

Selective patterning of Si-based biosensor surfaces using isotropic silicon etchants

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ABSTRACT

Ultra-sensitive, label-free biosensors have the potential to have a tremendous impact on fields like medical diagnostics. For the majority of these Si-based integrated devices, it is necessary to functionalize the surface with a targeting ligand in order to perform specific biodetection. To do this, silane coupling agents are commonly used to immobilize the targeting ligand. However, this method typically results in the bioconjugation of the entire device surface, which is undesirable. To compensate for this effect, researchers have developed complex blocking strategies that result in selective patterning of the sensor surface. Recently, silane coupling agents were used to attach biomolecules to the surface of silica toroidal biosensors integrated on a silicon wafer. Interestingly, only the silica biosensor surface was conjugated. Here, we hypothesize why this selective patterning occurred. Specifically, the silicon etchant (xenon difluoride), which is used in the fabrication of the biosensor, appears to reduce the efficiency of the silane coupling attachment to the underlying silicon wafer. These results will enable future researchers to more easily control the bioconjugation of their sensor surfaces, thus improving biosensor device performance.

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1. Introduction

Label-free detection of antigens, bacteria, and viruses for medical diagnostics requires the development of both highly sensitive and selective biosensors [1,2]. Numerous high sensitivity biodetection platforms have been developed, leveraging fundamentally different physical principles, including mechanical, optical, and electrical signal transduction mechanisms. For example, by monitoring the mechanical vibrational frequency of silicon microcantilevers, highly specific detection of prostate specific antigen (PSA), at clinically relevant levels, has been demonstrated against a background of human serum proteins [3]. Additionally, by tracking electrical signals, an In_2O_3 nanowire FET sensor, which was integrated on a silicon substrate, has shown detection of N-protein, a SARS biomarker [4]. Lastly, by following the optical resonant frequency of silicon microring resonators, detection of a panel of cancer biomarkers has been confirmed [5].

All of these methods rely on combining a highly sensitive signal transducer with a biological targeting strategy, usually accomplished via the immobilization of a biological probe molecule to the transducer surface, to induce high selectivity toward a target of interest [1,2,6]. Choosing the appropriate targeting strategy, and hence the proper immobilization technique, is crucial, as many factors can affect the ability of the probe molecule to selectively

detect the biological target [7–9]. These include the orientation and density of the probe molecule on the transducer surface, the pH of the medium around the probe, the expected concentration of the target of interest (usually determined by biologically relevant conditions), the actual operating conditions required to produce a signal, and finally, the impact of the targeting strategy on the sensitivity of the signal transducer. Selecting the appropriate targeting strategy will help balance competing factors and lead to the development of highly sensitive and selective biosensors [10–12].

As biosensor platforms become increasingly involved, for instance, via multiplexing capabilities, the need to further refine these targeting strategies by performing selective patterning of probe molecules onto the transducer surface becomes paramount [13–16]. Selective patterning's advantage is that it allows for highly targeted detection, increasing sensitivity by eliminating possible signal shifts in optical devices due to residual probe molecules [14–16]. For many applications, patterning may be accomplished by spotting the surface using ink-jet or microcontact printing to less than 100 nm resolution [17], but this method can potentially damage the underlying sensing device, particularly if that surface is patterned with small-scale features [18]. Alternatively, novel surface chemistries, which can avoid mechanical damage of the transducer surface that can occur during spotting, have been created to allow for spatial patterning using combinations of blocking agents and functional probe molecules [19–21]. For on-chip biosensors created from the common silicon/silica material system, organosilane coupling agents, such as glycidoxypolytrimethoxysilane (GOPS/

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GPTMS), aminopropyltrimethoxysilane (APTMS), and carbonyldiimidazole (CDI), are commonly used to tether various probe molecules to the surface [12]. To achieve selective patterning, a process involving a blocking agent is required to prevent the substrate from being functionalized in the inactive regions of the biosensor platform [13]. After functionalizing the biosensor platform in the active regions, the blocking agent is typically removed from the substrate, often using a complicated chemistry. These processes to remove the blocking agent have the potential to either damage the signal transducer, thus negatively impacting its performance during detection, or leave blocking agents in place, resulting in potential interference with other chemical processes during the platform development or with non-targeted biological species during detection. Therefore, creating a selective patterning method that relies on neither complex chemistries nor mechanical spotting, but instead occurs simultaneously with the fabrication of the signal transducer itself (for instance, via proper material choice), would be highly beneficial for a variety of label-free biosensor platforms.

