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Exploiting the 3-Aminopropyltriethoxysilane (APTES) autocatalytic nature to create bioconjugated microarrays on hydrogen-passivated porous silicon☆

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ABSTRACT

Porous silicon (pSi) based microarrays are attractive because pSi: (i) can be modified in many ways, (ii) possesses a high surface area, and (iii) exhibits strong photoluminescence (PL). These characteristics make pSi-based microarrays candidates for a host of applications including sensing, optoelectronic devices, and photodetectors. Microarray fabrication requires a high-throughput approach to produce chemically modified, spatially isolated spots on a particular substrate. The most stable platforms are characterized by covalent attachment to the substrate. In this paper we exploit the autocatalytic nature of 3-aminopropyltriethoxysilane (APTES) to contact pin-print APTES directly onto as prepared, H-passivated pSi (ap-pSi) without the need for a formal oxidation step. We assess the APTES-derived spots by using PL and Fourier transform infrared spectroscopy (FT-IR) imaging and determine the spot size and spatial homogeneity. All APTES-derived spots exhibited two distinct regions; a silanized core surrounded by an oxidized halo. By decreasing the APTES concentration and increasing the acid concentration, the oxidized halo size decreased by 60%; however, the silanized core diameter remains APTES and acid concentration independent. Bioconjugation can be achieved to all APTES-derived features; however, the highest biomolecule loading was realized by using pure APTES. Together these experiments demonstrate an easy and simple strategy for creating protein microarrays on pSi.

1. Introduction

Microarrays are powerful tools for simultaneous multi-analyte detection, quantification, and high-throughput materials screening [1–8]. Spot size is an important factor in microarray development because it dictates spot density (D, spots/cm^2) and, ultimately, platform footprint [9–12]:

$$D = 100\left(\frac{1000}{CTC}\right)^2 \quad (1)$$

As seen in Eq. (1), D is limited by the center-to-center spacing (CTC) between non-overlapping spots within an array. Typically, CTC is given by the actual spot diameter multiplied by a factor of 1.2–1.4 (i.e., a 20–40% buffer between spot edges). As an example, microarrays that have 100-μm spot diameters yield D values between 5100 and 6900 spots/cm^2. Thus, for high density microarrays, producing spots with small diameters is paramount and doing so rapidly has obvious advantages.

Porous silicon (pSi) has served as a platform for chemical sensing, optoelectronic devices, and photodetectors [13–16]. The attraction arises, in part, because one can easily tune the pSi photoluminescence (PL) (e.g., emission maximum, excited-state lifetime, and quantum yield) by changing the nanocrystallite feature size and surface chemistry [17–19].

Since the late 1990's there has been growing interest in using pSi in microarray platforms because pSi is bio-compatible, it has a large surface area, and its surface can be readily modified [6,7,20]. The first generation pSi-based microarrays exploited pSi's high surface area for antibody immobilization, but relied on extrinsic fluorescent labels for detection [21,22]. More recently, researchers have developed pSi-based microarrays that permit label-free or multimode detection [23–30]. These approaches monitor changes induced by the presence of chemical and biological species by using Fourier transform infrared (FT-IR) absorbance, impedance, PL, or refractive index changes. While these newer approaches have moved away from extrinsic labeling, existing approaches can involve complex, multi-step fabrication protocols [23,26,27].

We seek to develop a label-free pSi-based microarray strategy that has high spot density and that is easy to create with few steps. Previously, we reported an approach to create microarrays on oxidized

☆ Dedicated to Professor Gary D. Christian on the occasion of his 80th Birthday. FVB is Prof. Christian's academic grandfather through Professor Linda B. McGown.

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pSi (ox-pSi) by exploiting simple silanization chemistry coupled with a high-throughput contact pin printing (CPP) [31]. In our original report [31] we used O_3 to oxidize as prepared, H-passivated pSi (ap-pSi) and produce surface Si-OH residues that were then chemically modified by contact pin-printing an organically-modified silane (ORMOSIL) [20,32–35]. In subsequent research we showed [36] that neat, contact pin-printed 3-aminopropyltriethoxysilane (APTES) reacted very rapidly (< 1 min) with ox-pSi to create well-defined APTES-derived spots on ox-pSi. However, because APTES is autocatalytic [33,36–38] and produced an abundance of EtOH/H_2O during hydrolysis, condensation, and polycondensation [33,36] we discovered that the area immediately outside the APTES-derived spots exhibited substantial additional oxidation that arose from the local EtOH/H_2O release [36,39–41].

In this paper we evaluate APTES that has been contact pin-printed directly onto ap-pSi. We report how the APTES and acid concentration affect the resulting spot size, functional group spatial distribution, and bioconjugation potential. Here, we find that APTES causes the ap-pSi to rapidly undergo in situ oxidation at the pin contact/APTES delivery site followed by silanization, avoiding the need to create ox-pSi prior to CPP.

