtaining mass spectra of multiple samples from DIOS surfaces. To create photopatterns on the DIOS surface, radiation from a fiber-optic light source (model I-150 Coherent Inc.) is passed through a printed mask and two achromatic lenses (Coherent Inc., part 23-9723, f = 80.0 mm, diameter 50.0 mm) and then focused on the silicon surface. A 150-W tungsten filament bulb and 1-m flexible light guide with adjustable lens at the end was used with typical light intensity of ∼50 mW/cm^2). Since p-type silicon is significantly less affected by light intensity during etching, photopatterning was typically performed on the n-type wafers, producing sharp reproducible patterns on porous silicon surfaces.

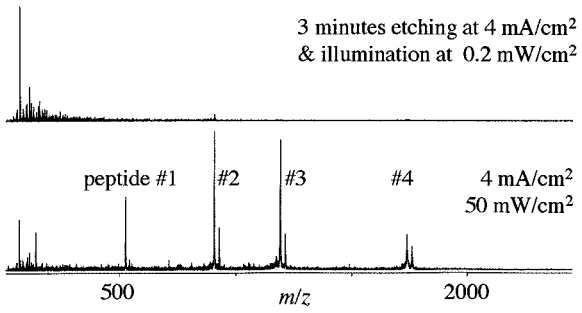

Figure 1. DIOS-MS of a mixture of four peptides (~1 pmol each) applied to porous silicon after etching two identical (0.005−0.02 Ω·cm resistivity n-type silicon wafer) surfaces under different illumination conditions (0.2 vs 50 mW/cm² illumination).

Table 1. Conditions Explored for Optimization of DIOS Surfaces

parameter	range tested
silicon type	n-type (⟨111⟩, ⟨100⟩); p-type (⟨111⟩)
resistivity	0.001−0.005 (n-type), 0.005−0.02 (n-type), 0.5−2.0 (n-type), and 20−30 Ω·cm (n-type); 3−6.6 Ω·cm (p-type)
etching solution	mixtures of 48% aqueous HF (w/w) and EtOH in the ratios (aqueous HF:EtOH) 30:70, 50:50, and 70:30 (n-type); 30:70 (p-type)
etching current	4, 6, 10, 20, 40, 80, and 120 mA/cm² (n-type); 80 mA/cm² (p−type)
etching time	0.5, 1, 2, 5, 15, and 20 min (n-type); 1 min (p-type)
irradiation	0.2, 1, 2, 5, 10, 20, 50, 100, and 150 mW/cm² (n-type); 0.001, 0.2, and 40 mW/cm² (p−type)

RESULTS AND DISCUSSION

Porous Silicon. *Silicon Etching.* Etching parameters such as silicon crystal orientation, light intensity, dopant type, dopant level, current density, etching solution, and etching time are all known to affect porous silicon morphology.[7,9,11−13] Generally, pore diameter for n-type (P-, As-, or Sb-doped) silicon increases with increasing resistivity (decreasing density of dopant atoms). Thus, lightly doped n-type (n^-) Si wafers normally give a mixture of macro (>50-nm diameter)-, meso (2−50 nm)-, and micro (<2 nm)-pores when etched under visible light illumination, while heavily doped n-type (n^+) Si wafers produce only meso- and micropores.[14,15] The reverse is true for p-type (boron-doped) materials where pore diameter decreases with increasing resistivity. Pore depth is generally proportional to etching time for both n- and p-type materials. Overall porosity depends on current density, light intensity, and HF concentration.[14] We have varied each of the above etching parameters in an effort to produce porous silicon materials with optimal DIOS performance. The resulting procedures are somewhat different from those normally used in generating photoluminescent porous silicon material.