Recently, our laboratory developed an approach for the covalent bioconjugation of probe molecules to the surface of ultra-sensitive optical biosensors (silica microtoroids fabricated on a $\langle 100 \rangle$ silicon substrate) based on the facile combination of organosilane coupling agents and *N*-hydroxysuccinimide (NHS) ester chemistry [6,22]. This highly efficient and robust bioconjugation method is universally applicable to any silica-based signal transducer and results in uniform monolayers of the probe molecule on the transducer surface. This paper presents the results of combining this facile bioconjugation technique with a XeF_2 gas phase etching technique, applied during signal transducer fabrication, to create a simple and novel method to selectively pattern the surface of silica/silicon biosensors, without requiring a *posteriori* modification.

2. Materials and methods

The detection platform is the on-chip microtoroid, a Whispering Gallery Mode optical resonator (Fig. 1a). This device is made from $\langle 100 \rangle$ silicon wafers covered with a $2\ \mu\text{m}$ thermal oxide (Montco Silicon, p-doped). The fabrication of this device is accomplished in three simple steps: (a) silica patterning via typical lithographic and buffered oxide etchant (BOE) techniques to form 100–200 μm diameter pads of silica on the silicon surface, (b) selective dry-etching of the silicon, via a pulsed XeF_2 etcher, thus undercutting the silica pads to form silica microdisks supported on silicon pillars, and (c) reflowing, via a CO_2 laser (Synrad, 50 W), to form silica microtoroids [23]. Among the various gas phase, automated Si-selective etchers, XeF_2 etchers are extremely simple and robust, accepting numerous types of substrates and material combinations. Therefore, they are routinely found in cleanroom and nanofabrication facilities. However, due to simplicity, KOH remains the more commonly used Si-etchant when fabricating microcantilever and nanowire sensors [24,25]. The motivation for this substitution is that XeF_2 is a gas-phase, isotropic etch whereas KOH is a liquid, anisotropic etch. However, both etchants are highly selective for silicon over silica [26].

A fabricated toroid is then functionalized with probe molecules using a simple three-step process shown in Fig. 1: (a) O_2 plasma is used to form hydroxyl groups on the silica surface (200 mTorr, 120 W, 2 min); (b) an organosilane coupling agent, aminopropyltrimethoxysilane (APTMS, Aldrich), is coupled to the surface via hydrolysis and condensation reactions with the surface hydroxyl groups using vapor deposition techniques (aspirator vacuum, 15 min), thus aminating the surface; and (c) the primary amines are reacted with NHS ester groups attached to the probe molecule of choice, forming stable amide bonds between the probe and the surface. Unlike previous methods, which were typically based on

physisorption rather than covalent bonding, this technique did not negatively impact the optical performance of the sensor, allowing it to maintain its sensitivity during detection.

As can be observed from the fluorescent labeling studies (used to confirm the bioconjugation of the probe molecules to the signal transducer surface Fig. 1), the biomolecules selectively patterned the sensor surface and preferentially bound to the silica signal transducer, rather than the underlying silicon substrate. This selectivity is clearly a benefit; however, it is also extremely surprising, because the silicon should have a native oxide layer on the surface and therefore should behave in a similar manner to the silica during functionalization [27]. In fact, in other integrated sensor devices, uniform surface functionalization has been observed as a result of this native oxide layer [28–30]. Therefore, to verify that selective patterning was occurring, several possible mechanisms for the lack of fluorescent labeling, as quantified by regions of varying fluorescent intensity, were investigated, including the quenching of the fluorescent signal from the dye by the silicon (a possible experimental artifact, rather than actual patterning), actual patterning during the bioconjugation step due to differences in surface roughness between the substrate and device, or due to differences in silicon etching conditions (for instance, comparing KOH etching with XeF_2 etching), and lastly, actual patterning during the bioconjugation step due to chemical passivation (or activation) of the silicon substrate (or silica transducer surface) by the XeF_2 etchant. Due to comparisons with other systems that do not see this type of patterning, our initial hypothesis centered on the use of the XeF_2 etchant as the probable cause of this unique behavior.

