ABSTRACT

We demonstrate the temperature mediated applications of a previously proposed novel localized dielectric heating method on the surface of a silicon field effect transistor and perform modeling and characterization of the underlying mechanisms. Two important applications spanning different temperature ranges are shown: a heat-mediated DNA exchange reaction and the complete desorption and reattachment of chemical functionalization from the transistor surfaces for biosensor platforms. Our results show that the use of silicon transistors can be extended beyond electrical switching and field-effect sensing to performing ultra-localized temperature controlled chemical reactions on the transistor itself.

KEYWORDS: Sensors, FET, heating, fluid, localized

INTRODUCTION

After demonstration of their use as ultra-sensitive biosensors[1], silicon field-effect transistors have found many applications in biology including DNA or protein assays, early cancer diagnosis[2-3], and lab-on-a-chip components. Because nearly all bio-chemical reactions are temperature dependent, lab-on-a-chip devices often require precise control of fluidic temperature. We recently reported a technique for the dual use of silicon field effect biosensors as localized heating elements[4]. The applications for such a heating technique extend from localized biochemical reactions on chip to densely integrated sensors for the simultaneous detection of many distinct analytes. In this work, we present two such applications. We performed a temperature-mediated exchange reaction on the silicon transistor surface with high spatial confinement, a step towards biochemical reactions on chip, such as local polymerase chain reaction (PCR) on chip with integrated sensing[5]. We also demonstrate the patterning of distinct probes on two sensing elements in close proximity. The precise patterning of a selective functional layer with nanoscale spatial resolution is critical for dense arrays of biosensors. Current techniques are not self-aligned[6-10], lack high spatial resolution[11], are limited to specific chemistries [12-13] with unknown reliability[14], or are limited in their operational environment[15]. A versatile, self-aligned and high-spatial resolution technique is needed.

EXPERIMENTAL

Unless otherwise noted, all chemicals were purchased from Sigma-Aldrich and were used without further purification. Peptide nucleic acid (PNA) was purchased from and purified via high performance liquid chromatography (HPLC, >95%) by Applied Biosystems. PNA was characterized by mass spectroscopy; theoretical: (m/z) 2961.90, observed: (m/z) 2961.2. Locked nucleic acid (LNA) was purchased from and purified via HPLC by Sigma-Prologi. LNA was characterized by MALDI-MS; LNA6-BHQ theoretical: (m/z) 2622, observed: (m/z) 2642; LNA8 theoretical: (m/z) 2662, observed: (m/z) 2660. All DNA was purchased from and purified via HPLC by Sigma Genosys, a part of Sigma Aldrich. All fluorophore and amine modifications were performed by Sigma Genosys as well.

RESULTS AND DISCUSSION

To demonstrate the utility of the heating technique for bio-chemical applications, we have carried out a temperature-mediated exchange reaction as a model reaction on the surface of transistors (Fig. 1a). The scheme involves selectively exchanging a higher affinity molecule (8-mer LNA) with a lower affinity molecule (6-mer LNA) already bound to a surface capture probe (PNA) by enhancing the local reaction rate due to the local temperature. We monitor the reaction by modifying the PNA probe with a fluorophore, and modifying the 6-mer LNA with a black hole quencher (BHQ). The decrease in the fluorescence intensity indicates the presence of the 6-mer LNA-PNA duplex due to the quenching effect. The recovery of the fluorescence indicates the presence of the 8-mer LNA-PNA duplex.

The spatially localized reaction on the surface of a device was carried out by covalently attaching the lysine-modified PNA molecule (FAM-PNA8) and subsequently incubating with the 6-mer LNA (LNA6-BHQ) to form a duplex on the surface, resulting in decreased fluorescence compared to the PNA alone. With the application of 50°C on the device (20 µm) for 20 minutes in the presence of the buffer solution containing 8mer LNA (LNA8), the heat-mediated exchange reaction took place on the devices resulting in a net increase in the fluorescence intensity on the device (Fig. 1b, c).

The heating technique can also be utilized for carrying out reactions at superheated temperatures by applying higher voltages. As one potential application of superheating, we demonstrate the regeneration of the surface of the sensor by decomposing the species attached on the surface via electrically addressable heat to the target device (Fig. 2a, b). The temperature-mediated removal of the molecules is consistent with earlier reports that DNA oligomers covalently attached to silane self-assembled monolayers on oxidized silicon substrates and glass slides are unstable over 80°C [16]. By at-
taching both the probe and the complementary target on the surface, we studied the removal of the molecules from the surface.

The heat-mediated desorption of the molecules can also be used to perform selective functionalization of surfaces, which is very important for the realization of dense arrays of nano-biosensors. Attachment of two distinct DNA probes was demonstrated on adjacent devices by decomposing the species attached on the surface via electrically addressable heat to the target device, followed by repeating the molecule attachment protocol with a distinct probe molecule (Fig. 2c, d). The selectivity of the attachment to the heated device suggests that the fluorescence intensity decrease after the heating is due to the complete removal of previously immobilized molecules from the heated devices. Further, refunctionalization experiments with the omission of the initial step of the functionalization process did not result with the immobilization of the new probe (Fig. 2e). Two sets of chips (Set A and Set B) were functionalized and desorbed, following an identical procedure to that outlined in the manuscript. The chips were first functionalized with a linear probe sequence D modified with a HEX fluorophore. Later, one device on the chip was heated to remove the previously immobilized probe, and the device was refunctionalized with linear probe sequence C modified with a FITC fluorophore. The refunctionalization of chips in Set A and Set B was carried out identically, with the exception of an omission of the 3% 3-aminopropyltrimethoxysilane in the silanization buffer for Set B. The results are shown in Figure 2e. The figure demonstrates the refunctionalization of Set A with the FITC labeled probes, while Set B registered no increase in the fluorescence level (some red fluorescence is observed in the heated device, and some green intensity is registered in the non-heated device as a result of bleed through of fluorescence through the filters). These experiments suggest that the surface is available for silanization after heating, since all the chemistry steps, including the silanization, are needed to refunctionalize the surface of the devices.
species from devices. c: Schematic demonstrating the procedure for decomposition and refunctionalization of devices.
d: Fluorescence micrographs showing the selective removal and refunctionalization of the devices, resulting in the patterning of 2 distinct probe molecules. e: Control experiment showing that the refunctionalization requires the silanization step to be successful.

CONCLUSION
In summary, our results indicate a high potential for wide ranging applications and high amenability of this method for further scaling down both the dimension of and the separation between devices, and increasing the spatial and temporal resolution of the technique. These field effect sensors with localized heating capability can be used to develop novel tools to be used in lab-on-a-chip systems such as highly integrated PCR systems, active sensor surfaces that can be regenerated, creating localized heat-mediated incisions on single cells, and dense sensor arrays for multiplexed and label free detection of biomolecules.

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