To test the sensitivity of DIOS-MS to changes in the method of porous silicon preparation (and therefore to the porous silicon structure), a mixture of peptide samples including MRFA tetrapeptide (523.3 Da), des-Arg-bradykinin (903.5 Da), Lys-bradykinin (1187.7 Da), and flock house virus capsid β−protein (fragment 16−31; 1735.9 Da) were used in positive ion mode. An example is shown in Figure 1, illustrating the analysis of the analyte mixture from porous silicon prepared with two different irradiation levels; spectra were judged on the basis of qualitative assessments of reproducible signal-to-noise levels and the appearance of background ions. The peptide mixture was deposited in a spot size of ~0.5-mm diameter. Table 1 summarizes the range of conditions that were tested for the preparation of porous silicon. Two sets of conditions were found to give the best "fresh" or "underivatized" (meaning neither oxidized by prolonged exposure to air nor modified by covalent attachment of alkenes or alkynes; see below) DIOS-MS surfaces: (a) 0.005−0.02 Ω·cm resistivity n-type silicon wafers etched at 4 mA/cm² for 1 min under moderate (50 mW/cm²) white light intensity (designated surface 1) and (b) 0.5−2 Ω·cm resistivity n-type silicon wafers etched at 20 mA/cm² for 5 min under ambient (~0.5 mW/cm²) diffuse light (surface 2). Generally, the etching solution consisted of a 1:1 mixture of 50% aqueous HF and absolute ethanol. Substantial deviation from these "optimum" etching conditions can be tolerated without a significant loss in performance. These conditions are milder than those used to prepare photoluminescent porous silicon materials, and we have found that photoluminescent samples are not effective DIOS surfaces. The characterization of active DIOS materials is described below.

In addition to the identification of a useful set of etching conditions and the role of illumination described below, the following observations were made in these experiments.

(1) Long etching times (>5 min) under illumination resulted in very fragile porous silicon surfaces and intense low-mass background ion signals.

(2) Little effect on DIOS performance was observed when the HF concentration was lowered (to 14%) or raised (to 34%) from the standard solution (24%) at constant current density. This is consistent with the relative insensitivity of porosity, pore depth, and pore diameter to changes in HF concentration that has been noted previously.[15]

(3) Changing the organic solvent used in the anodization procedure from ethanol to methanol, 2-propanol, or butanol did not significantly change the DIOS surface properties. However, storing the porous silicon in ethanol was typically better than the other solvents with respect to obtaining DIOS signals (see below).

(4) We have observed similar performance in DIOS-MS analysis of standard peptide samples on porous silicon prepared from both ⟨100⟩ and ⟨111⟩ silicon wafers using the same standard etching conditions, suggesting that Si crystal orientation does not play an important role in DIOS performance. Since the crystal orientation has been reported to affect the pore shape and directionality but not pore size,[12,16] these results suggest that pore size and overall porosity are the most important parameters for DIOS.

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(5) The upper mass limit for peptide samples appears to be somewhat dependent on the nature of the porous silicon platform and the sample composition. Currently, polypeptides with m/z values as high as 18 000 have been observed in DIOS, but routine analyses typically are performed on species below 2500 Da. Efforts to raise the mass ceiling by changing the morphology of the porous silicon material are underway.

For some analytes, the signal-to-noise ratio is compromised by the appearance of "background" ions (typically <300 Da) in DIOS-MS analysis. Accurate mass measurements of these ions below m/z 200 reveal them to be singly charged species possibly derived from aliphatic hydrocarbons, which are known to be adsorbed by porous silicon from the air or while under oil pump vacuum.[17] The most common background ion, observed at m/z 70, has not yet been identified. It is possible that hydrocarbons may play a role in the laser desorption process through vaporization. We have observed that the evacuation time for the mass spectrometer source is longer in the presence of a dried DIOS surface than for MALDI plates with cocrystallized matrix and analytes and that the evacuation time increases with the length of time that the DIOS surface is exposed to air. Investigation of the role of adsorbed small molecules, and other aspects of the DIOS mechanism, are underway and will be reported in future reports.

Pre- and Postetching Treatments. A variety of preetching treatments were tested to see if the background ions arose from impurities on the silicon wafers before anodization. These procedures included sonication in ethanol, washing in chloroform followed by ethanol and water, and immersion in a 2:1 mixture of sulfuric acid and hydrogen peroxide for 30 min followed by 5% aqueous HF for 15 min. Acid treatments were followed by washing in a 1:1:5 mixture of ammonium hydroxide, hydrogen peroxide, and water for 10 min, followed by rinses with ethanol and water. In no case was a significant difference in background ions observed relative to untreated samples. However, to make sure that surfaces are cleaned of organic contaminants before etching, an ethanol wash is advisable.