A series of experiments were performed that mimicked the standard process for fabricating and functionalizing the optical sensor (Fig. 2). Two wafer sample sets were prepared using $\langle 100 \rangle$ silicon with $2\ \mu\text{m}$ of thermal oxide as the substrate. The first set of samples followed the standard fabrication process described above (resulting in a silica on silicon surface), while the second set of samples had the oxide completely removed using buffered oxide etchant (resulting in a silicon surface with a native oxide layer). The wafers were then diced into $1\ \text{cm}^2$ samples, and the two sample sets were further subdivided: one set of silicon wafers was exposed to XeF_2 etching, again mimicking the standard fabrication process, while another was exposed to KOH etching (note that neither chemistry appreciably etches silica) [26]. Each set contained five diced samples. The XeF_2 etching of the wafers consisted of twenty pulses at 80 s each, at a pressure of 2800 mTorr, in a custom XeF_2 etcher. The KOH etching was done in a 40 wt% KOH solution at $90\ ^\circ\text{C}$ for 14 min with constant stirring.

Following the respective silicon selective etching steps, each set of samples underwent surface functionalization. First, O_2 plasma treatment was used to form hydroxyl groups on the surface. This process creates a monolayer of covalently bound oxygen on the surface of the wafer [31]. The bonds of this monolayer are highly strained and readily react with available atmospheric H_2O to form hydroxyl groups on the surface. The hydroxyl groups were reacted with the silane coupling agent, APTMS, in a vacuum desiccator for at least 15 min to form a linker between the inorganic substrate and an organic end group (in this case, a primary amine).

3. Characterization and data analysis

To determine the efficacy of the binding reaction, fluorescent imaging was performed on the amine-functionalized surfaces using fluorescein isothiocyanate (FITC) dye, which readily reacts with primary amines. Atomic Force Microscopy (AFM) measurements were also taken to compare the surface roughness of the samples. Finally, to characterize the surface composition of the wafers, X-ray photoelectron spectroscopy (XPS) was performed at