2. Materials and methods

2.1. Chemicals and reagents

The following chemicals and reagents were used: p-type B-doped < 100 > Czochralski prepared silicon wafers (8–12 Ω cm, 660–690 μm thick, Silicon Quest International); HF (48–51%, ACROS); pentanes (99.7%), HCl (36.5%), and $Na_2HPO_4{\cdot}7H_2O$ (Fisher Scientific); GaIn eutectic, glutaraldehyde solution (GA, 25% in water), and bovine serum albumin-fluorescein isothiocyanate conjugate (BSA-FITC, ≥ 7 mol FITC: mol albumin) (Sigma); $NaH_2PO_4{\cdot}H_2O$ (J.T. Baker); 200 proof ethanol (Decon Labs); and APTES (> 95%) (Gelest). (Safety note: HF is a weak mineral acid that is extremely corrosive, attacking glass. It has a pungent odor and can irritate the throat and nose at > 3 ppm. Contact with live tissue can cause necrosis and pain. Safety gloves and goggles are used while working with HF. Only Teflon vessels are used with HF in the current research.)

2.2. pSi fabrication

As-received crystalline Si (cSi) wafers were cut into 1.8 cm × 1.8 cm squares using a glass slide cutter (OM Laboratory, Chigasaki). The wafer matte surface was then cleaned with silicon carbide sandpaper (1200 grit) to improve contact to a copper sheet which served as the anode collector. To further facilitate collector-to-anode (Si wafer) contact, a thin layer of GaIn eutectic was applied to the wafer matte side. The wafer was then placed within a custom-made Teflon etching cell and the cell was filled with etching solution (1:2 (v: v) HF: ethanol). A platinum wire loop lowered into the etching solution served as the cathode. A 21 mA/cm^2 etching current density was maintained throughout the etching process by using a DC power supply (Keithley, model 2400-LV).

After etching for 2.5 min, the power supply was turned off and the etching solution was carefully removed from the etching cell. The ap-pSi was then removed from the cell and soaked for 2.5 min in each of the following solutions (20 mL) in the order listed: 1:2 (v: v) H_2O: ethanol, ethanol, and pentanes. The final ap-pSi material is delicate; failure to follow the above solvent rinsing/washing sequence leads to heavily cracked ap-pSi. The ap-pSi was then placed in an evacuated desiccator (< 150 Torr) for at least 15 min to remove residual solvent. For long-term storage all pSi samples were kept in an evacuated desiccator.

Table 1
Composition of APTES-based solutions used for CPP on ap-pSi.

Name	APTES (mL)	EtOH (mL)	HCl in H_2O (mL)
100% APTES	1.00	0	0
90% APTES	0.90	0.10	0
80% APTES	0.80	0.20	0
70% APTES	0.70	0.30	0
60% APTES	0.60	0.40	0
50% APTES	0.50	0.50	0
40% APTES	0.40	0.60	0
30% APTES	0.30	0.70	0
20% APTES	0.20	0.80	0
10% APTES	0.10	0.90	0
18% APTES	2.68	12.37	0
Sol 1	2.68	10.56	1.81[a]
Sol 2	2.68	10.56	1.81[b]
Sol 3	2.68	10.56	1.81[c]

[a] 0.67 M stock.
[b] 1.34 M stock.
[c] 2.00 M stock.

2.3. Contact pin-printing

We previously reported on CPP ORMOSILs onto ox-pSi [31,36] and hydrosilylating agents onto ap-pSi [12] by using a ProSys 5510 pin printer (Cartesian Technologies). For the current research the CPP conditions are as follows: 200 μm diameter solid tungsten pin (Point Technologies); print chamber relative humidity, 37 ± 5%; temperature 290–300 K; pin velocity, 10 mm/s; pin acceleration, 400 mm/s^2; pin-to-surface contact time, 7 ms; and *z*-distance travel, 44 mm. A dry print was included in each CPP cycle as a blank to assess background oxidation and any pin-induced damage. Table 1 reports the APTES-based solution compositions used in this research. Neat APTES was printed as-received from the manufacturer. The 10–90% APTES solutions were prepared in EtOH. All APTES sols were prepared following a previously described approach [42]. Briefly, while stirring, the following were added sequentially: APTES, EtOH, and HCl. Afterwards, the sol was stirred for an additional 1 h. (Note: 2.0 M HCl stock solution was the upper concentration limit tested. Above this concentration, sols rapidly gelled/precipitates formed resulting in poor spot-to-spot reproducibility.)

After CPP, the deposited material was allowed to react under ambient conditions for 2 h. The microarrays were then rinsed by using the same solvent sequence described earlier. Microarrays were stored in the dark under ambient conditions. (Note: These microarrays were all robust; the APTES-derived spots did not exhibit any flaking.) Control experiments demonstrate no detectable spot spreading during rinsing.

2.4. BSA-FITC bioconjugation

All BSA-FITC immobilization steps were carried out in aqueous phosphate buffer (0.1 M, pH = 7.00). Briefly, APTES microarrays were pre-treated by immersion in 2.5% GA solution for 1 h followed by triplicate rinsing with aqueous buffer. The GA-treated microarray was then incubated in 50 μM BSA-FITC at room temperature for 1 h followed by triplicate rinsing with aqueous buffer. Bioconjugated microarrays were immediately immersed in buffer and imaged following preparation.

2.5. Instrumentation

The APTES-derived microarrays were assessed by profilometry, PL, and FT-IR spectroscopy. Spot heights were determined by using an Alpha Step IQ surface profiler (KLA Tencor) with a 100 μm/s scan rate, 1000 Hz sample rate, and 0.1 μm *x*-direction spatial resolution. PL measurements were performed by using an epi-luminescence