Postetch washing with the following solutions or solvents did little to reduce background ions: (a) chloroform, (b) chloroform and ethanol, (c) 2-propanol, (d) 2-propanol and ethanol, (e) ethanol under nitrogen gas flow, (f) aqueous ammonium citrate followed by water then ethanol, and (g) ammonium citrate solution followed by water, ethanol, and 5% aqueous HF. Even extreme postetching procedures with hydrogen peroxide, concentrated HCl, and aqueous HF provided no significant improvement. However, we have found that soaking freshly etched porous silicon wafers in ethanol reduces the intensity of the higher-mass component of these background ions (notably m/z 155, 242, and 277, which are of unknown origin) and thereby improves the detection limits for compounds of interest. Typically, the signal-to-noise ratio improves after 1 day of storage in ethanol with no further enhancement thereafter. Therefore, we simply store underivatized porous silicon wafers in ethanol for long-term use (i.e., more than 8 h after production). It should be noted that the structure of photoluminescent, free-standing porous silicon material (very different from the wafers generated for DIOS applications) has been found to respond to changes in solvent.[18]

Storage of porous silicon samples in air for extended periods of time, or brief exposure to ozone or aqueous hydrogen peroxide, results in oxidation of surface groups to oxide (Si−O−Si) and hydroxide (Si−OH) moieties [stretching vibrations: 1090−1031 (Si−O), 2200, 2260 cm^{-1} (OSi−H). Si−O−C bending vibration 1176 cm^{-1}].[19] In general, DIOS performance degrades with increasing surface oxidation. However, simply dipping an oxidized material into the etching solution (or a 5% aqueous HF solution) for 1 min removes the oxidized layer and regenerates a hydride surface detectable by IR spectroscopy.[20] Indeed, the procedure whereby silicon wafers are etched, oxidized, and then treated with HF (termed "double etching") gives porous silicon surfaces that show better DIOS performance with higher concentrations of analytes and analyte mixtures (Figure 2). Modest changes in pore morphology resulting from the second HF treatment have been detected, as discussed below.

The double-etched surfaces have a higher threshold for desorption (i.e., require a higher laser fluence), yet contrary to common MALDI experience, the mass peak resolution is not degraded. Most significantly, these surfaces retain the ability to provide good DIOS-MS spectra after prolonged exposure to air even without ethanol immersion; the origin of this effect is not yet understood. Furthermore, the doubly etched surfaces work well on small molecules and tend to have a higher loading capacity (higher concentrations of sample and more complex mixtures) than the singly etched surfaces. A demonstration of the doubly etched surface properties are shown in Figure 2 for the analysis of a trypsin digest of bovine serum albumin. Both singly and doubly etched fresh surfaces produced good mass spectra, but only the doubly etched porous silicon provided quality DIOS performance after air exposure for 5 days. The relative intensity of the mass spectra can vary, but close examination show that all three surfaces generate the same ions. Note that less background ions (below m/z 200) are observed in the aged doubly etched surface.

Characterization of Porous Silicon Surfaces. Preliminary investigation of the structure of surface 1 has been performed primarily with scanning electron microscopy (SEM). SEM analyses of the porous silicon surface optimized for DIOS have revealed a surface with macrosized pores spaced ∼100 nm apart. The pores are approximately 70−120 nm in diameter with a depth of up to 200 nm (Figure 3). The double-etched surfaces show wider openings and therefore more cylindrical pores, but the pore depths are similar to the single-etched precursors. Compared to standard photoluminescent preparations,[8,20] these DIOS-active materials have much larger pores, a much thinner porous layer, and lower overall porosity. The ultrathin nature of the porous layer allows the DIOS wafers to be scored on the nonetched side and cleanly broken with little or no shattering. It also makes it difficult to measure porosity by the standard gravimetric method. From the SEM data, porosities of these materials appear to be approximately 30−40%.