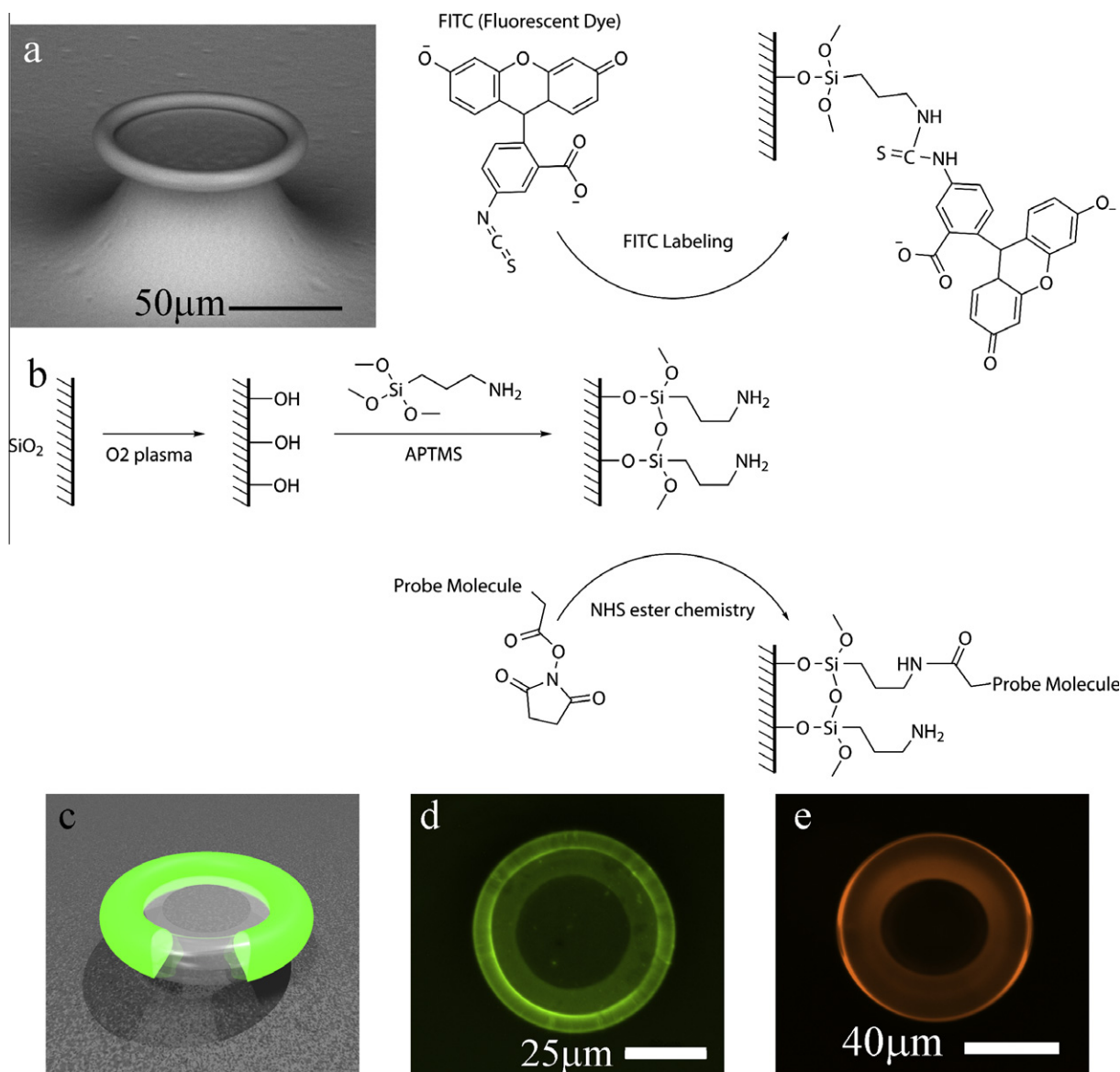


Fig. 1. (a) Scanning Electron Micrograph of the microtoroid optical device. (b) Bioconjugation protocol, which includes the hydroxylation, amination, and biotinylation via NHS Ester chemistry. Fluorescent imaging can be performed using a labeled probe molecule. (c/d/e) From right to left: (c) rendering of a Fluorescein isothiocyanate (FITC)-labeled toroid indicating where the dye molecule binds on the device, (d) fluorescent image of a FITC labeled device, and (e) fluorescent image of a Texas Red labeled device. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

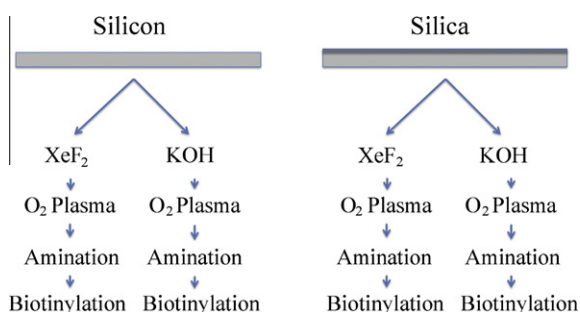


Fig. 2. Overview of the experimental procedures to elucidate the underlying mechanism of the selective patterning. Several controls are present in these experiments. For example, an alternative silicon etchant (KOH) was studied and all experiments were performed on both a bare silicon wafer and a wafer with 2 μm of thermal oxide on the surface.

three steps throughout the process: after etching, after plasma treating, and after amination.