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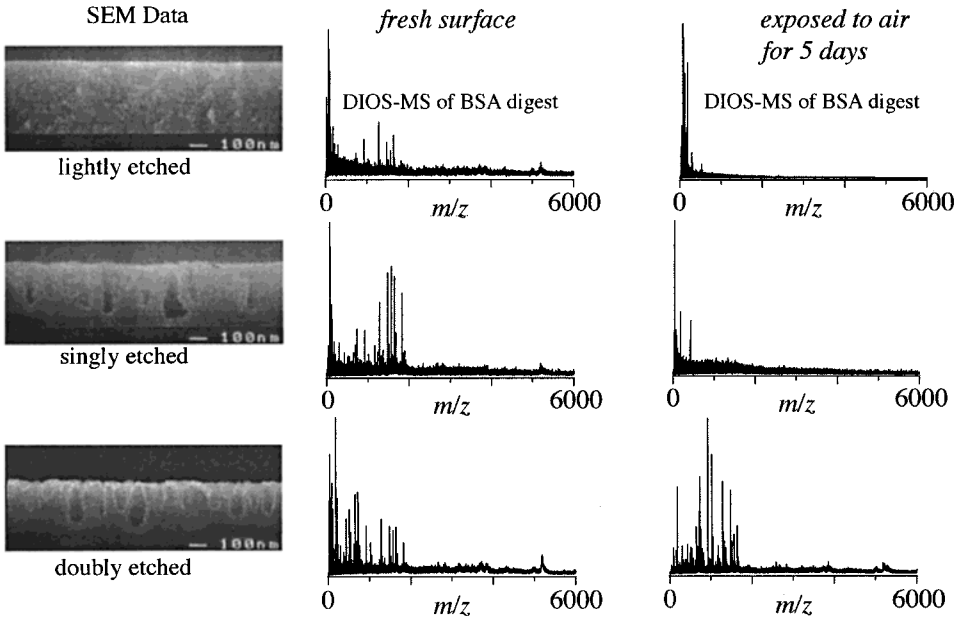

Figure 2. Comparison of DIOS performance of single- and double-etched porous silicon surfaces. SEM and DIOS-MS analyses before and after 5-days exposure to air. The surfaces were prepared using 4 mA/cm^2 current density (surface 1) and were irradiated with a light intensity of 0.2 (top), 45 (middle), and 45 mW/cm^2 (bottom). The analyses were performed on a digest of 500 fmol of bovine serum albumin (BSA). Note that the doubly etched surface still performed well after 5-days exposure and continued to perform equally as well even after 3-weeks exposure to air (no further time points were taken). SEM and XPS data on the singly and doubly etched surfaces suggest that a layer of oxidation more readily occurs on the singly etched surface.

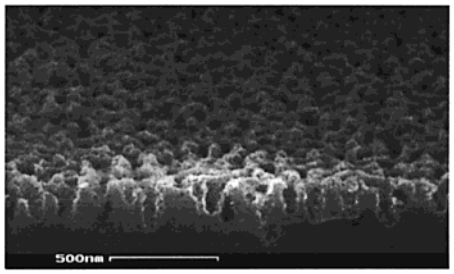

Figure 3. SEM analysis of the "double-etched" porous silicon surface prepared from low-resistivity n^+-Si material (0.001−0.005 Ω·cm).

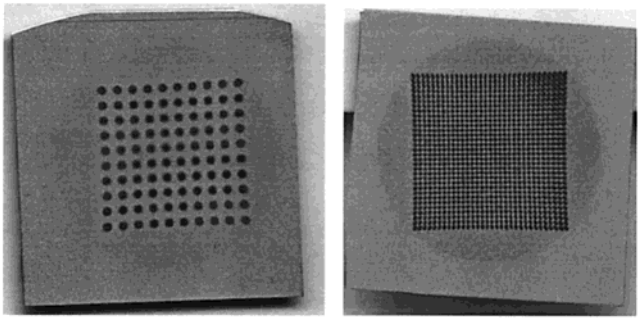

Figure 4. Porous silicon wafers suitable for DIOS having 100 positions (left) and 1000 positions (right) etched on a 3 cm × 3 cm surface. The patterns are generated by illumination through a simple transparency mask. Translucent areas on the transparency translate to dark active areas on the DIOS surfaces. The silicon wafers used in these studies were typically 0.5 mm thick.

It has been suggested that etching of silicon generates a layer of material at the surface resembling siloxene [$(Si_6H_6O_3)_n$], a material that is photoluminescent in bulk.[21] To determine whether it acts as a matrix, siloxene in powder form was prepared by the literature method and placed on the standard gold MALDI sample plate. Analytes that were then deposited on the siloxene material were not detected by laser desorption MS.

The freshly etched porous silicon surface is dominated by silicon hydride (Si−H) groups, which can be detected and monitored on the medium-resistivity (0.5−2.0 Ω·cm) surface 2 by diffuse reflectance IR spectroscopy [with characteristic stretching frequencies at 2085 (Si−H), 2110 (SiH_2), and 2140 cm^{-1} (SiH_3); and bending vibrations at 810 (Si−H) and 904 cm^{-1} (Si−H_2)]. The very low resistivity material (surface 1), having a high concentration of dopant atoms, is opaque to IR radiation, even in diffuse reflectance mode.

Photopatterning. The anodization of n-type silicon is strongly dependent on white light irradiation of the surface.[7,13] Thus, surfaces etched under ambient light versus a strong fiber-optic light source behave very differently in DIOS and have very different structures (Figures 1 and 2). We also take advantage of the ability of light to stimulate etching to create etched patterns of porous silicon by use of a simple mask. To create patterned grids, spots, and other shapes on the DIOS surface during etching, the light is passed through a computer-generated image printed on a transparency and two achromatic lenses focused on the n-type silicon wafer. This relatively simple procedure produces sharp, reproducible patterns of porous silicon on the bulk silicon surface (Figure 4). The use of low current densities facilitates this process since hydrogen bubble formation is minimized; such bubbles can induce heterogeneity in etching at the light/shadow boundaries. A complementary type of photopatterning has been demonstrated by Stewart and Buriak in the covalent derivatization of porous silicon following the etching step.[22] Photopatterning can also be achieved with lithography as is widely used in the semiconductor

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industry.[19,23] Photopatterning is particularly useful for DIOS applications because it aids visualization of sample spots that might ordinarily be difficult to see because of the small amount of material deposited and can be useful for automated analyses.

Chemical Derivatization. It has been known for several years that derivatization of the porous silicon hydride surface with organic functionality serves to stabilize the structure toward oxidation by air or degradation by acid and base,[9,24−27] and the Buriak laboratory has published two very convenient reactions for the purpose. Thus, hydrosilylation of alkynes and alkenes with the fresh porous silicon surface can be accomplished with the aid of Lewis acid catalysts[28] or by a simple photolysis procedure.[22] These techniques are mild and convenient, providing for rapid reactions at room temperature. Previous methodologies employing Grignard reagents[24] and alkyllithiums[25] are quite useful; radical reactions[26] and electrochemistry[29] have also been employed. Alcohols may be likewise attached by a variety of methods.[30] Thus, the variety of known porous silicon derivatization techniques allow for the incorporation of a broad range of functionality.

We also find that the covalent attachment of alkenes and alkynes by hydrosilylation (in the presence or absence of catalyst) stabilizes the materials toward oxidation and degradation. We have performed extensive studies of the effect of surface derivatization on DIOS efficiency. In general, the overall DIOS performance of such materials is not dramatically different than that of the "fresh" (Si−H) porous silicon surface. However, surface derivatization affords the opportunity for multistep chemical synthesis on porous silicon, allowing us to build molecules of interest for monitoring reactions or noncovalent binding events. Covalent modifications of the porous silicon surface also changes the chromatographic properties of the material. The results of these studies will be reported separately.

Applications. *Analytes.* For reasons of convenience, analytes have been typically dissolved in water or water/methanol solutions for DIOS-MS analysis. Freshly etched DIOS surfaces are hydrophobic, thus, aqueous samples bead up, whereas samples dissolved in predominantly nonpolar solvents spread out too much on the hydrophobic porous silicon wafer. Aliquots (0.5−1.0 μL; containing 0.05−100 pmol of analyte) are deposited directly onto the porous surfaces and allowed to dry before DIOS-MS analysis. In contrast to MALDI analysis with a matrix material, analyte spots created in this manner do not spread out, remaining in an area of ∼0.5 mm diameter.