One of the primary concerns was that the selectively patterning observed in Fig. 1 was due to fluorescence quenching and that the bioconjugation was present throughout the sample, thus making the apparent patterning an artifact rather than actual patterning. Fluorescence quenching has been observed on silicon wafers previously [32,33]. Therefore, to verify that fluorescence quenching was not occurring and that selective patterning was taking place, fluorescent microscopy images were taken of aminated silica and aminated silicon samples, which were processed using either KOH or XeF₂ and subsequently labeled via the covalent interaction of the primary amines with the fluorochrome FITC. This labeling location mimics where the bioconjugation step would occur. All images were taken with identical microscopy parameters (gain, intensity, exposure time, etc.). In order to normalize the data, background samples were created by repeating the surface functionalization process, without the silane linkage step. Removing the silane linkage step ensures that the intensity measured for the background samples is a result of the intrinsic “brightness” (reflection) of the

wafer, chemisorption, and physisorption, and not the chemical bonding of the probe molecule.

As can be observed in Fig. 3, the fluorescence intensity for the silica and silicon samples etched with KOH is nearly identical, indicating that the density of the probe molecule is the same for both the silica and the silicon. Additionally, this confirms that the silicon is not quenching the fluorescence signal from the FITC probe molecule. However, there is a significant intensity difference between the XeF₂-etched silica and silicon samples, with minimal binding occurring on the silicon. Therefore, this fluorescence data verify that fluorescence quenching is not occurring and that actual patterning is occurring, most likely due to the use of XeF₂ during etching.

To determine whether this patterning was the result of fabrication-induced surface roughness due to the KOH and XeF₂ etching systems, AFM measurements were taken on the sample sets. The root-mean-squared (rms) roughnesses on a bare SiO₂ wafer (not exposed to any etchants) and the silicon wafer were 1.79 nm and 1.58 nm, respectively. For the wafers exposed to either etchant, the rms increased, in proportion to the exposure time or etch depth. For example, a KOH-etched wafer with a 10 µm etch depth had an rms of 8.765 nm, and a XeF₂-etched wafer with a 10 µm etch depth had an rms of 11.844 nm. Therefore, there is no appreciable difference in the surface roughness between the KOH and XeF₂ etched samples. As a result, surface roughness was eliminated as a cause of selective patterning.

To probe the potential for chemical passivation (or activation) due to etching with XeF₂, XPS measurements of the silicon wafer were performed at several steps in the fabrication process, including the XeF₂-etching (Fig. 4). XPS reads to a surface depth of approximately 100 Å and offers a significant amount of information about the surface chemistry. As can be seen in Fig. 4, after XeF₂ etching, the wafer shows a fluorine peak, due to the presence of fluorine created during the etching process. XeF₂ etching is known to add several fluorosilyl layers, approximately 10–20 Å thick in entirety, to the surface [34]. This etching mechanism begins with XeF₂ attacking the dangling bonds of the Si dimers by means of atom abstraction [35,36]. After saturating the dangling bonds, at about a monolayer of coverage, fluorine begins to attack the Si–Si σ -dimer and σ -lattice bonds [37]. This process creates the fluorosilyl layers, consisting, in succession, of SiF, SiF₂, and SiF₃, with each layer being created as a consequence of the previous

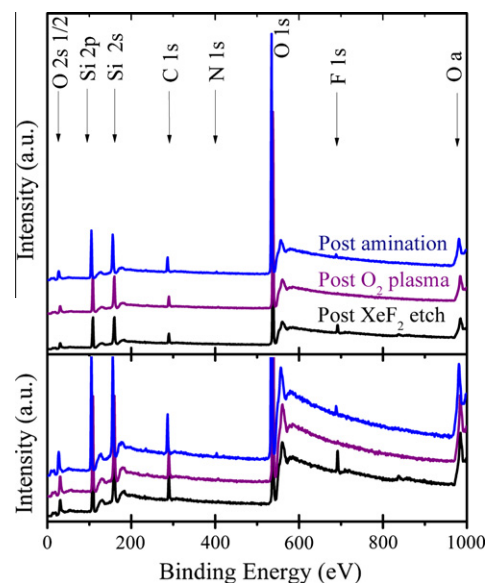


Fig. 4. XPS for a silicon wafer etched with XeF₂. Top: three different samples are plotted for straightforward comparison: post-XeF₂ etch, post-O₂ plasma treatment, and post-amination. Bottom: to reveal the details of the spectra, the data presented in the top graph are re-plotted. The post-XeF₂ etch wafer shows a strong fluorine peak, which is masked during O₂ plasma, and slightly returns after amination.