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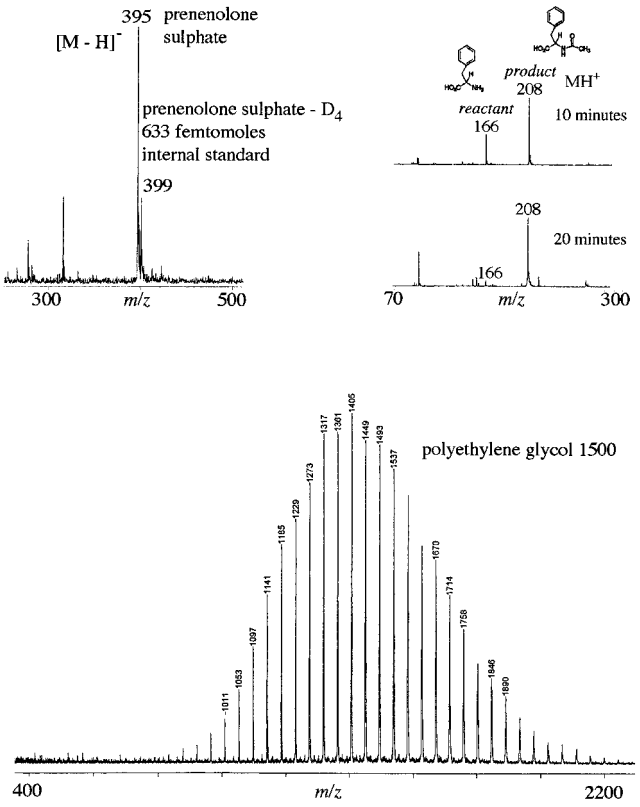

Figure 5. Examples of DIOS-MS spectra including a sulfated steroid (negative ionization mode (1 pmol), on-plate monitoring of the acetylation of an amino acid (positive ionization mode, 0.5-μL reaction volume, 50 pmol), and the identification of the components of a poly(ethylene glycol) sample of high polydispersity (positive ionization, 50 pmol).

A variety of compounds including peptides, natural products, small organic molecules, and polymers have been detected by desorption/ionization on porous silicon mass spectrometry with little or no fragmentation. Three examples are shown in Figure 5; note that DIOS-MS can be performed in both positive and negative modes on compounds that differ widely in polarity and that range in mass from 100 to 3000 Da. In general, it appears that these trends match those observed for electrospray ionization mass spectrometry (ESI-MS); thus, compounds with ionizable functionalities (amines and carboxylic acids) or groups that can bind alkali metal ions are efficiently detected with both techniques. Extending the work described below, we are continuing to explore the relationship of surface structure and derivatization to DIOS sensitivity and selectivity. It is also noteworthy that no silicon-containing adducts or fragments have been observed in DIOS-MS spectra, indicating the inert nature of the porous silicon material.

Quantitative Analysis. DIOS has been used for quantitative analysis of small molecules with internal standards similar in chemical structure to the molecules of interest. For example, Figure 6 shows a comparison of the calibration curves of sulfated pregnenolone and a d_4 analogue by nanoelectrospray (upper line) and DIOS (lower line). Both analyses were performed in the negative ion mode. Nanoelectrospray analysis was performed on a triple quadrupole with precursor ion scanning with the needle voltage set at −720 V and the orifice voltage set between 95 and 115 V. DIOS was performed in the linear mode with a 20-kV

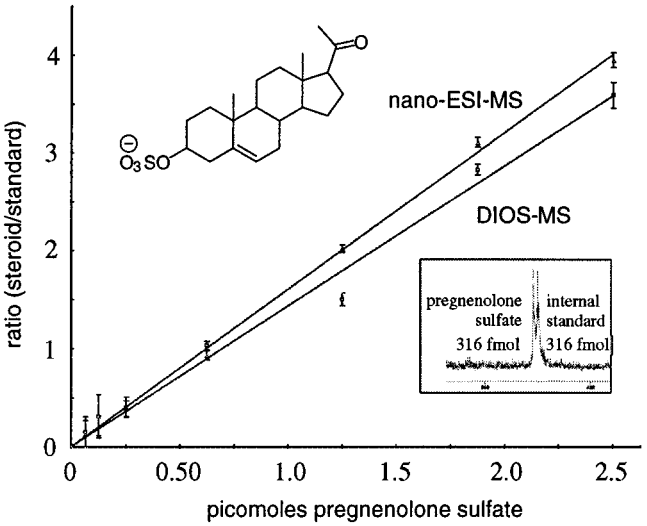

Figure 6. Quantitative analysis of a sulfated steroid using DIOS-MS and nanoESI precursor ion scanning.

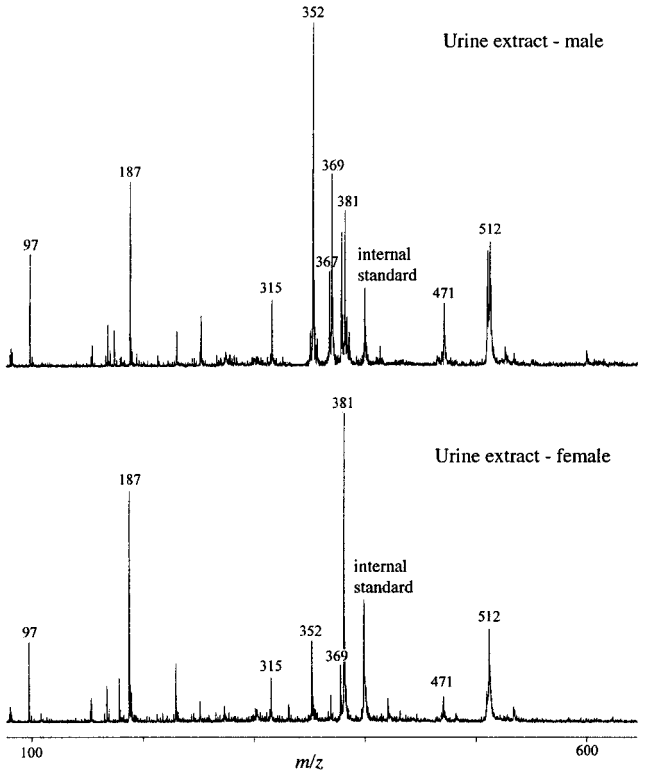

Figure 7. Measurement of conjugated steriods from male and female urine sample extracts. The amount of the internal standard was 316 fmol of pregnenolone.

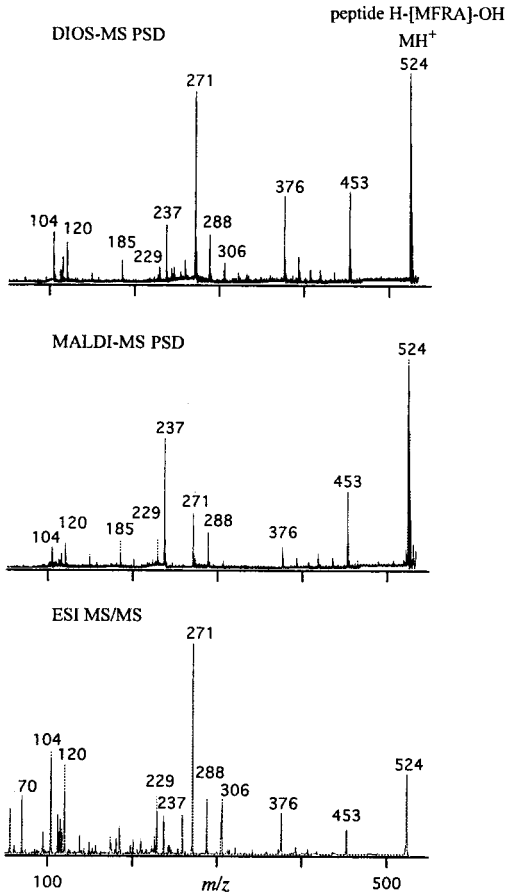

Figure 8. Comparison of DIOS and MALDI postsource decay data as well as electrospray triple quadrupole tandem mass analysis of the peptide H-MFRA-OH. In the analysis of smaller molecules (m/z <500) by MALDI-PSD (not shown), interference from background ions was observed in the fragmentation spectrum.

extraction voltage and a time-delayed ion extraction of 100 ns. DIOS provides comparable linearity to nanoESI-MS/MS in the concentration range of 65 fmol/μL to 2.5 pmol/μL; nanoESI-MS/MS has proven to be an effective technique for such analyses.[31] Precursor ion scanning in the MS/MS mode was needed in order to achieve the necessary signal-to-noise ratio with nanoESI in the mass range of interest; whereas DIOS readily provided reliable signals of the intact analytes. The potential to directly acquire clean DIOS-MS signals from small molecules allowed for the quantitative measurements.