layer's saturation. Once formed, leaving groups consisting primarily of SiF₄ and Si₂F₆ enable the etch process [38,39]. Thus, while this peak appears to be removed by the O₂ plasma, the monolayer of covalently bound oxygen deposited could simply be temporarily masking outermost portion of the fluorosilyl layer. This is suggested by the reappearance of a small fluorine peak (Fig. 4) post-amination. In all cases, XPS shows that the amination is either unsuccessful (lack of amine peak) or very poor (very low amine peak).

From these data, it appears that the cause of the selective surface patterning during bioconjugation may be the presence of the underlying fluorosilyl layer. This layer can interfere with the first step in the bioconjugation process (the hydroxylation of the surface), thereby “blocking” all subsequent steps. Specifically, the fluorosilyl layers, by means of either steric hindrance or fluorine's unique properties (high electronegativity), could prevent the linkage of the organosilane to the surface. Regardless of the mechanism, the simple expedient of using XeF₂, in place of KOH, as the silicon etchant is a facile, universal way to selectively pattern the surface of silica/silicon signal transducers.

4. Conclusion

In summary, we have demonstrated that XeF₂ can be used to reduce or completely block the bioconjugation of silicon surfaces, when the surface chemistry relies on the presence of hydroxyl groups to bridge the inorganic substrate and the biological probe molecule. We have developed a mechanism to potentially explain this behavior and verified it through XPS and fluorescence microscopy, as well as a comparison study with KOH. From the data, we concluded that the reduction in the density of hydroxyl groups on the silicon surface enables the selective patterning of silicon surfaces. This simple method, which can be applied during transducer fabrication, is an excellent alternative to conventional spotting or surface chemistries that promote surface patterning. The use of this technique will allow researchers to develop more targeted bioconjugation strategies, such as selectively conjugating targeting ligands to a sensor surface and attaching blocking moieties to the

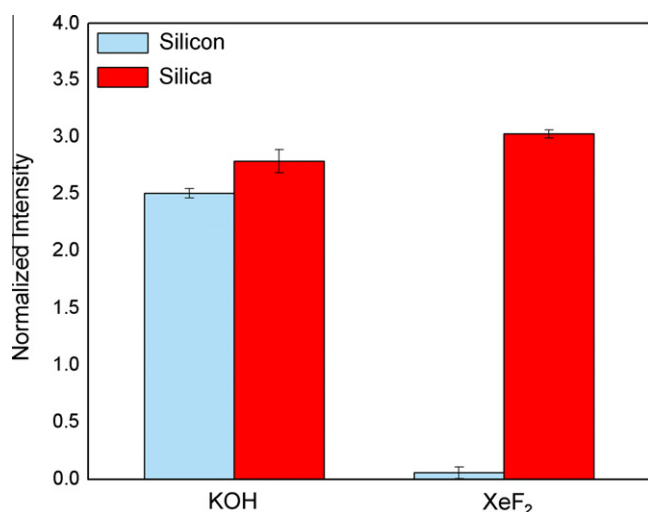


Fig. 3. Normalized intensity data from fluorescent microscopy. From the relative intensities of the fluorescence, which is an indication of density of amine groups on the surface, it is clear that selective patterning does not occur when KOH is the silicon etchant. However, the patterning does occur with XeF₂ is the etchant.

substrate in a single step. As this method can be used to improve the performance of any non-silicon transducer that is fabricated on a silicon substrate, it will find applications throughout the sensing and diagnostics communities [1,2].

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