The successful use of the steroidal internal standards in Figure 6 was further used in the simple analysis of sulfated and other steriod conjugates (Figure 7) in human urine samples. The samples were subjected to a simple two-step liquid/liquid extraction procedure previously described by Siuzdak and co-workers.[31] Briefly, 100 μL of urine was extracted with 600 μL of diethyl ether/hexane (90:10 v/v). The aqueous phase was then further extracted by adding 600 μL of chloroform/butanol (50:50 v/v) and collecting the organic phase. The collected fraction was dried and resuspended with in 70%/30% methanol/water with the addition of the testosterone-d_9 sulfate internal standard. Such modest purification methods may be generally needed in such cases to rid biological samples of high concentrations of salts and other contaminants. In the example of Figure 7, the same amount (316 fmol) of the same internal standard (pregnenolone-d_4) was used as in the calibration curve, and samples from males and females were found to contain different amounts of testosterone sulfate (m/z 367), Other conjugated steriods such as androsterone sulfate, 3-hydroxy-5-androstan-17-one sulfate, or 17-hydroxy-5-androstan-3-one sulfate (m/z 369) and 3-hydroxy-17-oxoandrost-5-en-19-al sulfate (m/z 381) were assigned based upon their known masses. On the basis of an experimentally determined extraction recovery

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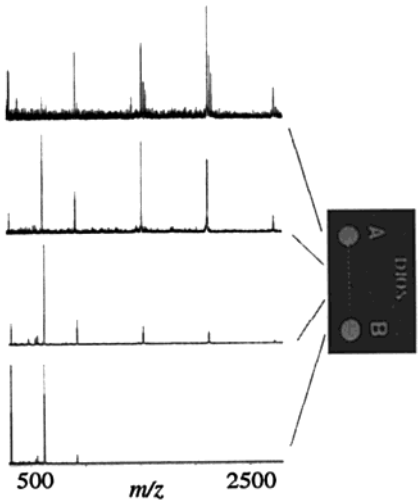

Figure 9. Porous silicon as a medium to separate different components. A mixture of compounds (from an Agilent calibration mixture) was placed at point A and allowed to spread across the sample plate to point B. DIOS-MS was used to analyze the partially separated components at 0, 3, 6, and 10 mm from point A to point B.

of 83%, the concentration of testosterone sulfate was found to be 487 ng/mL in the male urine sample. Interestingly, DIOS post-source decay analysis of the peak at m/z 352 revealed a loss of 79 Da (corresponding to PO_3^-) and no other fragmentation, suggesting that the signal originated from a phosphorylated compound. Other steriods, in addition to testosterone sulfate, were also identified on the basis of known masses of conjugated steriods and further confirmed by electrospray tandem mass spectrometry.

Postsource Decay. PSD measurements can be used for molecule structure analysis from the DIOS surfaces in a manner very similar to that observed with MALDI. However, the DIOS technique can accommodate small molecules whereas MALDI often cannot, due to low-mass interference of matrix-derived ions. Analysis of identical samples by PSD with MALDI and DIOS, and collision-induced dissociation with an electrospray triple quadrupole instrument, reveal similar fragmentation patterns with the ESI-MS/MS technique showing more fragments, especially in the low-m/z region (Figure 8).

Chromatography. Like many high-surface-area materials, porous silicon has the ability to separate molecules according to their physical characteristics[32] and is easily chemically modified. Given its ability to support direct desorption/ionization mass spectrometry, the use of porous silicon as a microscale chromatographic platform is potentially useful. As shown in Figure 9, we have found that a mixture of compounds (from a proprietary Agilent electrospray calibration mixture) undergoes a crude separation as the drop containing the analytes spreads across the freshly etched DIOS plate. The lower mass components of the mixture migrate farther than the larger components and can be detected directly by DIOS after the solvent has evaporated. For this reason strong signals for small hydrophilic compounds are often found on the outer edges of dried sample droplets.

CONCLUSIONS

The initial stages of development described here show the DIOS-MS technique to have broad potential for the direct analysis of biomolecules and small organic structures. The formation and treatment of the porous silicon surface determines the quality of the DIOS spectra obtained from that surface, and characterization of surface morphology has started to illuminate correlations between porous silicon structure and its DIOS-MS utility. This work, as well as other investigations of the desorption and ionization mechanisms of DIOS, is ongoing in our laboratories. In addition to the direct desorption/ionization properties of DIOS, the unique properties of porous silicon can allow for the inclusion of specific surface modifications to meet select chemical, biochemical, and analytical needs. Such modifications are crucial to our current efforts to apply DIOS-MS to proteomics, solid-phase syntheses, and combinatorial library analysis. Combined with improvements in chromatographic properties, DIOS may provide an important component of laboratory-on-a-chip devices.

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